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INDIAN NATIONAL SCIENCE ACADEMY SPONSORED REPORT

ON

NEW ANTIMALARIAL DRUG DISCOVERY IN INDIA AND FUTURE STRATEGY FOR MALARIA CONTROL

PROJECT PRINCIPAL INVESTIGATOR

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PREFACE

This Status Report on ”Antimalarial Drug Discovery in India” has been sponsored by the Indian National Science Academy, in order to focus on the country’s progress in the field of Antimalarials Drug Discovery for malaria control in India, specifically for the development of new powerful antimalarials for controlling/eradicating the emerging MDR virulent strains of *Plasmodium falciparum* and *P. vivax* relapsing malaria. The other objective was to highlight the development of emergency treatment for the patients dying of complicated/comatose cerebral malaria complications specially to save millions of children dying of malaria. Even after hundred years of Sir Ronald Ross’s Noble Prize winning discovery in India, showing that malaria is transmitted by mosquitoes, there was urgency to develop safer gametocytocidal (transmission blocking) drugs which can be used as safe primaquine substitutes in malaria treatment. First priority at CDRI was to develop Reliable Technologies for Drug discovery at CDRI including the MDR screening experimental models which can select most active antimalarials for treatment of multidrug resistant malaria patients resistant to available drugs.

During the last five years nearly Thirty Two Malaria Eradication Agenda have been published by WHO/International Malaria Control Agencies/Malaria Experts with the ultimate goal of malaria eradication. Only few Institutions in the country have been engaged in development of antimalarials. Central Drug Research Institute (CDRI) a premier CSIR Institute was able to establish the Full Drug Discovery Experimental Technology. Several International Authorities have, however, strongly expressed the views that with the present antimalarial armamentarium it may not be possible to achieve malaria control or prevent malaria related mortality because of the: (i) Inadequacy of available drugs to control the emerging multidrug resistant strains of malaria, (ii) Control complicated *P. falciparum* and comatose cerebral malaria in children under five years which causes high mortality, (iii) Lack of safe anti-relapse/radical curative antimalarials which could be used as primaquine substitutes, (iv) Lack of safe gametocytocidal drugs to block transmission of *P. falciparum* and sterilize the gametocyte carriers which remain undiagnosed in malaria endemic areas, (v) Lack of safe causal prophylaxis drugs against malaria,(vi) Non-availability of safe antimalarials for pregnancy, (vii) Search of safe 8-

aminoquinolines (to replace primaquine) which do not cause methaemoglobinaemia, cyanosis and haemolytic anaemia, (viii) And to discover more effective antimalarials for emerging artemisinins resistant and MDR *Plasmodium falciparum/P. vivax* which are threatening the International efforts to eliminate multidrug resistant malarias. In spite of Billions of Dollars donated by International Community for Antimalaria Research, African children under five years are still dying of complicated malaria.

I wish to record that a safe 8-aminoquinoline (developed as primaquine substitute) at CDRI (Dutta *et al*. 1989; Puri *et al*. 1989) and marketed in 2000, under trade name CDRI 80/53 (Bulaquine=Elubaquine) has the potential (i) Anti-relapse (radical curative) activity against relapsing *P. vivax* malaria, (ii) Gametocytocidal activity (transmission blocking drug for *P. falciparum*) and (iii) Causal prophylaxis action. This is the safest anti- relapse drug discovered till date (i.e. April,2016).

Another 8-aminoquinoline Tafenoquine (WR 238605-synthesized by WRAIR) a broad spectrum antimalarial was initially developed through primate antimalarial screening (Milhous *et al.* 1988a,b) jointly by CDRI (M.M. Dhar, S.K. Puri and G.P. Dutta) and WRAIR Staff, Washington, DC. This new drug can be developed for use in India by CDRI as well as Internationally by US (WRAIR).

A fast-acting blood schizontocide drug (α/β-Arteether, 3 injection dose) is a valuable emergency treatment for both uncomplicated/severe complicated/MDR/cerebral malarias (Dutta *et al.* 1987, 1989) which is now internationally marketed in several African countries, and has been included in Indian National Malaria Programme of Govt. of India and reserved as emergency treatment for Hospital use in comatose malaria cases. Govt. of India has given tax exemption for manufacturing this drug by the Pharmaceutical Industry in the country. Ministry of Science & Technology have awarded Jai Vigyan Recognition to both Arteether and Bulaquine. CSIR had put-up α/β-Arteether Tableau during the Republic Day Parade at New Delhi.

Besides several artemisinin derivatives (including artemisinin (first reported in 1989), α/β-Arteether, β-Artemether, Artelinic acid and 1,2,4-trioxanes) have been discovered as safe gametocytocides which can replace toxic primaquine in blocking malaria transmission. All these agents have been shown to kill the gametocytes in

vertebrate host within 24 hours, resulting in complete absence of oocysts development in the mosquito-midgut as shown by individual mosquito dissections.

Dihydroartemisinin patented internationally in sixteen countries by the CDRI/CIMAP (1999 and 2000) has been combined with Piperaquine by MMV. This combination has been approved by the World Health Organization (WHO) (2010), as fifth ACT for global control of uncomplicated malaria.

I wish to convey my gratitude to Indian National Science Academy, New Delhi for giving me an opportunity to prepare this status report on "New Antimalarial Drug Discovery in India". The focus of this report is to provide future strategy towards control/eradication of malaria and stop malaria related mortality.

(G.P. DUTTA)

15th September, 2014

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The Antimalarial Drug Discovery Programme of India was jointly initiated by the CSIR-Central Drug Research Institute (CDRI) and the Indian Council of Medical Research (ICMR) "Modified Plan of Operation for Control of Malaria" during 1976-77, as a component of National Malaria Eradication Programme (NMEP) of Ministry of Health, Govt. of India.

Since I joined the Central Drug Research Institute on 29th September, 1964, I had the privilege to work in close cooperation with seven successive Directors of CDRI including Professor Nitya Anand who provided valuable research facilities and encouragement to the Antimalarial Drug Discovery Programme. Thanks are also due to Professors Akhatar Hussain, Sushil Kumar, former Directors and Professor R.P. Sharna (CIMAP) for their valuable support. Special thanks are due to my colleagues Prof. S.K. Puri, Prof. Renu Tripathi, Head, Parasitology Division and late Dr. V.C. Pandey, Head, Biochemistry Division for their valuable contribution and support in experimental studies on drug development. Valuable support for clinical trials of antimalarials was provided by Dr. O.P. Asthana (CDRI), by Professor Neena Valecha, Director, NIMR and by late Dr. V.P. Sharma, MRC, Delhi.

I am grateful to US for award of NIH International Post Doctoral Fellowship (1962-63) under Professor Geoffrey M. Jeffery, Sc. D., former Scientist Director (1962), US Public Health Service, Malaria Laboratory, Columbia S.C. who was my mentor and introduced me to Chemotherapy of Malaria and Late Prof. William Collins for Human Malaria Transmission Studies.

I am also grateful to late Prof. P.C.C. Garnham, FRS, for bringing frozen malaria parasites from London School of Trop. Med. for malaria researches at CDRI.

I wish to thank International Agencies for technical help and financial grants/support to Antimalarial Research. I wish to express my deepest gratitude to Director General, WHO, Geneva for kindly providing the extensive published Technical Reports WHO Documents related to Global Malaria Control which I have the privilege to incorporate in discussion of malaria eradication programme and guidelines sponsored by WHO. World Health Organization also established WHO-TDR Collaboration "Preclinical Evaluation of Antimalarials and WHO Collaborating Centre CC59 at CDRI. Special thanks are also due to Col. David E. Davidson Jr. and W. Milhous & Heads of Division of Experimental Therapeutics, Walter Reed Army Institute of Research, Washington DC, US for long term Indo-US collaboration with CDRI.

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This Status Report on "Antimalarial Drug Discovery in India" has been sponsored by the Indian National Science Academy. I am indebted to Professor Raghavendra Gadagar, FNA, President, INSA and Professor Krishan Lal, FNA, Past President for their guidance and financial support of INSA in bringing out this report. Special thanks are due to Professor S.C. Lakhotia, FNA, Vice President, INSA Publications for valuable help and discussions in bringing out this publication.

G.P. DUTTA

15th September, 2014

CHAPTER-I

INTRODUCTION

(Malaria Resurgence, Emerging Multidrug Resistance, WHO & International Co-operation to Reduce Mortality and Discovery of Antimalarials to Focus on Malaria Eradication)

1. HISTORICAL ACCOUNT OF GLOBAL MALARIA ERADICATION AGENDA AND CONTROL STRATEGY IN INDIA

The Organization of Pharmaceutical Producers of India in collaboration with the Indian Council of Medical Research had organized the First International Malaria Symposium in India in 1977 in which the major focus was to evolve a strategy for Malaria Control in the face of expanding areas of Drug Resistant Malaria in India, South-East Asia, Africa and Southern America. The malaria related mortality was linked to wide-spread emergence and spread of insecticide resistance (specially the failure of DDT) to control vector *Anopheles* spp. in malaria affected countries. Among the participants of the OPPI-ICMR symposium, T. Lepes (1977) the WHO Expert on Malaria Control and Eradication Strategy had pointed out that the International Agency (Pan American Health Organization founded in 1902) was established to control yellow fever and interrupt transmission of malaria to malaria vectors. For the first time the project aimed at '**Eradication of Malaria**' was visualized and proposed by Hoffman (1917). This was followed by setting up Health Committee of League of Nations (established in 1924) which took a policy decision to set up the Malaria Commission for training man-power for achieving malaria control, with major emphasis to reduce mosquito densities which would be able to arrest the spread of the dreaded malaria infections. During 1930-1940, and World War II, the European and US Scientists had put major emphasis towards the development of synthetic antimalarials (blood schizontocides) for malaria control/prophylaxis and in spite of the toxicity problems, several 8-aminoquinolines were developed to prevent mosquito transmission. The very successful application of DDT in early forties successfully brought down the vector density which was effective in controlling the spread of malaria in India and other malaria affected countries. The successful application of the powerful blood schizontocide namely chloroquine, combined with wide-spread use of vector control measures through the use of DDT, was effective in bringing down the malaria transmission. The International Agencies decided in 1950 to put forward a **Global Strategy to Eradicate Malaria** through the large-scale use of chloroquine and extensive DDT sprays.

Dr. Lepes, Director General, World Health Organization and the Expert Committee on malaria proposed that (1) the Global Malaria Eradication could be

achieved by ending the transmission of malaria through the miraculous effect of DDT, and (2) by elimination of Reservoir of Infective Cases (Gametocyte carriers etc.) in a compaign limited in time and carried out to such a degree of perfection, that when it comes to an end, there is no resumption of transmission. It needs to be emphasized that the subsequent failure of chloroquine (drug discovered in 1934 by the German Scientist) was due to the emergence of chloroquine resistant strains of *Plasmodium falciparum* which was gradually spreading throughout South-East Asia from 1950 onwards.

According to Dr. Lepes, in spite of the miraculous effect of DDT on malaria transmission, it was recognized as early as 1950 that the **Malaria Eradication might not be feasible in all ecological areas such as Africa South of Sahara etc., and emphasis of WHO should be shifted to Malaria Control Programme with special emphasis on:**

(1) Reduction of mortality.

(2) Reduction of malaria transmission.

(3) Emphasis should be to control the emerging drug resistance of *P. falciparum*.

(4) To overcome expanding DDT resistance of vectors.

Dr. Lepes (Director General, WHO) finally concluded that the **Malariologists had lost faith in Global Malaria Eradication.**

Amongst the earliest epidemiological surveys on malaria situation in India, Sinton (1935) had reported that at least 100 million cases suffered from malaria in India (including Pakistan and Bangladesh), and out of these nearly one million deaths were reported chiefly due to *Plasmodium falciparum.* This mortality due to severe malaria infection was caused by *P. falciparum*, prior to introduction of chloroquine for malaria chemotherapy or the acquisition of drug resistance by the parasite as reported by Pattanayak (1977). In another survey, Chopra and Sinton (1933-35) reported that 100 million malaria cases in India, had resulted in economic loss of nearly Rs. 10,000 million due to malaria morbidity and loss of man-hours. During the post-partition period (1952), the number of malaria cases detected in India was estimated to be 75 million cases which accounted for economic loss of Rs.7,500 million per year.

With the objective of controlling malaria mortality and morbidity in India, the **Planning Commission of Govt. of India**, endorsed the setting up of **National Malaria Control Programme** in 1953.

Indoor insecticide spray was integral part of the Control Programme which provided very successful mosquito control up to 10 weeks after the spray. This strategy to use DDT along with chloroquine was very effective which provided high level of protection to the children by 1957. The infant parasite rate was brought down to 0.6 per cent. Encouraged by high efficacy of DDT insecticidal sprays, the **Govt. of India launched during Fourth Five Year Plan, the National Malaria Eradication Programme (NMEP) in 1958** to stop the spread of malaria through wide-spread chloroquine therapy together with mass DDT spray to eliminate the breeding of malaria vectors and also incorporate the measures to stop the breeding of larval stages in water bodies etc. From **1958 – 1965, the NMEP had achieved tremendous success and brought down number of malaria cases to about 100,000 cases with no malaria related mortality in India.**

2. MALARIA RESURGENCE

From 1965 onwards the malaria resurgence was recorded in the whole country because of emerging chloroquine resistance by *P. falciparum* accompanied with DDT resistance by malaria vectors in different parts of the country. The emergence of chloroquine resistance by *P. falciparum* in states of Assam, Meghalaya and Mizoram was a major factor in resurgence of malaria. Besides drug resistance of malaria parasites, the rural malaria vectors (*Anopheles culicifacies*, *A. philippinensis* and *A. balbacensis*) also had developed resistance to insecticides such as DDT, BHC and Malathion etc. The number of malaria positive cases started rising from 1,00,185 cases (in 1965) to 6,94,647 cases (in 1970) and to 58,30,3000 cases (in the year 1976). **Govt. of India Appointed Indepth Evaluation Committee** to assess the malaria situation in the country in 1974, and the committee recommended that due to resurgence of malaria situation in the face of chloroquine resistance of *P. falciparum* and increasing insecticide resistance of malaria vectors, the **Modified Plan of Operation for Malaria Control** was approved with effect from April 1977. The Committee also reported that under the present circumstances the Eradication of Malaria was not possible. The Indian Council of Medical Research was the nodal agency to implement the **Modified Malaria Control Programme** of NMEP (1976-1977).

It may be mentioned that according to assessment of T. Lepes (Director, WHO Malaria Division) "the emerging resistance of *P. falciparum* to 4-aminoquinolines was not being considered as an element hampering the progress of malaria eradication" (Lepes, 1977). In spite of above observation, Lepes had documented that "the resistance of *P. falciparum* to 4-aminoquinolines has spread considerably in the past twenty years in South-East Asia (affecting Vietnam, Laos, Kampuchea, Thailand, Burma, Peninsular Malaya, Chittagong Hill tract in Bangladesh and Assam in India, besides in South America, Colombia, Brazil, Venezuela).

Dutta *et al.* (1977) while discussing the problem of resurgence of drug resistant malaria parasites *P. falciparum* and *P. vivax* and resistance of malaria vectors to powerful insecticides like DDT and malathione etc., had pointed out that global malaria control was difficult to achieve in the face of emerging resistance to available drugs as shown by a review of clinical trial data from 1946 to 1974 (OPPI Symposium, 1977). He also quoted the WHO (1974) authorities who had expressed the opinion that "**it seemed impossible to eradicate malaria from the world and concluded that we should change the strategy of global eradication of the disease and aim at prevention of deaths"**. The author also quoted the Press Report of Dr. Halfdan Mahler (1975) (Director General, WHO) which stated "**For many years we tried to keep up a bluff that global malaria eradication was possible…. ". "It is very difficult to admit failure and it took us a very long time to admit that this organization was not able to put forward a programme for eradication"**.

3. DISCOVERY OF KNOWN ANTIMALARIALS

(1) Historical records show that the bark of quina-quina tree, now known as cinchona bark, was the first crude antimalarial to undergo extensive clinical use in Peru during 16^{th} century as a traditional medicine for the treatment of periodic fevers characterized with chills and rigor. Because of the unique property of curing fevers, the chinchona bark had been introduced into other South American States, Spain, Italy, Europe, England and India between (1640-1657) (Covel, Coatney, Field and Singh, 1955). Quinine was the first active principle isolated in 1820 from the cinchona bark by the French Scientist and it continues to be a drug of

choice even today for treatment of cerebral malaria cases as well as for the control of drug-resistant *P. falciparum* infections.

(2) Another major discovery following quinine, was the isolation of Qinghasu (artemisinin) from *Artemisia annua* in 1972 by the Chinese Traditional Medicine Group which ultimately revolutionized the antimalarial Drug Discovery during the last four decades.

(3) The discovery of antimalarial endoperoxide moiety in Artemisinin by Chinese in 1979 opened up un-precedented field of development and discovery of new artemisinin derivatives (artesunate, artemether, arteether, artelinate) which have saved the lives of millions of malaria cases in African continent including children.

(4) During the last 25 years, the novel synthetic endoperoxides compounds (1,2,4-trioxanes) have been synthesized all over the world and will become most powerful antimalarial blood schizontocides for the control of multidrug resistant *Plasmodium falciparum*/*P. vivax* and *P. knowlesi* (monkey malaria) epidemics in humans.

(5) Soon after World war I, several synthetic antimalarials were discovered in quick succession, including pamaquine in 1925, mepacrine in 1930, chloroquine in 1934, primaquine and proguanil in 1945, amodiaquine in 1946 and pyrimethamine in 1951. Clinical studies on malaria conducted between 1945-1955 had clearly established that 8-aminoquinolines (primaquine and pamaquine) were poor blood schizontocides, nevertheless, they exhibited high tissue schizontocidal (anti-relapse) activity against *Plasmodium vivax* and *P. ovale* as well as strong gametocytocidal action against *P. falciparum* and *P. vivax*. On the basis of clinical studies, the remaining antimalarials including 4-aminoquinolies (chloroquine, amodiaquine), 9-amino-acridine (mepacrine) and cinchona alkaloid (quinine) were designated as highly active blood schizontocides effective against asexual blood stages but without any tissue schizontocidal action against relapsing stages of *vivax* malaria. Both proguanil and pyrimethamine were designated as causal prophylactic and primary tissue schizontocides active against pre-erythrocytic forms of *P. falciparum* as well as sporontocides, effective against

sporogonic forms developing in mosquitoes (Covell, Coatney, Field and Singh, 1955).

4. EARLY REPORTS OF EVOLUTION OF DRUG RESISTANCE BASED ON CLINICAL TRIALS

A survey of laboratory and field clinical trials with antimalarials from 1946-1974 shows the evolution of emerging drug resistance as evidenced by treatment failure to various antimalarials used as single drugs (mepacrine, proguanil, pyrimethamine, primaquine and chloroquine) as presented in Table 1. Failure of various antimalarials used in successive treatments of *Plasmodium falciparum* in clinical trials (judged as successive treatment failures from 1961-1976 (in Thailand, Colombia, Malaya, Viet-Nam, Bangkok, Phillipines, Sobah, Jaya, Cambodia, Burma and Panama) are summarized in Tables 2 and 3. Clinical trial failures of the blood stages of *P. vivax* treated with proguanil, pyrimethamine and primaquine were also reported (Table 4). Numerous clinical trials failure/emergence of chloroquine resistance by blood stages of *P. vivax* have subsequently appeared in different countries as reported in literature by numerous investigators, include independent clinical trials against *P. vivax*. Several reports have recently appeared which document emergence of choroquine resistance by blood stages of *P. vivax* [Schwartz *et al.*, 1991; New England J. Med. 324, 927; Schuurkamp *et al.* (1992) Trans. R. Soc. Trop. Med. Hyg. 86, 121-122; Murphy *et al.* (1993) Lancet, 341, 96-100; Garg *et al.* (1955) Trans. R. Soc. Trop. Med. Hyg. 89, 656-7; Than *et al.* (1995) Trans. R. Soc. Trop. Med. Hyg. 89, 307-8; Baird *et al.* (1996) Trans. R. Soc. Trop. Med. Hyg. 90, 409-410]. The author strongly emphasized that our efforts to develop newer drugs for the control of multidrug resistant parasites can be jeoparadized because of the demonstrated ability of virulent parasites to readily acquire resistance to new drugs after they had been used for a short-time.

5. NATIONAL MALARIA ERADICATION PROGRAMME: MORTALITY AND FIELD DRUG RESISTANCE DATA FROM INDIA

At the time of initiating National Malaria Eradication Programme (NMEP, 1958), the malaria control strategy in India was highly successful because the malaria parasite

specially *Plasmodium falciparum* showed complete sensitivity to chloroquine/ amodiaquine (standard drugs) which was responsible for bringing down the number of malaria cases. The malaria vectors (*Anopheles* spp.), which transmitted the malaria infections, were also brought under effective control through the large-scale spray of insecticide DDT, which was very effective against the vectors (*Anopheles* spp.). The NMEP operations from 1958 onwards thus brought down incidence of malaria in India from 75 million malaria positive cases and 0.8 million malaria related deaths to 0.1 million malaria cases annually with **no malaria related deaths during 1965**. However, from 1965 onwards there was a resurgence of malaria primarily due to failure of malaria surveillance programme, the emergence of high level of DDT resistance by vectors responsible for malaria transmission, besides sporadic occurrence of *P. falciparum* strains resistant to chloroquine. By 1976, the peak level of 6.47 million malaria cases was recorded throughout the country which was accompanied with 0.75 million *P. falciparum* infections (NMEP data, Tables 5a). Besides mortality records, emerging resistance to several antimalarial treatments between 1978-1997 was also recorded in India (Table 5b).

In 1977, the Indian Council of Medical Research (Expert Groups) formulated "**Modified Plan of Operation**" directed towards bringing the malaria situation in the country under control and the data provided in Table 5a shows a progressive decrease of malaria cases from 1976 to 1998.

In spite of sustained NMEP and National Antimalaria Programme (NAMP) operations from 1990 to 1998, the total number of malaria cases in India had remained static ranging from 2.0-3.0 million, with *P. falciparum* incidence around 0.91 million and 653 deaths were recorded during 1998. However, the WHO estimates showed nearly 15 million malaria positive cases in India in 1998 with approximately 40% *P. falciparum* infections and mortality due to malaria was estimated around 19500 cases (quoted by Sharma, 1998). It is surprising that a high proportion of *P. falciparum* cases (nearly 1/3rd) are considered to possess some degree of chloroquine resistance in India. According to data generated by NAMP *in vivo* studies (Table 5b), *P. falciparum* resistance against chloroquine (RI to RIII) level was recorded in 3065 cases out of 12863 cases evaluated during 10 yrs. Period (1978 to 1998) and resistance against amodiaquine was recorded in 118 cases out of 333 cases evaluated. Resistance against second line of malaria treatment

(Sulfadoxine/Pyrimethamine combination) also showed moderate level of resistance in 24 cases out of 915 cases screened and against sulfalene/pyrimethamine combination in 2 out of 160 cases investigated (Table 5b). Besides very low level of resistance against quinine was also demonstrated in 15 cases out of 209 falciparum cases tested for *in vivo* quinine resistance. It may be pointed out that overall incidence/frequency of RII and III level of chloroquine resistance of *P. falciparum* has remained fairly low in India when data obtained between 1978-1997 is critically analysed (Shiv Lal, 1999 NAMP Report) (Table 5b). The conclusion drawn by WHO sources that nearly one-third of Indian falciparum cases are resistant to chloroquine/amodiaquine (see Sharma, 1998), could not be substantiated by data on supervised *in vivo* field drug sensitivity tests by Indian investigators/NAMP, teams. This information had been very important for formulating Anti-malaria Drug Policy of the Government of India.

6. MODIFIED PLAN OF OPERATION FOR MALARIA CONTROL IN INDIA (1976)

(Sponsored by National Malaria Eradication Programme (NMEP), Govt. of India).

1. The Indian Council of Medical Research invited all the Indian Scientists at ICMR Headquarters, New Delhi, to evolve Programme for Basic, Applied and Operational Research with a focus on Malaria Eradication Strategy for the country. Under the modified plan, seven task forces comprising of nearly 50 scientists were set-up to undertake research programmes directed towards Malaria Eradication Strategy and funds/grants were sanctioned immediately by ICMR, New Delhi to execute the project. The CSIR-Central Drug Research Institute, was made centre for (1) Chemotherapy (antimalarial drug development), (2) Seroepidemiology of malaria and (3) Develop biocides technology (based on *Bacillus thuriengiensis* and *B. sphericus* spore toxins for antilarval mosquito control. The modified plan of operation for malaria control started from 1977 was focused on the following:

   (1) Increased supply of insecticides (DDT, BHC, Malathion).

   (2) Set-up drug distribution centres, chloroquine supply at fever treatment depots.

   (3) Anti-larval operations.

(4) Fourteen research projects granted by ICMR for research on malaria.

(5) WHO support to treat cerebral malaria cases.

(6) Starting of *in vitro* anti-malarial screening of blood samples of malaria cases for emergence of drug-resistant *P. falciparum* cases in the field centres and assessment of RI, RII and RIII level of drug resistance in malaria endemic areas.

According to Patnaik, Director, NMEP, two major objectives of the Modified Plan of Operation of NMEP included (i) the recording of success of new malaria control measures and (ii) the monitoring of emergence of multi-drug resistance in *P. falciparum* in different endemic areas in the country.

The review of the malaria incidence in India as published by National Anti-Malaria Programme Report (Shiv Lal, 1999) also showed that malaria cases numbering 6.47 million in India in 1976 showed gradual decline under modified malaria plan from 6.47 million in 1978 to 2.18 million (with 247 deaths due to malaria) to 2.10 million cases (with 653 mortality) (in 1998), while the malaria incidence in 1998 reached to 2.5-3.2 millions cases (with additional malaria mortality of 1000). The increase in mortality was due to spread of multi-drug resistant *P. falciparum.*

Emergence of Drug-Resistant Malaria in India based on Field Treatment Data

Under the modified plan of operation, NMEP developed expertise to monitor the drug sensitivity/resistance in *P. falciparum* infected cases. The *P. falciparum* positive patients were treated with several anti-malarials at the standard doses and patient's drug response was evaluated in treated patients who showed resistance. The data on *in vivo* anti-malarial tests reported by NAMP (March, 1999) summarizes the cumulative data from tests conducted between 1978-1997. The MDR against *P. falciparum* in Indian cases, treated with chloroquine, amodiaquine, sulfadoxin-pyrimethamine, sulfalene-pyrimethamine, mefloquine, quinine showed the epidemic spread of MDR malaria in different regions of Indian continent. Both RI and RII level of resistance of *P. falciparum* was recorded by the tests conducted between 1978-1997 (Table 5b). The spread of MDR *P. falciparum* in Indian patients was confirmed.

Besides India, high level of MDR *P. falciparum* resistance to a variety of antimalarials, had already appeared in several countries as shown by failure of clinical response of patients to *P. falciparum* treatment between 1996-1998. Review of MDR data among *P. falciparum* cases had established the spread of MDR malaria in Indian border states.

7. DRUG DISCOVERY AND EMERGENCE OF DRUG RESISTANCE IN MALARIA AND STRATEGY FOR ACT OPTIONS FOR CONTROL OF MDR MALARIA

In recent reports from the World Health Organization the global malaria incidence has been estimated to be nearly 300 million cases of malaria besides very high mortality (nearing 670,000 deaths) attributed to malaria (WHO, 2011; WHO, 2013). A review of **antimalarials drug discovery** presented in Table 6, clearly show that powerful antimalarials (blood schizontocides) were discovered between 1880 to 1990 including quinine (discovered in 1880), mepacrine (1930), chloroquine (1934), proguanil (1944), primaquine (1945), amodiaquine (1946), pyrimethamine (1952), pyronaridine (1970), mefloquine (1971), Metakelfin (1975), Fansidar (1975), Fansimef (1980), quinine + tetracycline (1970) and halofantrine (1990) for control of *Plasmodium falciparum.* Within a short span of their clinical use, the malignant form of parasite (*P. falciparum)*, which causes mortality, had the ability to **develop resistance against most of these drugs** as shown in clinical trials data (Tables 6 and 7). The discovery of another powerful antimalarial by **Chinese Scientists in 1979 (include artemisinin (Qinghaosu)-**a natural product from *Artemisia annua* and several fast-acting semisynthetic artemisinin derivatives (artesunate, artemether, artelinate and arteether) by the Chinese, Indian and U.S. Scientists) had powerful impact on the control of drug resistant *P. falciparum* and played significant role in saving human lives and prevented mortality of millions of children in Africa and South-East Asia from 1979 (year of artemisinins discovery) to around 2010. These drugs successfully arrested the rising trends of malaria (both uncomplicated and complicated/severe *P. falciparum)*. In order to protect these valuable antimalarials against emergence of drug resistance, World Health Organization supported by World Bank/International Agencies/and Scientists/Clinicians from malaria endemic countries, jointly developed the concept of using combination drug therapy comprising of

two to three drugs (with different modes/sites of action on plasmodia) termed artemisinin based combination treatment (ACTs) and non-ACT drug treatments (i.e. drug combinations without artemisinins) with a major objective to control the emerging foci of multidrug resistance malarias (for both MDR *P. falciparum* and emerging chloroquine resistant *P. vivax*). The WHO (2010) have already approved five ACTs and one non-ACT combinations for treatment of MDR *P. falciparum*. Besides two dozen non-ACT drug combinations of standard (conventional) antimalarials are undergoing clinical trials with the objective to control the spread of MDR malarias and reduce malaria mortality. Nosten and White (2007) Am. J. Trop. Med. Hyg. 77, 181-92 reported: (i) Artesunate-Sulfadoxine-Pyrimethamine, (ii) Artesunate-Amodiaquine, (iii) Artemether-Lumefantrine, (iv) Artesunate-Mefloquine, (v) Artesunate-Chlorproguanil-Dapsone, (vi) Artesunate-Atovaquone-Proguanil, (vii) Artesunate-Pyronaridine, (viii) Dihydroartemisinin-Piperaquine. ACT combinations. Yeung *et al.* (2004): Am. J. Trop. Med. Hyg. 179-186 (i) Coartem^{R} : Artemether + Lumefantrine, (ii) Artekin^{R} : DHA + Piperaquine, (iii) Artesunate + Mefloquine, (iv) Artesunate + Amodiaquine, (v) Sulfadoxine/Pyrimethamine, (vi) Chloroquine + Sulfadoxine/Pyrimethamine, (vii) Artemisinins, (viii) Arteether injection + Sulfadoxine/Pyrimethamine/Metakelfin, (ix) Artesunate – Lumefantrine + Fansidar, (x) Artesunate – Lumefantrine + Mefloquine, (xi) Artesunate – Lumefantrine + Quinine + Tetracycline/Doxycycline, (xii) Arteether + Mefloquine. WHO combinations (2003): (i) Artesunate oral, (ii) Dihydroartemisinin, (iii) Artemether oral, (iv) Artemether injection, (v) Artesunate injection iv/im, (vi) Artemotil injection, (vii) Artesunate + Meflqouine oral, (viii) Artesunate + Amodiaquine oral, (ix) Artesunate + Fansidar oral. Ogbonna *et al.* (2008) Trans. Roy. Soc. Trop. Med. & Hyg. 102, 64-67 (registered co-formulated ACT) (i) Artemether-Lumefantrine, (ii) Artesunate-Mefloquine, (iii) Artesunate-Amodiaquine, (iv) Artesunate-Fansidar. IPCA – (i) Lumerax-80, (ii) Larinate kit.

During the last 15-20 years the efficacy of **artemisinins (monotherapy)** for control of malignant *P. falciparum* in China, South-East Asia and African Continent, started diminishing in clinical trials primarily due to **emergence of artemisinins resistant *P. falciparum*.**

In the face of emerging drug resistance the International Clinical Scientist from Tropical Malaria endemic countries had continued to assess various drug combinations to cure multidrug resistant *P. falciparum* in clinical cases and they had reported the clinical efficacy of selected drug combinations which could control *P. falciparum*.

Hien *et al.* (1992) reported Artemisinin + Mefloquine cured 100% patients of *P. falciparum*. Li *et al.* (1984) reported 100% cure with Artemisinin + Mefloquine + Sulfadoxine + Pyrimethamine. Shwe *et al.* (1989) and Win *et al.* (1992) achieved 100% cure with Mefloquine + Artemether. Shwe *et al.* (1988) used Artemether + Mefloquine + Quinine, while Win *et al.* (1992), Looareesuwan *et al.* (1992b,c) and Looareesuwan *et al.* (1996) were successful in achieving 100% cure rates against multidrug resistant *P. falciparum* in different countries/*P. falciparum* strains. Around 1999-2000, the **World Health Organization/International Clinical Scientists** in malaria endemic countries, realized the impending danger of emerging artemisinin resistance in clinical trials in which **artemisinin/artesunate/artemether/ dihydroartemisinin** etc. were found to **fail in clinical trials when these agents were used as monotherapy for *P. falciparum* treatment**. At this stage the **World Health Organization Clinical Groups realized that artemisinins monotherapy had failed to control MDR *P. falciparum* resulting in the increase in malaria mortality exponentially**. The **WHO (1998) issued an International Alert to stop using artemisinins monotherapy for malignant form of *P. falciparum* throughout the malaria endemic regions of the globe**. The above cited clinical studies prior to 1998 by International Medical Scientists seems to have favoured the **World Health Organization decision (2000 Recommendation)** to introduce "**Artemisinin Based Combination Treatment Policy**", as the most potential drug combinations for efficient control and treatment of the multidrug resistant, uncomplicated *P. falciparum* malaria. **World Health Organization recommendations (2006 and 2007)** further made it obligatory for all National Governments/National Antimalaria Control Agencies in malaria endemic countries, **to introduce ACTs** as the most potential drug combinations for efficient malaria treatment, control of mortality and elimination of multidrug resistant *P. falciparum*. The **five ACT combinations** recommended by WHO for multidrug resistant *P. falciparum* malaria treatment and control are listed below:

(i) Artesunate + Amodiaquine

(ii) Artesunate + Mefloquine

(iii) Artesunate + Sulfadoxine + Pyrimethamine

(iv) Artemether + Lumefantrine

(v) Dihydroartemisinin + Piperaquine

According to WHO guidelines, Artemisinin derivatives cannot be recommended as monotherapy for *P. falciparum* (uncomplicated malaria or complicated severe malaria) because of danger of emergence of artesunate resistance (Sahr *et al.* 2001; Luxemburger, 1998).

Cases of artemisinin resistant *P. falciparum* have already been reported from several South-East Asian countries (Noedl *et al.* , 2005, 2008; Afonso *et al.* 2006; Dondorp *et al.* 2009; Inselburg, 1985; Eckstein-Ludwig *et al.* 2003). Another ACT combination Coartem (**a combination of Artemether + Lumefantrine**) used for control of *P. falciparum* in Africa was **reported to fail** in treated cases in Africa because of emergence of Coartem resistance (Hastings and Ward,2005). Martensson *et al.* (2005) and Siswath *et al.* (2005) also reported a significant increase in mutation of *P. falciparum* Pf mdr1 86N was observed in treated cases, indicating **emerging resistance** in clinical cases treated with **Coartem** (ACT combination of Artemther + Lumefantrine).

Resistance of *P. falciparum* to **parenteral artemether (IM),** another component of ACT (Artemether + Lumefantrine) has been documented from **Mumbai by Gogtay *et al.* (2000a,b)**. Three cases of artemether resistance (480 mg IM dose given in 5 days) as shown by recrudescence of sever *P. falciparum* were documented by Gogtay *et al.* (2000a). Gogtay *et al.* (2000b) also reported *P. falciparum* probable **resistance to artemether in Mumbai trials.** Further molecular genetic studies on *P. falciparum* isolates exposed to *P. falciparum in vitro* identified point mutations of the SERCA-type PfATPase 6, suggesting Artemether resistance of *P. falciparum* (Jambou *et al.* , 2005). In another clinical study on *P. falciparum* treatment in Jharkhand, Singh (2002) had documented a case of MDR malaria showing resistance to three artemisinin derivatives (Arteether, Artemether, Artesunate besides Chloroquine).

The World Health Organization Report (2013) has also focused on the problems of **emerging threat of *P. falciparum* developing artemisinins/ACTs resistance** in several South-East Asian countries. They have also argued that it **may not be feasible to**

control artemisinin resistance with the available ACTs, and such *P. falciparum* cases may be prescribed a **sixth non-ACT combination** comprising of **Atovaquone +Proguanil (Malrone)** for the treatment of these cases. Priority should be given to promptly diagnose such resistant cases for appropriate treatment. It seems pertinent to record here, the emergence of *P. falciparum* **resistance to Atovaquone-Proguanil (Malarone) which had been document** by several investigators between 2002-2006 (Fivelman *et al.*, 2003; Farnert *et al.*, 2003; Schwartz *et al.* 2003; David *et al.*, 2003; Happi *et al.*, 2006; Bergy, 2006). Fivelman *et al.* (2002) and Farnet *et al.* (2003) reported Malrone treatment failure in *P. falciparum* mlaria, Schwartz *et al.* (2003) reported genetic confirmation of atovaquone-proguanil resistant *P. falciparum* (in Africa), David *et al.* (2003) reported atovaquone-proguanil resistance (in Africa), while Happi *et al.* (2006) and Berry *et al.* (2006) reported that the **cytochrome gene mutation** in *P. falciparum* isolates from Africa were implicated in **Malarone resistance.**

Pyrimethamine resistance in *P. falciparum* has also been reported in several clinical studies. Roper *et al.* (2004) reported intercontinental spread of pyrimethamine *P. falciparum* resistant. Cowman *et al.* (1988) had reported amino acid changes linked to pyrimethamine resistance in dihydrofolate reductase-thymidylate synthase gene of *P. falciparum*. Peterson *et al.* (1988) also had reported a point mutation in dihydrofolate reductase-thymidylate synthase gene in pyrimethamine resistant *P. falciparum*. Likewise, Lynch *et al.* (2008) also reported emergence of a dhfr mutation conferring high level of drug resistance in *P. falciparum* from South-West Uganda.

Piperaquine another component of ACT has been vulnerable to emergence of resistance (Basco and Ringwald, 2003). The authors report that slowly eliminated tail of PQP (piperaquine) would provide a selective filter for PQP resistant parasites to be selected.

The World Health Organization Report (2006) documents wide-spread **sulfadoxin-pyrimethamine** resistance and chloroquine resistance in South-Africa. Nepal also recorded 56-87% resistant *P. falciparum* in 1989. The molecular characterization of drug-resistance markers in *P. falciparum* from Kilifi (Kenya) identified dhfr haplotypes conferring SD/Py resistance (Certain *et al.*, 2008). It seems that out of five ACT's listed

above, judicious choice has to be made for selection of most effective ACT which can reduce mortality due of complicated/severe malaria.

8. EMERGENCE OF DRUG RESISTANCE

Drug resistance in malaria is not a recent problem and the first report of resistance dates back to 1910 in South America, where the *P. falciparum* was reported to show decreased response to quinine**.**

Chloroquine resistance

Chloroquine, a candidate compound belonging to 4-aminoquinoline group was synthesized as early as 1934 in Germany by Andersag, which was, however, used clinically ten years after its synthesis. The evolution of drug resistant strains of *P. falciparum* threatened the efficacy of chloroquine which was witnessed by drug failure reports published from South America and Thai-Cambodian border. Drug resistant foci had spread to South American countries, South-East Asia (Young and Moore, 1961; Harinasuta *et al.* , 1962; WHO, 1984; Wernsdorfer, 1991; Segurado *et al.* , 1997; Pinichpongse *et al.* , 1982; Nosten *et al.* , 1991; Looareesuwan *et al.*, 1992a; Shapira *et al.*, 1993; Luxemberger *et al.*, 1996, 1997). In India, on the contrary, chloroquine resistance was reported in M.P. and Rajasthan (Singh *et al.*, 1995; Ghosh *et al.*, 1989, 1992; Mohapatra *et al.*, 1989; Shukla *et al.*, 1995; Narayan, 1997; Dua *et al.*, 1997; Singh and Chaturvedi, 1997). In other South-East Asian countries like Indonesia, Myanmar, Pakistan, Sri Lanka, Malaysia too chloroquine resistant *P. falciparum* was reported (Baird *et al.* , 1997a; Fryauff *et al.* , 1998; Dandreo *et al.* , 1976; Hakim *et al.* , 1996).

Reports of chloroquine resistance had been published in African continent (Fogh *et al.*, 1979), South America (Menon *et al.*, 1987). *P. falciparum* infection in West Africa also developed resistance to chloroquine (Oduola *et al.*, 1989; Sowunmi and Oduola, 1995), from Nigeria (Sowunmi and Oduola, 1997) and also from Gambia, Malaya and Uganda (Sowunmi and Salako, 1992; Brewster and Greenwood, 1993). 100% chloroquine resistance had been reported from Senegal (Onori *et al.*, 1982; Sokhna *et al.*, 1997). From Cameroon region also *P. falciparum* had developed chloroquine failure in 50-60% cases (Louis *et al.*, 1992).

Resistance against anti-folate group of drugs

Sulfadoxin-pyrimethamine (SP), (synergistic dihydrofolate reductase inhibitor) and proguanil were successfully used to control chloroquine resistant malaria and a causal prophylactic agent. Resistance to this drug combination appeared in 1980s from Thailand besides cross-resistance between proguanil and pyrimethamine was recorded (Hurtwiz *et al.* , 1981; Black *et al.*, 1982; Pinichpongse *et al.*, 1982; Karbwang and Harinasusta, 1992). RII and RIII levels of resistance recorded in India was 10-20% of total resistant cases. From Pakistan, Malaysia, Indonesia, etc. resistance to SP was also reported (Hakim *et al.*, 1996; Rowland *et al.*, 1997).

In African subcontinent too reduced efficacy of SP combination, the second line reserve antimalarial drug against *P. falciparum* was reported (Hess *et al.*, 1982; Menon *et al.*, 1987; Garicavidal *et al.* , 1989; Lege-o-guntoye *et al.*, 1990), besides Tanzania (Edoh *et al.*, 1997) and Solomon Islands (Hess *et al.* , 1997).

Mefloquine resistance

Mefloquine, one of the most active drug was synthesized in early 1940's. In spite of its strong photosensitizing action (Whiselogle, 1946), the drug was introduced for clinical use in 1971.

Resistance against mefloquine was first reported from South-East Asian countries, especially from Thailand in early 1980's from where RII category of resistance had been reported (Boudreau *et al.*, 1982; WHO, 1984; Wernsdorfer, 1991; White and Olliaro, 1996). At 15 mg/kg mefloquine dose, 80% of drug failure was reported with RIII level of resistance in Eastern Thailand Region (WHO, 1996). Mefloquine resistance was recorded from Thai-Myanmar and Thai-Cambodian border. Strains of *P. falciparum* highly resistant to chloroquine showed cross-resistance to mefloquine (Brasseur *et al.* , 1990; Brasseur *et al.*, 1992). From India, however, not much resistance to this drug which has a very long half-life, has been reported.

Resistance against amodiaquine

Amodiaquine which belongs to same class of 4-aminoquinoline as chloroquine, was basically developed in 1946, and found to be more effective than chloroquine (Hawley *et al.*, 1996; Olliario *et al.*, 1996). Agrunulocytosis, one of its major toxic

effects, was one of the main reason for its rejection, but with the increasing chloroquine resistance, the amodiaquine usage for treating drug resistant malaria also got a boost.

Failure of amodiaquine in controlling *P. falciparum* had been reported in Eastern and Northern Thailand (WHO, 1984). From Brazil in South America, resistance has also been reported (Segurado *et al.*, 1997). It has been in use for treating chloroquine resistant malaria in Africa (Olliaro and Massano, 1997). From India, 10% RII and 7% RIII level of resistance of *P. falciparum* against amodiaquine had been recorded (NMEP, 1998).

Resistance against halofantrine

Halofantrine was developed by U.S. to combat chloroquine resistant falciparum malaria. However, strains resistant to halofantrine have been reported (Basco *et al.* , 1991; Brasseur *et al.* , 1993). Its 82% cure-rate from Kenya against multi-drug resistant malaria had been reported (Ana-Bwani *et al.*, 1996). A high level of *P. falciparum* resistance against halofantrine had been reported from China (Liu-Dequan *et al.*, 1998).

Halofantrine produces serious toxic effects such as black-water fever, intravascular haemolysis, resulting in haemoglobineurea, haemoglobinaemia and these toxic effects are similar to quinine therapy (Orlando *et al.*, 1996). Its cardiac toxicity causes lengthening of Q-T interval (Touze *et al.*, 1975) and it shows uneven distribution in various organs.

Resistance against Quinine

Emergence of quinine resistant *P. falciparum* dates back to 1910 in South America where quinine 10 mg/kg was not found adequate to treat falciparum malaria (Nocht and Werner, 1910; Nieva, 1910). Growing quinine resistance against *P. falciparum* had been reported (WHO, 1984; Pukrittayakamee *et al.*, 1994; Jelinek *et al.*, 1995). Increasing level of quinine resistance had been reported from Thai-Cambodian border, Thai-Kampuchean border and Brazil (Sylvia *et al.* , 1986; Segurado *et al.* , 1997). Quinine 600 mg every 8 hourly for 7 days produced 90% cure rate in Thailand but the additional tetracycline (at 250 mg/kg every 8 hr for 7 days) produced 95% cure rate (Looareesuwan *et al.*, 1992; WHO, 1994; Patenotte *et al.*, 1995; Metzer *et al.*, 1995; Bunnag *et al.*, 1996). Indian reports also show 30 mg/kg quinine together with 250 mg/kg tetracycline for 7 days was able to control resistant infections (Singh *et al.*, 1995; Shukla

et al. , 1995; Dua *et al.* , 1997). From African continent also declining efficacy of quinine had been reported (Brasseur *et al.*, 1992; Pasvol *et al.*, 1992; Haruki *et al.*, 1998). However, quinine in Africa is still the drug of choice for treating resistant *falciparum* infection.

Global Incidence of *P. vivax*

Nearly 40% of malaria cases all over the world are due to *P. vivax*. Emerging chloroquine resistance in *P. vivax* has been reported by a large number of scientists from different continents (Luzzi *et al.*,1992; Rombo *et al.*, 1987; Collins & Jeffery, 1996; Rieckman *et al.*, 1989; Murphy *et al.*, 1993; Looareesuwan *et al.*, 1997; Purkittayakamee *et al.*, 1998). *P. vivax* blood stages are reported to have developed resistance to full dose of chloroquine (1500 mg) (Baird *et al.* , 1991, 1996, 1997). Soto *et al.* (2001) also reported the emergence of *P. vivax* clinically resistant to chloroquine in Colambia. Schuurkamp *et al.* (1989) also reported a case of mixed *P. falciparum* and *P. vivax* infection where both the parasites had developed resistance to 4-aminoquinoquine.

Dormant hypnozoites in the liver which cause relapses of *P. vivax* are cured with standard primaquine (15 mg x 14 days) and provide radical cure (Collins and Jeffery, 1996). Chesson strain of *P. vivax* shows nearly 30% relapses after standard primaquine dose (15 mg x 14 days), indicating emerging primaquine resistance (Bunnag *et al.* , 1994). Nearly 18% relapse rate in *P. vivax* case after primaquine (15 mg x 14 days) was recorded (Looareesuwan *et al.*, 1997). Primaquine resistant *P. vivax* has also been reported from Myanmar, Indonesia (Baird, 1991, 1997).

Artemisinin resistance

The leads provided by Chinese traditional medicine based on clinical use of qinghaosu (aretemisinin) had given a new hope to tackle the problem of drug resistance in malaria (Anon. 1979). In this study, the cure rate of *P. falciparum* cases treated with artemisinin ranged from 74.3-89.5%, chloroquine resistant cases showed 88.9-96.8% and cerebral malaria cases showed 92.9% cures in Chinese trials (Anon, 1979). A further report on clinical trials in China showed that two derivatives of artemisinin namely artemether and artesunate were potentially useful for treatment of cerebral malaria and the cure-rate reported with sodium artesunate was 86.7%, artemether 94.1% and

artemisinin used for comparison gave a cure rate of 89.3% (Guoqiao *et al. ,* 1982). Although the parasite clearance rate with these artemisinin derivatives was faster than standard chloroquine or quinine therapy, the recrudescence rate in drug-resistant areas was still high (up to 35-52%) with artemether and artesunate and mortality rate continued to be high (18.4%) (Bunnag *et al.* , 1992; Karbwang *et al. ,* 1992; Murphy *et al.* , 1996). A minimum 5-7 days treatment would be necessary to improve the cure-rates which would be operationally difficult in the field. The regulatory toxicological clearance of artemisinin, artemether and artesunate according to Western standards had not been currently achieved as per World Health Organization Reports. However, the half-life of clinically used derivatives is short (0.5-1.4 h for artesunate, 11.1-13.2 h for artemether, and 4.0-4.4 h for artemisinin) and this could be partly responsible for recrudescence of *P. falciparum* in multiple drug-resistant areas (White, 1994). Attempts are also continuing to contain the emerging high level of mefloquine resistance by combining meflqouine (750-1250 mg) with artemether (300-600 mg). Although cure rates improve marginally with meflqouine + artemether combinations (96.4-100%), there are increasing episodes of neurological sequelae and severe toxic manifestations due to mefloquine use in this combination (Murphy *et al. ,* 1996; Karbwang *et al. ,* 1995; Shwe *et al. ,* 1988, 1989) which compromise the safety of these drugs.

Chronological appearance of resistance of *P. falciparum* to chloroquine, sulfadoxine/pyrimethamine, mefloquine, quinine and resistance of *P. vivax* to chloroquine, had been summarized by WHO database compiled in 2011 (Table 7). Clinical trials conducted in various South-east Asian countries showed emergence of confirmed resistance as reported by WHO Health Database (2011). According to this document, chloroquine resistance *P. falciparum* was first reported from Bangladesh (in 1970), sulfadoxine-pyrimethamine from India (in 1973), mefloquine from India (in 1979), quinine also from India (in 1986) and *P. vivax* chloroquine resistance from clinical case from Indonesia (in 1989).

9. WHO (2013) : Q + A ON ARTEMISININ RESISTANCE

According to WHO report (April 2013) artemisinin (isolated from *Artemisia annua* (sweet wormwood) and its derivatives have the ability to quickly reduce *Plasmodium*

parasites from the blood circulation of malaria patients following treatment with ACTs (artemisinin base combination therapies). WHO recommended ACTs as the first-line treatment for uncomplicated *P. falciparum*. According to WHO, the large-scale treatment with ACTs undertaken in 2005 covered 11 million ACT treatment courses and the total ACT treatments in 2011 covered 278 million treatments, resulting in significant decline in malaria cases in endemic countries resulting in protection of uncomplicated *P. falciparum* cases. WHO have so far recommended five different ACT combinations for treatments and control of blood stages of *P. falciparum*. The WHO have cautioned that in areas where the artemisinin resistance has already appeared, artemisinin component may not be able to clear all the parasites within three days. The longer acting combination drug in ACT treatment, can still produce curative effect in *P. falciparum* cases.

The WHO (2013) have cautioned the South-East Asian countries (Cambodia, Myanmar, Thailand and Vietnam) where **artemisinin resistance had already appeared** and documented by WHO, that the artemisinin resistant component of ACTs might not be effective in clearing all the resistant parasites (*P. falciparum*) within three days (i.e. latest by third day of treatment). However, the patients could still be cured as part of a longer treatment regimen, provided that the patients are treated with an ACT combination containing a partner antimalarial drug to which the malarial parasite is still sensitive in that geographical area. The WHO have strongly advocated that **artemisinin-based monotherapies should not be used alone in order to prolong the effective use of ACT combinations** (Resolution World Health Assembly, 60.18, 2007). The WHO have also strongly emphasized the **need to stop ongoing malaria transmission to contain emergence of artemisinin resistance**. The WHO (2013) have finally expressed the view that "**if resistance were to spread to – or emerge in – India or sub-Saharan Africa**, the public health consequences could be dire, as **no alternative antimalarial medicine is available** at present with the same level of efficacy and tolerability as ACTs". The WHO (2013) have further warned that "there is, therefore, **a limited window of opportunity to avert a regional public health disaster**, which could have severe global consequences". **In author's opinion, limited clinical trials with 3 dose α/β-arteether (IM) alone, may be better choice to evaluate** in areas where artesunate/artemisinin resistance has been reported. Pharmacokinetic study on **IM α/β-arteether (30:70)** has established that the

drug has longest t½ β (33 h) in comparison to artesunate. Following 3 or even 4 dose treatment with α/β-arteether (150 mg IM injection daily x 3 or 4 days), the arteether blood levels would be giving protection against MDR *P. falciparum* infections beyond 120h after 3-4 dose course. **α/β-Arteether injection has dual advantage as it also possesses transmission blocking activity against *P. falciparum* by killing the stage I, II, III gametocytes.**

The Global plan for artemisinin containment (GPARC) (Jan. 2011) was drawn (1) to protect the efficacy of ACTs and special emphasis should be (2) to invest in "Artemisinin related research", and (3) intensified malaria control should be focused in high transmission areas to limit the spread of malaria. Another WHO recommendation (25 April, 2013) was focused on "**Emergency Response to Artemisinin Resistance in the Greater Mekong Subregion"**. Another high level malaria summit hosted by Govt. of Australia (31 Oct.-2 Nov. 2012) in Sydney was focused on "**Consensus on Malaria Control and Elimination in the Asia-Pacific"**. Another WHO sponsored meeting was focused on "**Declaration of the 7th Asia Summit on Regional Responses to Malaria Control and Addressing Resistance to Antimalarial Medicines"** on 20 Nov. 2012 in Cambodia.

The WHO (2013) while discussing the consequences of artemisinin resistance in the Greater Mekong Subregion, have cautioned the countries that "**there is a real risk of parasites developing resistance to all available medicines"**. According to the WHO assessment "the ACT options in Western Cambodia have now become so limited" and that a **non-ACT drug combination (Atovaquone-Proguanil**) is currently used as the first-line treatment for control of resistant *P. falciparum*". This Atovaquone-Proguanil combination was used earlier for malaria chemoprophylaxis.

The WHO (2013) have cautioned the "National malaria control programmes need to consider the possibility of emerging resistance to both the artemisinin compound and the partner (longer acting) drug" e.g. piperaquine. Further they have expressed the opinion that "If resistance to the partner (longer acting) drug exists, it is more likely that resistance to artemisinin will also develop and vice versa.

Professor Krishna and Professor Kremsner (2013) have focused their opinion on the implications of the terms "artemisinin specific WHO term "ACTs resistance" which

appears to be threatening the global malaria eradication programmes. They advocate that the "**Treatment failure of artemisinin combination therapy (TFACT)"** would be valid description for the general term "**artemisinin resistance**". It is important to determine whether the generally used term "ACT resistance" could be due to resistance either to the artemisinin component or to the second longer acting (partner) drug component, or it implies resistance to both the artemisinin component as well as to the second longer acting antimalarial component.

Professor Krishna and Professor Kremsner (2013) have recommended to develop reliable molecular markers for piperaquine which is considered as important longer acting component of DHA-piperaquine ACT sponsored by WHO (2010). The work of Fairhurst, R.M. *et al.* (2012) have argued that "Defining artemisinin resistance is a work in progress and currently no consensus exists on the standard definition; thus claims of artemisinin resistance should be considered with caution".

10. WORLD HEALTH ORGANIZATION (2012) "MALARIA ERADICATION: WHO REGIONAL OFFICE, SOUTH-EAST ASIA REGION" REPORT

The WHO (2012) report records that malaria has been successfully eradicated from the following regions of Asia and there is no malaria in: (1) Hong Kong, (2) Bangkok, (3) Kuala Lumpur, (4) Jakarta, (5) Singapore, (6) Rangoon, (7) Phnom-Penh, (8) Manila, and most other major urban areas, (9) Urban areas of Papua New Guinea, (10) Some urban areas in India, and Pakistan, (11) Large cities in Latin America (with exception of Guayaquil Ecuador), (12) South-East Asia are malaria free, (13) Karnataka State is also malaria free. According to the WHO (2012) report, the proportion of infected *Anopheles* mosquitoes in Western Kenya may exceed 20%, whereas in Latin America and Asia less than 1% of the *Anopheles* mosquitoes are malaria infected.

WHO Malaria Mortality Report Worldwide (2012)

According to **WHO records** that vector borne diseases including malaria, kills more people than all wars and plagues combined. Moreover, **malaria kills more children than any infectious disease in the world.** Epidemiological surveys in 2010 showed that nearly **100 countries were still malarious**, in **which 40% population was at risk of infected with malaria, together causing 500 million acute episodes every**

year. According to official WHO estimates **one million deaths occurred** due to malaria episodes affecting more children in Subsaharan Africa. Major malaria related infections **40% to 60% are due to *P. falciparum* and the rest are caused by *P. vivax*.**

The Indian mortality data recorded by National Antimalaria Programme (NAMP) for the year 2000 was 932 deaths due to malaria and for the year 2010 the mortality was 1018 deaths according to NAMP mortality record reported in the Indian population (see Table 8). In authors' opinion, the NAMP Malaria Control Programme in India has been successful in controlling both malaria infections and mortality. The Indian NAMP mortality data is specifically based on all India mortality data due to certified deaths due to malaria.

11. WORLD HEALTH ORGANIZATION REPORT (2012): SUCCESS STORIES IN MALARIA CONTROL FROM THE SOUTH-EAST ASIA REGION

According to this report the application of bioenvironmental control strategy have been incorporated in the integrated malaria control strategy of the NAMP (Govt. of India), together with Rapid/Diagnostics for Malaria, and the appropriate antimalarial strategy (under supervision of National Antimalaria Programme, NAMP) together with New Antimalarial Drug Development had been able to reduce malaria burden in country. Following the NAMP Antimalaria Programme, the malaria which was on the rise in areas like Mangalore, was brought under control. This **WHO SEA Regional Report dated 2012 also documents the progress in Drug Development in India specifically Antimalarial Research leading to:**

(i) **α/β-Arteether (30:70 ratio) a racemic mixture** of ethyl derivative of Artemisinin developed by the Central Drug Research Institute and CIMAP, Lucknow. Multicentric trials for uncomplicated and complicated *P. falciparum* in over 500 patients have shown a recrudescence rate of 8%. The injectible formulation (150 mg/ampoule) is given IM for 3 days. The drug was **marketed in India since 1997** by Themis Chemicals, Mumbai. α/β-Arteether has been introduced as fast acting blood schizontocide in the treatment of severe/multidrug resistant *P. falciparum* (This drug was first published in 1987-1988 as fast-acting

blood schizontocide by author (Dutta *et al.* , 1987, 1988) and also patented by CSIR-CDRI and CIMAP in 1990.

(ii) **Azithromycin (oral)** a macrolide antibiotic has shown delayed blood schizontocidal activity against multidrug resistant *P. yoelii nigeriensis* infection in experimental murine malaria (published by author see Tripathi *et al.* 2005). Clinical trials had been undertaken by Malaria Research Centre (ICMR), New Delhi.

(iii) Bulaquine – a new anti-relapse (CDRI-80/53) designated as **Elubaquine, WHO INN (2000)**, was **first published in 1989** by the author (Dutta *et al.* 1989) Central Drug Research Institute, Lucknow. This new 8-aminoquinoline is a **new safer anti-relapse antimalarial.** The drug has successfully completed phase I, II, III and IV clinical trials and was **marketed in India in year 2000**. The drug was granted **U.S. Patent 7183291 (Feb. 27, 2007), EP 1055437 on 13th Oct. 2004; Mumbai 501/MUM/May 5, 2000 (Last update 9th Jan. 2007 WHO, SEA Regional Report).**

Table 1: Evolution of drug resistance in *Plasmodium falciparum*

Resistance to	Treatment	Area	Source
Mepacrine	Clinical	New Guinea	Fairly *et al.* (1946)
Proguanil	Clinical	Indonesia	Adams & Seaton (1949)
			Van Goor & Lodens (1950)
	Clinical		Van Goor *et al.* (1950)
	Clinical	Malaya	Robertson *et al.* (1952)
	Clinical	Assam	Gilroy (1952)
	Clinical	New Guinea	Gunther *et al.* (1952)
	Clinical	Indochina	Canet (1952)
	Clinical	Malaya	Field *et al.* (1954)
Pyrimethamine	Clinical	Kenya	Jones (1954)
	Prophylaxis	Tanganyika	Clyde & Schute (1954)
	Prophylaxis	Tanganyika	Clyde (1957)
	Prophylaxis	Kenya	Jones (1958)
	Clinical	Tanganyika	Clyde *et al.* (1958)
	Experimental	-	Burgess & Young (1959)
Primaquine	Experimental	-	Arnold *et al.* (1955)
Chloroquine	Experimental	-	Young (1961, 1962)
	Clinical	Colombia	Moore & Lanier (1961)
	Clinical	Phillipines	Nakabayashi *et al.* (1974)

Dutta, G.P. (1977) OPPI-ICMR International Malaria Symposium.

Table 2: Evolution of multiple drug resistance in *P. falciparum*

Resistance to	Treatment	Area	Source
Chloroquine, hydroxychloroquine, amodiaquine, mepacrine, pyrimethamine, proguanil	Experimental	Thailand	Powell *et al.* 1964 Young *et al.* 1962 Jeffery *et al.* 1963
Chloroquine, Amodiaquine	Expeimental	Thailand	Young 1962
Chloroquine, mepacrine, chloroquine, mepacrine, pyrimethamine, proguanil	Experimental Experimental	Colombia Cambodia & Malaya	Young 1961, 1962 Contacos *et al.* 1963
Chloroquine, proguanil	Experimental	Malaya	Montgomery & Eyles 1963
Chloroquine, amodiaquine	Experimental	Panama	Young & Johnson, 1972
Chloroquine, amodiaquine, proguanil, pyrimethamine, quinine	Experimental	Burma	Clyde *et al.* 1973
Chloroquine, amodiaquine, proguanil, pyrimethamine, trimethoprim, sulfadiazine, quinine, sulfametopyrazine	Experimental	Vietnam	Willerson *et al.* 1974

Dutta, G.P. (1977) OPPI-ICMR International Malaria Symposium

Table 3: Multiple drug resistant strains of *P. falciparum*

Strain designation	Isolated/ Originated from	Resistance to drugs	References
Thailand (JHK)	Thailand	Chloroquine, hydroxy-Chloroquine, amodiaquine, mepacrine, pyrimethamine, proguanil	Powell *et al.* (1964). Bull. Wold Hlth Org. 30, 29
Colombia Strain	Colombia	Chloroquine, amodiaquine, hydroxyl-chloroquine	Young (1961). Am. J. Trop. Med. Hyg. 10, 689 Young (1962). Trans. R.. Soc. Trop. Med. Hyg. 56,252
Cambodian II	Cambodia	Chloroquine, proguanil	Contacos *et al.* (1963). Trans. R. Soc. Trop. Med. Hyg. 57,417
Malayn I	West Malaysia	Mepacrine, pyrimethamine	Contacos *et al.* (1963). Trans. R. Soc. Trop. Med. Hyg. 57,417
Malayan II	West Malaysia	Chloroquine, proguanil	Contacos *et al.* (1963), Trans. R. Soc. Trop. Med. Hyg. 57,417 Montgomer and Eyles (1963). Trans. R. Soc. Trop. Med. Hyg. 57,409
Malayan III	West Malaysia	Chloroquine, proguanil, pyrimethamine	Contacos *et al.* (1963). Trans. R. Soc. Trop. Med. Hyg. 57,417
Viet Nam (SN)	Viet Nam	Chloroquine, hydroxyl-chloroquine, amodiaquine, mepacrine, proguanil, quinine	Powell *et al.* (1964). Bull. Wld Hlth Org. 31, 379
Malaya (camp.)	West Malaysia	Chloroquine, hydroxyl-chloroquine, amodiaquine, mepacrine, pyrimethamine, proguanil	DeGowin and Powell (1965). Am. J. Trop. Med. Hyg. 14, 519
Viet Nam (CV)	Viet Nam	Chloroquine, pyrimethamine, quinine	Eppes *et al.* (1966). Milit. Med. 131, 844
Thailand II Malayan IV	Thailand West Malaysia	Chloroquine, proguanil, pyrimethamine	Chin *et al.* (1966). Am. J. Trop. Med. Hyg. 15, 823
Viet Nam I (SV-I)	Viet Nam	Chloroquine, proguanil, quinine	Chin *et al.* (1966). Am. J. Trop. Med. Hyg. 15, 823
Thailand (Man)	Bankok (Thailand)	Chloroquine, pyrimethamine, quinine	Clyde *et al.* (1969). Milit. Med. 134, 787
Malaya (Pool) Malaya (Tay)	West Malaysia	Chloroquine, proguanil, pyrimethamine, quinine	Clyde *et al.* (1969). Milit. Med. 134, 787
Viet Nam (Brai)	Viet Nam	Chloroquine, quinine	Clyde *et al.* (1969). Milit. Med. 134, 787
Viet Nam (Smitgh)	Viet Nam	Chloroquine, amodiaquine, proguanil, pyrimethamine, quinine	Clyde *et al.* (1970). J. Am. Med. Ass. 213, 2041
Viet Nam (Marks)	Viet Nam	Chloroquine, amodiaquine, proguanil, pyrimethamine, quinine	Rieckmann (1972). J. Am. Med. Ass. 217, 573 Willerson, Dr.Jr. *et al.* (1974). Milit. Med. 139, 175 (1974)

Table: contd….

Strain designation	Isolated/ Originated from	Resistance to drugs	References
Phillipines (per)	Phillipines	Chloroquine, pyrimethamine, quinine	Clyde *et al.* (1971). J. Trop. Med. Hyg. 74, 101
Panama strain	Panama	Chloroquine, amodiaquine	Young and Johnson (1972). Am. J. Trop. Med. Hyg. 21, 13
Cambodia (Buch)	Cambodia	Chloroquine, amodiaquine, quinine	Rieckmann *et al.* (1972). Proc. Helminth. Soc., Wash. 39, spec. issue, 339
Burma (Thau.)	Burma	Chloroquine, amodiaquine, pyrimethamine, proguanil, quinine	Clyde *et al.* (1972). Trans. R. Soc. Trop. Med. Hyg. 66, 369
Sobah (Kal.)	Sobah	Chloroquine, amodiaquine, proguanil, pyrimethamine	Clyde *et al.* (1973). J. Trop. Med. Hyg. 76, 226
Indonesia (Whit)	Jaya	Chloroquine, amodiaquine, pyrimethamine, quinine	Clyde *et al.* (1976). J. Trop.Med. Hyg. 79, 38

Puri, S.K. and Dutta, G.P. (1982). *Progress in Drug Research* **26**, 167-205

Table 4: Evolution of drug resistance in blood stages of *P. vivax*

Resistance to	Treatment	Source
Proguanil	Experimental Experimental Clinical	Seaton & Laurie 1949 Laurie & Seaton 1949 Cooper *et al.* 1950 Field *et al.* 1954
Pyrimethamine	Experimental Experimental	Hernandez *et al.*1953 Young & Burgess 1959
Primaquine	Experimental	Arnold *et al.* 1961

Dutta, G.P. (1977) OPPI-ICMR International Malaria Symposium

Table 5a: Malaria incidence in India (year-wise) based on NMEP and NAMP reports

Year	Total Malaria cases (in millions)		Total malaria deaths (year-wise)
1976	6.47	(0.75 P.f.)	59
1984	2.18	0.65 P.f.)	247
1990	2.02	(0.75 P.f.)	353
1991	2.12	(0.92 P.f.)	421
1992	2.13	(0.88 P.f.)	422
1993	2.21	(0.85 P.f.)	354
1994	2.51	(0.99 P.f.)	1122
1995	2.93	(1.14 P.f.)	1151
1996	3.04	(1.18 P.f.)	1010
1997	2.66	(1.01 P.f.)	879
1998	2.10	(0.91 P.f.)	653
1998*	2.5-3.2	(0.8 P.f.)	1000
1998*	15.0	(40% P.f.)	19,500

Data based on National Antimalaria Programme Report (Shiv Lal, 1999)
*NMEP data and WHO estimates cited by Sharma (1998).

Table 5b: Emerging malaria drug resistance against *P. falciparum* cases in India on the basis of National Anti-Malaria Programme (Cumulative data from 1978 up to 1997)

In vivo				
No. of cases tested	S	S/RI	RI	RII
Chloroquine treatment				
12863	3814	5984	1932 (15.0%)	653 (5.07%)
Amodiaquine treatment				
333	116	99	61 (18.3%)	35 (10.5%)
Sulfa pyrimethamine-combination treatment (a) Sulfadoxin/Pyrimethamine				
915	256	639	10 (1.09%)	6 (0.65%)
(b) Sulfalene/Pyrimethamine				
160	135	23	2 (1.25%)	0 Nil
Quinine treatment				
209	171	23	13 (6.22%)	2 (0.95%)

R = resistant

S = sensitive, RI = low level of resistance, RII = moderate level of resistance and RIII = high level of resistance

Data reproduced from: National Anti-Malaria Programme (March, 1999)

Table 6: Emergence of antimalarial resistance against malaria

Evaluation of clinical trials data against malaria *P. falciparum* cases show emergence of resistant strains amongst most of the standard drugs

Resistance against *P. falciparum*	Discovery year	Year
Quinine	1880	1910
Chloroquine	1934	1957
Proguanil	1945	1949
Pyrimethamine	1951	1954
Sulphadoxine	1975	1982
Quinine + Tetracycline	1970	1974
Piperaquine		2003
Amodiaquine	1946	1961
Mepacrine	1930	1946
Halofantrine	1990	1992
Metakelfin		1981
Pyronaridine	1970	1982
Atovaquone		1995
Atovaquone + Proguanil		2002
Mefloquine	1971	1982
Fansimef	1980	1985
Pyrimethamine + Sulphadoxine (Fansidar)	1975	1982
Artesunate		1998
Artemether		200
Artemether + Lumefantrine		2005
Primaquine	1945	
Metakelfin	1975	
Pamaquine	1925	

Antimalarial discovery and year of emergence of drug resistance (Dutta, G.P.)

Table 7: World Health Database (2011)

First report of drug resistant malaria cases in SEA region

Countries	*P. falciparum*				*P. vivax*
	CQ	SP	MEF	QN	CQ
Bangladesh	1970	1985			
Bhutan		1985	1990		
India		1973	1979	1986	1991
Indonesia	1973	1979			1989
Nepal	1972	1997			
Myanmar	1969	1986	1997		1993
Sri Lanka	1984				
Thailand	1962	1984	1990		

(CQ : Chloroquine; SP: Sulfadoxine-pyrimethamine; MEF: Mefloquine; QN: Quinine)

Courtesy: WHO Report (2011)

Table 8: Malaria epidemiological situation and indicators in India from 2000 to 2011

Year	Popula -tion in Crores	Blood smears examined	Positive cases	*P. falciparum* cases	*P. falciparum* %	ABER	API	SPR	SFR	Deaths
2000	97.02	8,67,90,375	20,31,790	10,47,218	51.54	8.94	2.09	2.34	1.21	932
2001	98.45	9,03,89,019	20,85,484	10,05,236	48.20	9.81	2.12	2.31	1.11	1005
2002	101.39	9,16,17,725	18,41,229	8,97,446	48.74	9.04	1.82	2.01	0.98	973
2003	102.71	9,91,36,143	18,69,403	8,57,101	45.85	9.65	1.82	1.89	0.86	1006
2004	104.09	9,71,11,526	19,15,363	8,90,152	46.47	9.33	1.84	1.97	0.92	949
2005	108.28	10,41,43,806	18,16,569	8,05,077	44.32	9.62	1.68	1.74	0.77	963
2006	108.28	10,67,25,851	17,85,129	8,40,360	47.08	9.95	1.66	1.67	0.79	1707
2007	108.75	9,49,28,090	15,08,927	7,41,076	49.11	8.73	1.39	1.59	0.78	1311
2008	111.96	9,73,16,158	15,26,210	7,75,526	50.81	8.69	1.36	1.57	0.80	1055
2009	115.01	10,33,96,076	15,63,574	8,39,877	53.72	8.99	1.36	1.51	0.81	1144
2010	116.73	10,86,79,429	15,99,986	8,34,364	52.15	9.31	1.37	1.47	0.77	1018
2011	119.49	10,89,69,660	6,65,004	6,65,004	50.74	9.12	1.10	1.20	0.61	753

ABER: Annual Blood Smear Examination Rate
API: Annual Parasite Incidence
SPR: Slide Parasite Rate
SfR: Slide falciparum Rate
Malaria Epidemiology in India (2000-2011). NAMP Report

CHAPTER-IIA

TECHNOLOGY FOR ANTIMALARIAL DRUG SCREENING AND DEVELOPMENT

1. HISTORICAL ACCOUNT OF DEVELOPMENT OF EXPERIMENTAL MODELS FOR ANTIMALARIAL SCREENING

Primary antimalarial screening with rodent test system for identification of new potential blood schizontocides had been going on at the former Malaria Research Institute of Govt. of India, Delhi, between 1950-1965 but no new lead had been identified mainly because there was no programme for synthesis of new compounds and also absence of established secondary screening primate malaria models and facilities for animal pharmacology and regulatory toxicology including sub-acute toxicity facilities. Only a few pharmaceutical concerns in the country like Ranbaxy, IPCA and Dr. Reddy's Lab. had initiated research and developmental activity which could lead to the development of new antimalarials.

Following the resurgence of malaria in the country between1965-75, the "Modified Plan of Operation for Control of Malaria" a new strategy, was adopted by the Indian Council of Medical Research in 1976-77 to establish a National Task Force on Chemotherapy of Malaria. On behalf of CSIR, the Central Drug Research Institute became the nodal agency to initiate new drug development programme for the country. The highest priority was given at CDRI in 1977 to establish fool proof antimalarial screening protocols which could identify potential antimalarials for detailed preclinical development.

Quinine was the first active principle isolated in 1820 from the cinchona bark by the French Scientist and it continues to be a drug of choice even today for treatment of cerebral malaria cases.

Soon after World War I, several synthetic antimalarials were discovered in quick succession, including pamaquine in 1925, mepacrine in 1930, chloroquine in 1934, primaquine and proguanil in 1945, amodiaquine in 1946 and pyrimethamine in 1951. Clinical studies on malaria conducted between 1945-1955 had clearly established that 8-aminoquinolines (primaquine and pamaquine) were poor blood schizontocides, nevertheless, they exhibited high tissue schizontocidal (anti-relapse) activity against *Plasmodium vivax* and *P. malariae* as well as strong gametocytocidal action against *P. falciparum* and *P. vivax* (Covell *et al.*, 1955).

The tremendous efforts were made to develop experimental drugs screening animal models between 1941-1975 (as reported in Table 9). Amongst the large number of antimalarial screens, special attention was paid to develop experimental models:

(1) Avian malaria

(a) Blood schizontocidal activity

(i) *P. cathemerium-canary*

(ii) *P. lophurae*-duckling

(iii) *P. gallinaceum*-chick

(b) Tissue schizontocidal activity

(i) *P. cathemerium*-canary

(ii) *P. gallinaceum*-chick

(2) Rodent malaria

(a) Blood schizontocidal activity

(i) *P. berghei*-mice

(b) Tissue schizontocidal activity

(i) *P. berghei*-mice

(ii) *P. yoelii*-mice

(3) Simian malaria

(a) Blood schizontocidal activity

(i) *P. cynomolgi* B-rhesus monkey

(ii) *P. knowlesi* W1-rhesus monkey

(iii) *P. knowlesi* W1-Assamese monkey

(b) Tissue schizontocidal activity

(i) *P. cynomolgi* B-rhesus monkey

(4) Human malaria in non-human primates

(a) Blood schizontocidal activity

(i) *P. falciparum-Aotus* monkey

(5) Human malaria in volunteers

(a) Blood schizontocidal activity

(i) *P. vivax*

(ii) *P. falciparum*

(b) Tissue schizontocidal activity

(i) *P. vivax*

(ii) *P. falciparum*

Primary Antimalarial Screening Models

The International efforts by experimental malaria experts had carried out extensive studies using the above cited animal models in order to identify new antimalarial entities which could be develop a safe drugs for malaria. *Plasmodium relictum* – canaries model developed by Roehl (1924) was first used for large-scale blood schizontocidal screening by German Scientists. Later on under the U.S. Army antimalarial screening programme nearly 15,000 new compounds had been screened against *P. gallinaceum* – chick model and the results were published by Wiseloogle (1946a,b) in "A survey of antimalarial drugs". The avian screens (*P. relictum* and *P. gallinaceum*) are of limited value because the blood induced infections with the parasites show recrudescence after treatment with standard blood schizontocides like chloroquine, mefloquine and quinine. Avian malarias are known to develop exo-erythrocytic phase in endothelial cells of capillaries of brain/lung etc. following inoculation with blood stages and these EE-stages are not sensitive to the action of known blood schizontocides.

With the discovery of rodent malaria parasite *P. berghei* Vincke and Lips (1948), the emphasis on large-scale primary screening of potential compounds was shifted from *P. relictum/P. gallinaceum* screens to *P. berghei* and related rodent parasites like *P. vinckei, P. yoelii* and *P. yoelii nigeriensis* etc. Initial studies with rodent sporozoite and blood schizontocidal screening tests led to the discovery of pyrimethamine in 1950 which has activity both as causal prophylactic and blood schizontocide. Besides, the rodent blood schizontocidal screens have led to the discovery of several new long-acting sulphones like dapsone (DDS) and acedapsone (DADDS) and sulphonamides such as sulphadoxin. During the last five decades, nearly 300,000 new compounds had been screened for blood schizontocidal activity against *P. berghei* by Rane rodent malaria screen (Osdene *et al.* 1967) under contracts with Walter Reed Army Institute of Research, Washington. Out of this massive effort of primary screening of potential antimalarials, two new drugs identified as mefloquine (WR 142-490) and halofantrine had emerged.

Although both *P. gallinaceum* – chick and *P. berghei* – Swiss mice models are available for primary screening of potential antimalarials, most of the investigators at CDRI new prefer to use MDR rodent blood schizontocidal screen as primary screening model for large scale screening since this model is dependable in predicting potential blood schizontocidal activity both with known blood schizontocides as well as new compounds such as artemisinin derivatives.

2. ANTIMALARIAL *IN VITRO* DRUG SENSITIVITY OF *PLASMODIUM FALCIPARUM* STRAINS TO MONITOR EMERGING DRUG RESISTANCE

Fidock *et al.* (2004) *in vitro* screening protocols

Fidock *et al.* (2004) have described in detail the protocols for conducting antimalarial screening of potential antimalarial compounds. The protocols for antimalarial drug screening described by Fidock and colleagues represent the pooled expertise of several laboratories engaged in antimalarial-drug discovery which include the protocols jointly developed and shared by London School of Hygiene and Tropical Medicine; Swiss Tropical Institute; Medicines for Malaria Venture, Geneva; University of California and Albert Einstein College of Medicine, New York. The protocols briefly described below are evidence of commitment of Research Collaborators engaged in Drug Discovery/ Screening of new molecules.

1. **A protocol for antimalarial efficacy testing *in vitro***: Initially the *P. falciparum* standard ten strains (Dd2, W2, HB3, 3D7, D6, D10, CAMP, FCB, 7G8, and K1) isolated from different malarial endemic countries were cultured *in vitro* to record their resistant patterns to various antimalarials. This information on reference strains will be very vital for other scientists who would like to screen new compounds *in vitro* against these standard *P. falciparum* isolates (data on drug sensitivity and resistance pattern of various strains and the country of isolation of strains is briefly described in Table 10a). The Desjardin's *et al.* (1979) step-wise procedure is fully described by Fidock *et al.* (2004) for the benefit of investigators. *P. falciparum* strains from different countries have also been cultured *in vitro* to determine the percentage of strains which had acquired resistance to standard antimalarials. The data presented in Table 10b show the

percentage of resistance of *P. falciparum* isolates from patients to different antimalarials as shown by *in vitro* drug sensitivity tests during the period 1987-1998. The data on drug resistance as evidenced by *in vitro* tests, show that nearly 90-100% *P. falciparum* isolates, had acquired resistance to chloroquine and amodiaquine in China-Amozonian region as well as in French Guiana in *in vitro* tests. It may be also pointed out that overall chloroquine resistance in most of the countries was well established (Table 10b).

3. PROTOCOLS FOR *IN VIVO* ANTIMALARIAL SCREENING IN EXPERIMENTAL ANIMALS

(i) In primary biological screen: The authors prefer to use screening against *P. berghei* (ANKA strain) infection in NMRI mice. Four dose schedule of the test compound has been adopted in the primary screening in *in vivo* test. Further protocols for *in vivo* screening are also fully described by the authors, which may be consulted for details.

(ii) Secondary Biological Assessment: Four dose levels of drug i.e. 100, 30, 10 and 3 mg/kg are tested in 4 dose schedule.

(iii) Tertiary Biological Screen: This screen involves *in vivo* screening against drug-resistant lines of the rodent malaria parasites.

The authors refer to several drug-resistant strains of rodent malaria parasites which have been selected over the last five decades by Professor Wallace Peters (Northwick Park Institute for Medical Research, Middlesex, U.K.; Formerly London School of Hygiene and Tropical Medicine, U.K.). Large number of experimentally selected drug resistant rodent malaria strains published by Professor Peters (1998), are also reported in US Patent No. 58934505 by Peters, which are reproduced in Table 11. These include four resistant strains of *P. berghei, P. yoelii nigeriensis* (N67, NIG-chloroquine resistant); and six strains of *P. yoelii* showing resistance to major antimalarials. Professor W. Peter's book (1970) on "Drug Resistant Malaria" is a monumental life-time contribution to develop experimental protocols for New Antimalarial Drug Discovery. Experimental results of Professor Peters antimalarial researches are also published in series of large number of Research Publications on Chemotherapy of Malaria. The mention must also be made of similar studies on drug

resistant rodent malaria *P. berghei* N (normal strain) and twelve drug resistant *P. berghei* lines used for antimalarial screening by Barossi *et al.* (1988), which are included in Table 12. The drug resistant lines of rodent malaria parasites are used for *in vivo* screening of relative antimalarial activity of various artemisinin derivatives against the drug resistant lines of *P. berghei.*

As reported above by Peters (1998), and Brossi *et al.* (1988), the drug resistant strains of *P. berghei* resistant to various standard antimalarials were also selected at CDRI and these resistant lines of *P. berghei* are useful for *in vivo* screening of potential antimalarials using Peters (1970) relapse technique for selection of resistant lines and 4-day test for *in vivo* screening.

TECHNOLOGY DEVELOPED FOR ANTIMALARIAL DRUG SCREENING AND DISCOVERY AT CSIR-CENTRAL DRUG RESEARCH INSTITUTE, LUCKNOW

Selection of *P. berghei* Drug Resistant Lines

As reported in Table 13, drug resistant strains of *P. berghei* derived from parent strain *P. berghei* (NK67) could be selected by sequential exposure to various drugs administered by oral route. The chloroquine resistant strain was resistant in *in vivo* (4 day treatment) up to 128 mg/kg), pyrimthamine resistant strain up to 128 mg/kg, mefloquine resistant was up to 256 mg/kg, quinine resistance up to 450 mg/kg, primaquine resistance up to 70 mg/kg, sulfanilamide resistance up to 800 mg/kg, mefloquine + quinine resistance (up to 256 + 100 mg/kg) and mefloquie + pyrimethamine combination was also found to be resistant. These *P. berghei* resistant lines could tolerate increasing dose levels of drugs exposure to **sequential, interrupted sub-curative dose level of respective drugs** as given in the listed publications. Between successive mouse passages, the strains were cryopreserved in buffered glycerol and stored in liquid nitrogen.

Selection of highly multidrug-resistant lines of *P. yoelii nigeriensis*

The original strain of *P. yoelii nigeriensis* (N67) was kindly procured by late Professor P. C. C. Garnham from London School of Hygiene and Tropical Medicine and brought safely on August 21, 1976 to New Delhi airport, wrapped in bath towel containing pellets of Dry Ice (CO_2) for cryopreservation and safe arrival of frozen blood (2 vials) from U.K. The vials with cryopreserved *P. yoelii nigeriensis* were stored in Dry Ice container and brought to CDRI, Lucknow by the author and the strain was passaged into 20±2g random bred Swiss mice, for sequential passage/cryopreservation in Liquid N_2 container.

The strain *P. yoelii nigeriensis* (N67) showed innate low level of resistance to chloroquine administered orally. This strain was subjected to a series of sequential subcurative doses of drugs which led to the selection of lines resistant to individual doses of chloroquine 128 mg/kg, mefloquine 256 mg/kg and quinine 600 mg/kg (x 4 days oral drug schedules) (Table 13). Further work on selection of resistance was continued for 2 years and highly multidrug resistant strain of *P. yoelii nigeriensis* (designated MDR) was maintained in Swiss mice. Resistance of *P. yoelii nigeriensis* (MDR) to individual drugs as shown in Tables 13 and 14 was achieved and the strain was cryopreserved in multiple vials in liquid N_2. The original *P. yoelii nigeriensis* strain was very virulent with 100% mortality of 20±2 g Swiss (random bred) mice. The strain was passaged in mice by taking blood from cardiac puncture in citrate saline (ACD). Sub-passage of the resistant line was by intraperitoneal route after diluting the inoculum in sterile citrate saline. Mortality with this MDR line is 100% within 5-7 days. Parasitaemia record of Giemsa stained blood smears show 100% parasitaemia. *P. yoelii nigeriensis* MDR strain selected by the author around 1987 has undergone indefinite weekly serial passages in the last 25 years and extensively used by the author in all antimalarial drug discovery projects at CDRI. The level of resistance to various drugs/drugs-antibiotics/antibiotics recorded in 4-day treatment schedule between 1997-2000 is presented in Table 14. The strain has been made available to all Scientific Researchers engaged in researches on malaria chemotherapy. The strain maintains exceptionally high virulence, produces 100% mortality in Swiss mice and stained blood smears show 100 RBC infections. This **Rodent**

Malaria Parasite – *Plasmodium yoelii nigeriensis* (MDR strain) has been used for Drug Discovery (Antimalarial Primary Screening) at CDRI by the Author.

P. yoelii nigeriensis standard strain shows **innate chloroquine resistance** and it produced 100% mortality in Swiss mice.

***P. yoelii nigeriensis* MDR (multidrug resistant)** strain selected at CDRI is 100% fatal in Swiss mice. This strain was procured from the London School of Tropical Medicine and Hygiene. This strain was exposed to a large variety of standard drugs listed below and designated as: MDR *P. yoelii nigeriensis* (developed by the author) has been extensively used at CDRI for Drug Discovery of New Antimalarials.

***P. yoelii nigeriensis* (MDR Strain)** shows high level of multi-drug resistance to the following antimalarials, antibiotics and antimalarial-antibiotic combinations:

Chloroquine, Amodiaquine, Mefloquine, Mepacrine, Quinine, Quinidine, Halofantrine, Pyrimethamine, WR238605, Tetracycline, Demeclocycline, Doxycycline, Erythromycin, Ciprofloxacin, Norfloxacin, Chloroquine + Tetracycline, Amodiaquine + Tetracycline, Mefloquine + Tetracycline, Mepacrine + Tetracycline, Quinine + Tetracycline, Quinine + Oxytetracycline, Quinine + Doxycycline, Quinine + Erythromycin, Ciprofloxacine + Chloroquine, Norfloxacine + Chloroquine, Quinidine + Tetracycline, details of the resistance level of *P. yoelii nigeriensis* MDR to drugs antibiotics and drug antibiotic combinations using four days treatment schedule are presented in Table 14.

Table 9: Antimalarial drugs screening animal models developed by earlier investigators including screening in human volunteers.

Host-parasite combination	References
(1) Avian malaria	
(a) Blood schizontocidal activity	
(i) *P.cathemerium*-canary	Wiseloogle F.Y.(ed.): A survey of antimalarial drugs 1941-1945, Vol.1. Ann Arbor, Michigan, 1946a Wiseloogle F.Y.(ed.): A survey of antimalarial drugs 1941-1945, Vol.2. Ann Arbor, Michigan, 1946b Darrow, E.M. et al. Am.J.Trop. Med. Hyg. 1,927 (1952)
(ii) *P.lophurae*-duckling	Wiseloogle F.Y.(ed.): A survey of antimalarial drugs 1941-1945, Vol.1. Ann Arbor, Michigan, 1946a Wiseloogle F.Y.(ed.): A survey of antimalarial drugs 1941-1945, Vol.2. Ann Arbor, Michigan, 1946b
(iii) *P.gallinaceum*-chick	Wiseloogle F.Y.(ed.): A survey of antimalarial drugs 1941-1945, Vol.1. Ann Arbor, Michigan, 1946a Wiseloogle F.Y.(ed.): A survey of antimalarial drugs 1941-1945, Vol.2. Ann Arbor, Michigan, 1946b Coatney, G.R. et al. Proc. Soc. exp Biol. Med. 72, 587 (1949) Darrow, E.M. et al. Am.J.Trop. Med. Hyg. 1,927 (1952) Kazim M. et al. Chemotherapy 25, 222 (1979)
(b) Tissue schizontocidal activity	
(i) *P. cathemerium*-canary	Darrow, E.M. et al. Am.J.Trop. Med. Hyg. 1,927 (1952)
(ii)*P. gallinaceum*-chick	Coatney, G.R. et al. Proc. Soc. exp Biol. Med. 72, 587 (1949) Darrow, E.M. et al. Am.J.Trop. Med. Hyg. 1,927 (1952) Puri S.K. et al. Ind. J. Med. Res.73, suppl., 36 (1981)
(2) Rodent malaria	
(a) Blood schizontocidal activity	
(i) *P. berghei*-mice	Darrow, E.M. et al. Am.J.Trop. Med. Hyg. 1,927 (1952) Thurston J.P. Br. J. Pharmac. 8, 162 (1953) Lewis, C. J. Parasit. 54, 169 (1968) Kaddu J.B. et al. Ann. Trop.Med. Parasit. 68,41 (1974) Warhurst D.C. et al. Ann. Trop Med. Parasit. 70,253 (1976) Puri, S.K. and Dutta, G.P. Ind. J. Med. Res. 73, suppl., 29 (1981)
(b) Tissue schizontocidal activity	
(i) *P.berghei*-mice	Hill J. Ann. Trop. Med. Parasit. 69, 421 (1975)
(ii) *P.yoelii*-mice	Peters W. et al. Ann. Trop. Med. Parasit. 69, 311 (1975)

Table 9: continued….

Host-parasite combination	References
(3) Simian malaria	
(a) Blood schizontocidal activity	
(i) *P. cynomolgi* B-rhesus monkey	Powers K.G. Am. J. trop. Med. Hyg. 18, 485 (1969)
(ii) *P. knowlesi*-rhesus monkey	Schmidt, L.H. et al. Am. J. trop. Med. Hyg. 19, 1 (1970)
(iii) *P. knowlesi*-Assamese monkey	Powers, K.G. et al. Expl. Parasit. 40, 13 (1976)
	Dutta, G.P. and Singh, P.P. Proc. Asian Congr. Parasit. 6 (1978)
	Dutta, G.P. and Singh, P.P. Ind. J. Med. Res. 70, suppl., 91 (1979)
(b) Tissue schizontocidal activity	
(i) *P. cynomolgi* B-rhesus monkey	Schmidt, L.H. et al. Am.J. trop. Med. Hyg. 19,1 (1970)
	Garnham, P.C.C. et al. J. trop. Med. Hyg. 74, 32 (1971)
(4) Human malaria in non-human primates	
(a) Blood schizontocidal activity	
(i) *P. falciparum*-Aotus monkey	Powers,K.G. and Jacobs, R.L. Antimicrob. Ag. Chemother. 1, 49 (1972)
(5) Human malaria in volunteers	
(a) Blood schizontocidal activity	
(i) P. vivax	Clyde, D.F. et al. J. trop. Med. Hyg. 74, 278 (1971)
(ii) P. falciparum	Clyde, D.F. et al. J. trop. Med. Hyg. 74, 278 (1971)
	Rieckmann, K.H. et al. Am. J. trop. Med. Hyg. 20, 811 (1971)
	Miller, L.H. et al. Am. J. trop. Med. Hyg. 23, 565 (1974)
	Colwell,E.J. et al. J. Am. Med. Ass. 220, 684 (1972)
	Colwell, E.J. et al. Am. J. trop. Med. Hyg. 21, 144 (1972)
(b) Tissue schizontocidal activity	
(i) P. vivax	
(ii) P. falciparum	Clyde, D.F. et al. J. trop. Med. Hyg. 74, 278 (1971)
	Willerson, Jr. D. et al. Am. J. trop. Med. Hyg. 21, 857 (1972)
	Rieckmann, K.H. et al. Proc. Helminth. Soc., Wash. 39, spec. issue, 339 (1972)

Antimalarial drug screening models (Puri, S.K. and Dutta, G.P. 1982, *Progress in Drug Research* **26,** 167-205

Table 10a: Standard *Plasmodium falciparum* strains showing multiple drug resistance to various antimalarials as reported by Fidock *et al.* (2004) based on *in vitro* tests.

Name	Origin	Resistant to	References
Dd2	Indochina	CQ, QN, PYR, SDX	Noedl, H. *et al.* Trends Parasitol. 19,175-81 (2003)
W2	Indochina	CQ, QN, PYR, SDX	Wellems, T.E. *et al.* Nature 345, 253-255 (1990)
HB3	Honduras	PYR	Oduola, A.M. *et al.* Exp. Parasitol. 66, 86-95 (1988)
3D7	Apparently West Africa	--	Oduola, A.M. *et al.* Exp. Parasitol. 66, 86-95 (1988)
D6	Sierra Leone	--	Wellems, T.E. *et al.* Nature 345, 253-255 (1990)
D10	Papua New Guinea	--	Walliker, D. *et al.* Science 236, 1661-1666 (1987)
CAMP	Malaysia	PYR	Culvenor, J.G. *et al.* Exp. Parasitol. 63, 58-67 (1987)
FCB	Apparently SE Asia	CQ, QN, CYC	Schmidt, L.H. *et al.* Am. J. Trop. Med. Hyg. 26, 837-49 (1977)
7G8	Brazil	CQ, PYR, CYC	Joy, D.A. *et al.* Science 300,318-21 (2003)
K1	Thailand	CQ, PYR	Burkot, T.R. *et al.* Trans. R. Soc. Trop. Med. Hyg. 78, 339-341 (1984)

CQ, chloroquine; QN, quinine, PYR, pyrimethamine; SDX, sulfadoxine; CYC, cycloguanil

MDR of *P. falciparum* isolates *in vitro* (Courtesy Fidock *et al.* 2004)

Table 10b: Resistance of *P. falciparum* isolates collected from different countries, against various drugs *in vitro.*

Country	Drugs	Resistance (%)	Reference
China-Myanmar, China-Laos border	Chloroquine Amodiaquine Artemisinin derivative Pyronaridine	90-100% R 83.5-100% R 6.3-22.2% R 36.4% R	Yang Henglin et al. (1997)
China	Chloroquine Amodiaquine	86.6% R 86%	Liu Dequan et al. (1998)
Tamil Nadu (India)	Chloroquine	60% R	Dua et al. (1997)
Orissa (India)	Chloroquine Sulfadoxin-Pyrimethamine	25% R 62.5% R	Choudhary et al. (1987)
African region	Chloroquine Halofantrine	20% R 9% R	Quedraogo et al. (1998)
Tanzania	Chloroquine Sulfadoxin-Pyrimethamine SD-PY	30% 13% R 28/38 R	Alin (1997) Ronn et al. (1996)
Cameroon	Chloroquine	62.1%	Ringwald et al. (1996)
Brazil-Amazonian Region (S.America)	Chloroquine Amodiaquine	100% R 100% R	Segurado et al. (1997)
Mpumalanga S. Africa	Chloroquine	86% R	Neirop et al. (1996)
Tanzania	Pyrimethamine	28% R	Edoh et al. (1997)
French Gujana	Chloroquine Quinine	97% R 14% in (1995) R	Reynes et al. (1997)
Burkina Faso (W. Africa)	Chloroquine Halofantrine	19% R 9.6% R	Ouedraogo et al. (1998)

Resistance of *P. falciparum* isolates in vitro from different regions

Table 11: Drug resistance lines/strains of rodent malaria developed for antimalarial screening of potential compounds at London School of Tropical Medicine (U.K.)

Reference = [Peters (1998) US Patent No. 58934505]

1. *P. berghei* 173 N strain (drug sensitive) parent line
2. *P. berghei* P strain (Primaquine resistant)
3. *P. berghei* B strain (Cycloguanil resistant)
4. *P. berghei* PYR strain (Pyrimethamine resistant)
5. *P. berghei* ORA strain (Sulphonamide resistant)
6. *P. yoelii nigeriensis* (N67, NIG) (Chloroquine resistant)
7. *P. yoelii* NS strain (Chloroquine resistant)
8. *P. yoelii* MEF strain (Mefloquine resistant)
9. *P. yoelii* SH strain (from NS) (Halofantrine resistant)
10. *P. yoelii* ART strain (from NS) (Artemisinin resistant)
11. *P. yoelii* SPN strain (from NS) (Pyronaridine resistant)
12. *P. yoelii* SAM strain (from NS) (Amodiaquine resistant)
13. *P. vinckei petteri* strain (sensitive)

Drug resistant lines of rodent malaria developed for *in vivo* screening (Courtesy Peters, 1998)

Table 12: Blood schizontocidal activity of α-DQHS ethyl ether, Arteether, and QHS *in vivo*[a] against drug resistant strains of *P. berghei*.

Parasite	Primary resistance to	α-DQHS ethyl ether		Arteether		QHS	
		ED_{90}	I_{90}	ED_{90}	I_{90}	ED_{90}	I_{90}[b]
P. berghei N		1.3	1.0	0.5	1.0	2.3	1.0
P. berghei NS	Chloroquine	1.5	1.3	2.2	2.0	10.0	2.4
P. berghei RC	Chloroquine	16.5	13.8	8.2	7.5	630	150
P. berghei N/1100	Mefloquine	4.1	3.4	4.2	3.8	17.0	4.0
P. berghei N/HAL	Halofantrine	9.5	7.3	2.1	4.2	31.0	13.5
P. berghei QS	Quinine	13.8	10.6	1.4	2.8	24.0	10.4
P. berghei NPN	Pyronaridine	3.5	2.9	2.5	2.3	90.0	21.4
P. berghei P	Primaquine	2.5	2.1	0.8	0.7	12.0	2.9
P. berghei B	Cycloguanil	2.9	2/4	0.9	0.8	8.2	2.0
P. berghei PYR	Pyrimethamine	1.3	1/1	0.8	0.7	4.8	1.1
P. berghei ORA	Sulfaphenazole	0.8	0.7	0.8	0.7	7.5	1.8
P. berghei MEN	Menoctone	1.7	1.4	2.2	2.0	6.2	1.5
P. berghei ART	QHS	29.0	29.0	4.6	4.6	49.0	21.3

[a]Data from "4-day test". With the exception of the NS line, all lines are resistant to the maximum doses of the respective compounds tolerated by the host.
[b]Doses given subcutaneously on each of 4 consecutive days.
Brossi *et al*. Drug resistant lines of rodent malaria *P. berghei* used for evaluation of Artemisinins *J. Med. Chem*. 1988 **31**, 645-650

Table 13: Experimental selection of drug-resistant *Plasmodium berghei* strains for *in vivo* screening of potential antimalarials

***P. berghei* resistant to:**		
1.	Chloroquine -128mg/kgx4 days	Puri, Agarwal, Kazim & Dutta (1979). Indian J. Med. Res. 70 (suppl) 85-90
2	Pyrimethamine-128mg/kgx4 days	Agarwal, Puri & Dutta (1979). Indian J. Med. Res. 68, 577-582.
3.	Mefloquine-256mg/kgx4 days	Kazim, Puri & Dutta (1979). Indian J. Med. Res. 70 (Suppl.) 95-102.
4.	Quinine-450mg/kgx4 days	Kazim, Saxena& Dutta (1986). Indian J. Malariol. 23, 133-137.
5.	Primaquine-70mg/kgx4 days	Puri & Dutta (1979). Indian J. Med. Res. 70 (Suppl.) 79-84.
6.	Sulfanilamide-800mg/kx4 days	Kazim & Dutta (1980). Indian J. Med. Res. 72, 632-636.
7.	Mefloquine+Quinine-256+ 100 mg/kg	Saxena, Kazim,,Moitra & Dutta (1989). Indian J. Malariol. 26, 199-208.
8.	Mefloquine+pyrimetahaime	Puri & Dutta (1982). Indian J. Med. Res. 70 (Suppl.) 79-84.
***P. yoelii* nigeriensis resistant to:**		
1.	Chloroquine 128 mg/Mefloquine 256 mg/ Quinine 600mg/kg (x4 days) by oral route	Dutta & Pande (1986). Proc. 2nd Asian Congr. Parasitol. Lucknow, pp. 8-9.
2.	Fansi mef.	Dutta, Shukla, Bajpai (1986). Proc. 2nd Asian Congr. Parasitol. Lucknow, pp. 11-12

Antimalarial rodent malaria screening models established at CDRI.

Table 14: Present status of resistance pattern of *P. yoelii nigeriensis* (MDRII) strain to different anti-malarial in Swiss mice.

Drug/anti-biotics	Dose mg/kg (Oral) x days	Parasite response		Drug anti-biotics	Dose mg/kg (Oral) x days	Parasite response
Chloroquine	128x4	Resistant		Chloroquine + Tetracycline	128x4 250x4	Resistant
Amodiaquine	128x4	Resistant		Amodiaquine+ Tetracycline	128x4 250x7	Resistant
Mefloquine	128x4	Resistant		Mefloquine+ Tetracycline	128x4 250x7	Resistant
Mepacrine	128x4	Resistant		Mepacrine+ tetracycline	128x4	Resistant
Quinine	400x4	Resistant		Quinine+ Tetracycline	300x4 500x4	Resistant
Quinidine	400x4	Resistant		Quinidine+ Oxytetracycline	300x4 500x4	Resistant
Halofantrine	32x4	Resistant		Quinine+ Doxycycline	300x4 25x4	Resistant
Pyrimethamine	16x4	Resistant		Quinine+ Erythromycin	300x4 250x4	Resistant
WR238605	10x7	Resistant		Ciprofloxacine+	50x4	Resistant
Tetracycline	500x4	Resistant		Chloroquine	32x4	
Oxytetracycline	500x4	Resistant		Norfloxacine+ Chloroquine	50x4 32x4	Resistant
Demeclocycline	500x4	Resistant				
Doxycycline	25x4	Resistant		Quinidine+ Tetracycline	400x4 250x4	Resistant
Erythromycin	250x4	Resistant				
Ciprofloxacine	50x4	Resistant				
Norfloxacine	50x4	Resistant				

Multidrug-resistance level of *P. yoelii nigeriensis* (MDR) (Dutta, G.P. 2000 Patent)

CHAPTER-IIB

TECHNOLOGY DEVELOPED FOR ANTIMALARIAL DRUG SCREENING AND DISCOVERY

4. PRIMATE ANTIMALARIAL RADICAL CURATIVE SCREENING MODEL

Current status and need for new radical curative (anti-relapse) drugs

The only anti-relapse drug available for the treatment of relapses of *Plasmodium vivax* is primaquine which also exerts strong gametocytocidal action against *P. falciparum* gametocytes. Primaquine and other 8-aminoquinolines are known to produce a variety of adverse side-effects in human subjects such as methaemoglobinaemia with cyanosis, hemolytic anaemia in G-6-PD deficient individuals, hepatotoxicity, gastrointestinal distress, epigastric discomfort, anorexia, vomiting, nausea, headaches and derangement of leucocyte levels (Clyde, 1981). Because of its toxicity, primaquine is contraindicated in pregnancy and not recommended for use in infants and children as well as in individuals whose red cells possess genetic G-6-PD deficiency.

Although several other analogues of primaquine such as pamaquine, pentaquine, isopentaquine and quinocide had been clinically evaluated, these were found to be either less effective or more toxic than primaquine and were, therefore, superseded by primaquine which continues to be only radical curative agent in clinical use at present (Covell *et al.*, 1955; Davidson *et al.*, 1981). In view of toxic side-effects of primaquine, global efforts had been continuing for the last four decades to discover a safer anti-relapse drug for replacing primaquine, which would not produce significant levels of methaemoglobinaemia and hemolytic anaemia, and which could be administered to infants, children and pregnant women without any adverse effect. The CHEMAL group of the World Health Organization have also given top priority to study the pharmacokinetics, metabolism and toxicity aspects of primaquine with a view to identify some less toxic but more active metabolites of primaquine.

The search for new anti-relapse drugs was seriously hampered because of the non-availability of a suitable primate malaria model with a relapse stage in liver which could be used for tissue schizontocidal screening of potential primaquine analogues. The establishment of sporozoite induced *Plasmodium cynomolgi* infection in rhesus monkey through *Anopheles* vector by Schmidt *et al.* (1966) was the starting point for development and screening of new tissue schizontocides against this simian parasite which resembles *P. vivax* in its biological characteristics. *P. cynomolgi* B strain is of special importance

since it is known to be human transmissible and is considered ideal parasite for screening potential radical curative agents.

5. PRIMATE ANTIMALARIAL SCREEING MODELS DEVELOPED AT CSIR-CENTRAL DRUG RESEARCH INSTITUTE, LUCKNOW FOR SCREENING OF NEW ANTIMALARIALS

Protocols established for antimalarial screening against primate malaria at CDRI include the following test systems which led to the selection of new drugs:

1. Blood-schizontocidal activity (*P. cynomolgi* B-rhesus monkey, *P. knowlesi*-rhesus monkey, *P. coatneyi*-rhesus monkey and *P. fragile*-rhesus monkey (α/β-Arteether, DHA)
2. Anti-relapse activity (*P. cynomolgi* B-*Anopheles stephensi*-rhesus monkey (Bulaquine=Elubaquine)
3. Causal-prophylactic activity (*P. cynomolgi* B / *A. stephensi*-rhesus monkey (Bulaquine=Elubaquine, Ablaquine, Tafenoquine)
4. Gametocytocidal/sporontocidal activity (*P. cynomolgi / A. stephensi*-rhesus monkey (Artemisinin, α/β-Arteether, DHA and Bulaquine=Elubaquine and Tafenoquine)

The above experimental models have been successfully applied for the last thirty five years for the screening and identification of blood schizontocides, new 8-aminoquinolines as primaquine substitutes (for anti-relapse activity) and to develop new gametocytocidal (transmission blocking drugs). The primate screening protocols are also approved by the World Health Organization for recognizing CDRI as WHO collaborating centre for antimalarials screening (CC59 collaborating centres was established at CDRI). The details of the publications/technologies developed at CDRI for primate antimalarial programme are cited in the Table 15. Cyclic transmission of *Plasmodium cynomolgi* B through *Anopheles stephensi* (vector) has been continued since the primate screening was initiated. A large scale insectary for rearing *Anopheles stephensi* has been maintained successfully for transmission studies and a large task force of scientific and technical personnels have been trained over the above period. The primate antimalaria screening programme for anti-relapse (Radical Curative) tests with *Plasmodium cynomolgi* B

transmission through *Anopheles stephensi* has led to the discovery of two new anti-relapse drugs listed below:

Discovery of Tefnoquine as primaquine substitute (1988):

Milhous, W.K., Theoharides, A.D., Schuster, B.G., Puri, S.K., Dutta, G.P., Heisey, G.B., Kyle, D.E., Oduola, A.M.J., Dhar, M.M., Neiffer, M.H., Reid W.A. and Davidson, D.E., J.R. New possibilities in the development of malaria drugs-new alternatives to primaquine. XIIth Interntional Congress for Tropical Medicine and Malaria 18-20th Sept., 1988, Amsterdam.

A new anti-relapse compound from Walter Reed under Phase-I clinical trials (Activity identified at CDRI).

Discovery of CDRI 80/53 (Bulaquine/Elubaquine (1989) as safest anti-relapse/gametocytocidal (transmission blocking primaquine substitute):

Dutta, G.P., Puri, S.K., Bhaduri, A.P. and Seth, Manju (1989). Radical curative activity of a new 8-aminoquinoline derivative (CDRI 80/53) against *Plasmodium cynomolgi* B in monkeys. *American Journal of Tropical Medicine and Hygiene,* **41** (6), 635-637.

Puri, S.K., Srivastava, R., Pandey, V.C., Sethi, N. and Dutta, G.P. (1989). Methaemoglobin toxicity and haematological studies on malaria anti-relapse compound CDRI 80/53 in dogs. *American Journal of Tropical Medicine and Hygiene* **41,** 638-642.

6. PROTOCOLS USED FOR ANTIMALARIAL DRUG DISCOVERY

Work on the synthesis of new antimalarials for potential blood and tissue schizontocidal activity was initiated at CDRI in 1978 and the compounds were subjected to both MDR rodent and primate malaria screens. Several new potential 8-aminoquinoline compounds with anti-relapse activity against simian *P. cynomolgi* B – rhesus monkey model and fast acting blood schizontocides and transmission blocking agents had been identified during the period 1981-1985. Soon after identifying new blood schizontocides and anti-relapse primaquine analogues, the protocols were developed for LD_{50} bioassay for toxic dose, methaemoglobin toxicity, liver function and renal function tests and to monitor any adverse haematological changes related to drug toxicity. Beagle dogs from the Institute colony were used for toxicity evaluation using methaemoglobin production as most important parameter of 8-aminoquinoline toxicity. Systematic screening of the compounds through antimalarial screens, LD_{50} bioassay and methaemoglobin assay in beagles and impairment of haematological, liver function/renal functions has been operational since 1982 under the antimalarial screening programme in the Institute. Preclinical developmental of any new candidate antimalarial can now be completed within a couple of years after passing through the following protocols.

LD_{50} in mice and acute toxicity in primates.

Initial blood schizontocidal primary screening against multi-drug resistant *P. yoelii nigeriensis.*

Primate malaria secondary screens:

(i) Blood schizontocidal activity against *P. knowlesi/P. cynomolgi* B/*P. fragile.*

(ii) Anti-relapse activity against *P. cynomolgi* B.

(iii) Causal prophylactic activity against *P. cynomolgi* B.

(iv) Gametocytocidal/sporontocidal activity against *P. cynomolgi* B – *Anopheles stephensi* model.

(v) Cerebral malaria models (*P. knowlesi/P. fragile* rhesus model).

Methaemoglobin toxicity in beagle dogs including haematological/liver function/renal function tests.

General pharmacological studies in animal models.

Sub-acute toxicity studies were conducted for 30 to 90 days in two hosts including teratogenicity studies and carcinogenicity tests.

The above protocols for antimalarial drug development had been operational in the Institute and the data obtained were analyzed and submitted to the Ethical Committee for clearance. The data were compiled in the form of Dossier and submitted to "The Drugs Controller of India" for permission for conducting Phase I clinical trials in volunteers. After obtaining the DGCI clearance, the compound entered Phase I clinical trials for safety and tolerance in human volunteers, and subsequently the compounds were selected for further Phase II/III multicentric clinical trials.

7. PROTOCOLS FOR PRECLINICAL STUDIES ON NEW ANTIMALARIALS

7.1 Toxicity Dose Determination

LD_{50} dose determination is an important criterion to establish the safety/relative toxicity of potential tissue and blood schizontocides. LD_{50} of primaquine in Swiss mice is 80 mg/kg and in rats 93mg/kg. Like-wise the LD_{50} of chloroquine in Swiss mice is 369 mg base/kg and in rats 430 mg/base/kg.

Acute toxicity studies in rhesus show that 200 mg base/kg of primaquine by oral route is non-toxic, while 100 mg/kg by intraperitoneal route is toxic dose.

The therapeutic index of the potential antimalarials determined by calculating the ratio of LD_{50} and the curative dose provides an index of the relative safety of the new compounds.

A. LD_{50} in mice/rats

The acute toxicity was studied in Swiss mice weighing between 25-30g and rats weighing 40-50 g. The animals were fed laboratory pellet diet and water *ad libitum*. Graded doses of the drug primaquine/chloroquine were administered by oral route to groups of 5 mice and 5 rats each and mortality after 48 hr was recorded to determine the LD_{50} according to the method described by Horn (1956).

Toxic compounds like primaquine can be tested at doses of 21.5, 46.4, 100.0 and 215.0 mg base/kg in rodent hosts (Table 16 and 17). Compounds like chloroquine which

are apparently less toxic can be tested at doses of 46.4, 100.0, 215.0, 464.0 and 1000.0 mg base/kg (Tables 18 and 19). The mortality is recorded after 48 hrs.

Conclusions

LD_{50} in mice can provide very useful data to determine the relative safety of new potential compounds.

The results of LD_{50} dose determination with primaquine (a tissue schizontocide) and with chloroquine (a blood schizontocide) are summarized in Tables 16-19.

Primaquine

LD_{50} in Swiss mice = 79.4 mg base/kg

LD_{50} in rats = 92.6 mg base/kg

Chloroquine

LD_{50} in Swiss mice =369 mg base/kg

LD_{50} in rats = 430 mg base/kg

B. Acute Toxicity of Primaquine in Rhesus Monkey

Acute toxicity studies in 3 rhesus monkeys showed that oral primaquine administration upto 200 mg base/kg single dose was not fatal for the monkeys, although all the three treated monkeys showed onset of cyanosis. On the other hand, 200 mg base/kg administered via intraperitoneal route was fatal for 2/2 monkeys. One of the two monkeys administered 100 mg/kg primaquine also died within 24 hours.

The data on acute toxicity of primaquine in rhesus monkey show that 12 mg base/kg x 7 days is MTD of primaquine and its radical curative dose being 1 mg base/kg x 7 days, the therapeutic index of primaquine (MTD/MED) in rhesus monkeys is therefore 12.

7.2 Standardization Of Secondary Blood Schizontocidal Screening Against Simian Malaria

(A) *Plasmodium knowlesi* W1 Infection

a**) Course of *P. knowlesi* infection in different simian hosts**

1. *Macaca mulatta (Rhesus monkey)* had been extensively used by earlier investigators for blood induced *P. knowlesi infection.* The parasite develops

synchronously and shows high level of virulence for rhesus monkey. The blood induced infections are generally fatal after patency of 5-6 days and peak parasitaemia attained before death ranges from 30-90%. P. knowlesi rhesus monkey model has been extensively used for chemotherapeutic evaluation of blood schizontocides. The pathology produced by *P. knowlesi* in rhesus brain suggests that this is ideal test system to screen new compounds for efficacy against cerebral malaria.

2. Presbytis entellus (Indian langur) has been found to be a new host for P. knowlesi infection. Intravenous inoculation of *P.knowlesi* infected blood from rhesus monkey or langur produced fatal course of infection in 9 langurs *(P. entellus),* and they died after developing high parasitaemia. The present study is the first attempt to show that *P. entellus* can serve as an alternate host to M.mulatta for experimental studies with *P.knowlesi.*

3. Macaca assamensis (Assamese monkey) has been found to be susceptible to P. knowlesi infection. Intravenous inoculation of *P.knowlesi* infection in eleven *M. assamensis* shows that they die at a relatively lower parasitaemia as compared to *M. mulatta.* It is suggested that *M.assamensis* can serve as an alternate host for experimental studies with *P. knowlesi.*

4. Macaca radiata (Bonnet monkeys) when infected with low inocula of *P. knowlesi* have been found to develop a chronic non-fatal infection while exceptionally high inocula of parasites produced fatal infection.

Since the work of Sinton and Mulligan (1952), *P. knowlesi* is known to produce a fulminating infection in rhesus monkey and the model has been used for chemotherapeutic screening of the blood schizontocides. This host-parasite system has been used as secondary screen for developing new blood schizontocies. The course of blood induced infection of *P.knowlesi* in rhesus monkey as well as Assamese monkey. Bonnet monkey and Indian langur described below shows that these can be used as alternate hosts for chemotherapy.

MATERIALS AND METHODS

Strain of *P. knowlesi* (WI) received from (Late) Professor P.C.C. Garnham (U.K.) has been used for blood induced infection in 4 simian hosts. *M. mulatta, M.assamensis, M. radiata* and *P. entellus* were maintained in the Primate House of CDRI on standard

diet consisting of pellets (animal feed), bread, banana, gram, seasonal vegetables and fruit and water was given *ad libitum*. The animals were quarantined, chest x-rayed and Tuberculin tested. Their blood films were examined for any blood protozoa or filarial infection three times, at weekly intervals. The animals were kept under strict photoperiodicity of 12 hrs day and night. The animals were infected intravenously with 1 x 10^5-1 x 10^6 parasitized RBC and occasionally higher inocula were given. Blood smears stained with Giemsa were examined daily for recording parasitaemia.

RESULTS

The characteristics of blood induced *P. knowlesi* infection in four simian hosts namely *M.mulatta, M.assamensis, M. radiata* and *P. entellus* are summarized in Table 20. The schizogony is synchronous in all the hosts and the infection runs acute course with high level of parasitaemia (18-25%) in *M.assamensis* while *M. mulatta* and *P.entellus* develop 35-90% level of parasitaemia before death. *M. radiata* develops chronic infection (peak parasitaemia 4.5-8.5%) with low inoculum (1 x 10^6 or 10^7) but with very high inoculum (1.6 x 10^9) the level of parasitaemia attained by *M. radiate* is also high (60-80%) which proves fatal. The course of infection in 26 *M.mulatta* and the corresponding course of infection in *M.assamensis, M.radiata* and *P.entellus* are presented in Tables 20-22. In Assamese monkey (*M. assamensis*) also *P. knowlesi* has been found to produce a fatal infection, as in case of *M. mulatta.*

It is interesting to note that *P. knowlesi* infection in *Presbytis entellus* (a new experimental host for this parasite) followed a fatal course.

The course of *P. knowlesi* infection in bonnet monkeys has been compared with the infection in rhesus monkeys. The infection was consistently fatal in all the eight rhesus monkeys inoculated with parasite inocula ranging from 1 x 10^5 to 1 x 10^9 parasites with peak parasitaemia varying from 18.0-90.2 percent before death. In contrast to this, *P. knowlesi* produced either fatal or chronic non-fatal infections in bonnet monkeys depending upon the parasite inocula. An inoculum of 1.6 x 10^9 parasites produced fatal infection in two bonnets, whereas 1 x 10^7 to 1 x 10^8 parasites inoculated in other seven bonnets resulted in death of two bonnets and the remaining five developed chronicity extending over an observation period of more than 50 days. Small inoculum (1x10^5)

produced consistent chronicity in three bonnets. Similarly, one bonnet monkey inoculated with frozen parasites developed chronic infection. The peak parasitaemia in bonnets which developed chronic infection ranged between 4.5 and 8.5 percent. Two bonnets out of these died of severe anaemia without attaining high patasitaemia. Four bonnets which died of high parasitaemia showed a peak ranging 60.0-80.5 percent.

Mechanism of protection in bonnet monkeys which leads to chronicity of P. knowlesi infection still needs to be elucidated. The suppression of peak parasitaemia (4.5-8.5%) within 4-11 days particularly after very heavy challenge infections in bonnet monkeys, shows that this host in contrast to rhesus monkey, has a very efficient host defence mechanism to protect itself against infection.

CONCLUSION

P. knowlesi infection has been extensively used for screening potential compounds for fast acting blood schizontocidal activity. The infection runs acute course and the monkeys at peak parasitaemia develop haematuria due to renal complications. *P. knowlesi* rhesus monkey system is ideal experimental model for chemotherapy of cerebral malaria. The host shows parasite sequestration at high parasitaemia which causes plugging of brain capillaries and the model is comparable to *P. falciparum* infection in human brain.

P. knowlesi also produces fatal course of infection in langur (*P. entellus*) and assamese monkey (*M. assamensis*). Both these hosts can also be used for chemotherapeutic screening.

In bonnet monkey (*M. radiata*) the *P. knowlesi* infection after standard inoculum develops chronicity and the monkey survives by virtue of quick development of host immune response against *P. knowlesi*.

(b) Protocol for Blood Schizontocidal Screening Against *P. knowlesi*

Plasmodium knowlesi infection in rhesus monkey is a very useful experimental model to study the blood schizontocidal activity of standard drugs like chloroquine and mefloquine, and for screening of new potential blood schizontocides. Chloroquine (10 mg as well as 20 mg base/kg x 3 days administered orally/im) produces radical cure of P. knowlesi infection.

MATERIALS AND METHODS

P. knowlesi (W1 strain) used in this study was obtained through the courtesy of Professor P.C.C. Garnham on 21/8/1976. Counted number of parasitized erythrocytes ($1.0 \times 10^4 - 1.0 \times 10^7$) were given to initiate the infection by injecting the parasites in sephaneous vein of the monkey. Thin and thick smears were prepared and stained with Giemsa stain. Prasitaemia was recorded per 10^4 erythrocytes.

Mefloquine hydrochloride (Mefloquine^R) obtained from M/s Hoffmann-La Roche & Co. Ltd., was given orally as a water suspension by stomach tube at doses of 40, 80 and 160 mg/kg total dose (over a three day period). Chloroquine diphosphate (Resochin Bayer) was administered orally as well as im at 60 mg/kg total dose (base) over a three day period.

When the parasites could no longer be detected in the thin and thick blood smears, the iso-diagnostic method was used. Four ml of blood from the test monkey in citrate-saline was subinoculated into normal, uninfected and untreated monkey. Blood examination was done up to 50 days in most cases for detecting any patent infection.

RESULTS

Blood Schizontocidal Action of Chloroquine

Fifteen monkeys infected with *P. knowlesi* were given 20 mg/kg chloroquine (base im for 3 consecutive days) at various levels of parasitaemia ranging from 4.0-60.0% of the red cells infection and another group of 8 monkeys were given the same dose of chloroquine orally (Table 23). It was found that in almost all the cases, the parasites were cleared from circulation within 72 h after the administration of chloroquine. In one monkey (no. 7), however, in which the treatment was initiated at very high parasitaemia (60%), the parasites could be detected even up to 120 h after the initiation of therapy.

Subinoculation of blood (4.0 cc) from treated monkeys (4) into normal monkeys taken 30 days after the last drug administration showed no circulating parasites even up to 15 days postinoculation.

Mefloquine cleared initial parasiteamia at all the three doses used (Table 24). However, there was delayed recrudescence in all animals.

CONCLUSION

Chloroquine (10 or 20 mg base/kg x 3 days) administered either orally or im, produced radical cure when treatment was initiated at low as well as high parasitaemia. Our study suggests that *P. knowlesi* rhesus monkey model is ideal screening model for selecting fast acting blood schizontocides for preclinical development.

P. knowlesi W1 is resistant to melfoquine (40-160 mg/kg total dose given in 3 days)

B. Protocol for Blood schizontocidal Screening against *Plasmodium cynomolgi* B

Blood induced P. cynomolgi B infection in rhesus monkeys is a useful secondary screening model for screening of potential blood schizontocides. Chloroquine used as standard blood schizontocide was curative at 3 mg (base/kg x 7 days. No escalation of chloroquine curative dose was observed during the revalidation tests performed during the last 15 years after the initial dose determination, indicating that the sensitivity of the parasite to chloroquine remained unaltered.

Parasite

Blood induced *Plasmodium cynomolgi* B infection in rhesus monkeys was used to standardize the model for screening of potential blood schizontocidal agents.

Hosts

Rhesus monkeys of either sex, weighing 3-5 kg, obtained from animal suppliers were quarantined for 3 weeks, and certified to be tuberculin negative and free from any natural blood protozoan infections. The animals were maintained on standard diet comprising of bread, fruits and pellet, and water *ad libitum* was made available.

Drug Administration

Pure sample of chloroquine diphosphate has been used as the reference blood schizontocidal drug. Dose of chloroquine calculated to the drug base, (1 mg chloroquine base = 1.67 mg chloroquine diphosphate) was dissolved in distilled water.

Drug was administered separately in 10 ml volume via oral catheter followed by 5 ml water to flush the catheter. The treatment was administered once daily for seven consecutive days when asexual parasitaemia reached levels of approximately 5000/mm^3.

Parasitaemia recorded

Thick and thin blood smears prepared from ear-vein were stained with Giemsa stain and observed under oil immersion lens for the presence of parasites.

Determination of Chloroquine Curative Dose as Blood Schizontocide

For conducting the blood schizontocidal test, five groups of monkeys were infected with 1 x 10^5 parasitized RBC by i/v route and when the parasiteamia had reached 5,000-20,000/mm^3, each group was administered chloroquine dosages of 1.0, 3.0, 5.0, 7.0 and 10.0 mg/kg (base) for 7 days by oral route. Both thick and thin smears were examined daily to monitor the course of parasitaemia. Our results (Table 25) show that all monkeys treated with 3.0, 5.0, 7.0 and 10.0 mg/kg were cured radically and showed no recrudescence till day 50. The 8 monkeys showing recrudescence of parasitaemia at 1.0 mg/kg were retreated with the next higher dose i.e. 3.0 mg/kg x 7 days. This dose was again found to be curative and no recrudescence was observed till 50 days.

Revalidation of curative dose of chloroquine

Three batches of 5 monkeys were given blood induced infection from a sporozoite infected monkey in serial sporozoite passage number II. When the parasitaemia had reached approximately 5000/mm^3, they were administered chloroquine at 3.0, 5.0 and 7.0 mg/kg (base) x 7 days by oral route, and all the monkeys were followed for 50 days. All the three doses were again found to be curative (Table 26). The dose validation was again carried out using parasites obtained from infected monkeys in the XXXVth serial sporozoite passage conducted nearly 4 years after the first. Three monkeys each were treated with chloroquine at 1.0, 3.0 and 5.0 mg (base)/kg dose levels. Monkeys treated at lowest dose of 1.0 mg/kg showed recrudescence on days 11, 17 and 25, while monkeys at higher dose levels were protected.

Both initial tests and revalidation tests thus show that 3.0 mg/kg x 7 doses of chloroquine (base) has curative action on blood induced P.*cynomolgi* B.

C. *Plasmodium fragile* infection

P. fragile infection maintained in rhesus monkey (4-5 kg) by serial blood passage, is a good secondary blood schizontocidal screening model. The parasite has a 48 hour development cycle in the blood and is used as a biological model for human *P. falciparum* infection.

The curative chloroquine dose for blood induced *P. fragile* infection is 10 mg/kg x 3 doses by oral route. The parasite is equally infective for *Presbytis entellus*, which can also be used as secondary screening model.

Puri, S.K. and Dutta, G.P. (1994). *Presbytis entellus*, a new experimental host for *Plasmodium fragile*. J. Parasitology 80, 156-158.

D. *Plasmodium coatneyi* infection

P. coatneyi infection maintained in rhesus monkey (4-5 kg) by serial blood passage, is considered ideal for secondary blood schizontocidal screening. The parasite has a 48 hour cycle in the blood stage and is considered as a biological model for the human infection. The parasite shows marked sequestration, attains high level of parasitaemia and produces partial mortality in the untreated monkey. Infection is initiated in monkey with $5x10^4$ parasitized RBC by intravenous route and after patency chloroquine (20 mg base/kg) is administered orally for 3 consecutive days. This dose of Chloroquine is curative and no recrudescence of parasitaemia is observed when follow up is continued for 50 days.

Untreated *P. coatneyi* infection in rhesus monkey results in severe anaemia followed by death of some animals. It may be advisable to administer quinine sulfate (300 mg for 1-2 doses) when the primary parasitaemia reaches approximately 200 parasites/10^4 RBC.

Dutta, G.P., Puri, S.K, Kamboj, K.K., Srivastava, S.K. and Kamboj, V.P. (1984). Interactions between oral contraceptives and malaria infections in rhesus monkeys. *Bull. Wld. Hlth. Org*. **62**, 931-939.

7.3 Causal Prophylactic Screening against Simian Malaria

A. Cyclic Transmission and the Sporogonic Development

(i) Cyclic transmission of simian malaria parasite *Plasmodium cynomolgi* B through *Anopheles stephensi*

Plasmodium cynomolgi B is a unique simian parasite which is human transmissible and has the biological characteristics similar to human *P. vivax*. Untreated sporozoite induced infections with both these parasites, show a series of spontaneous

relapses which originate through the activation of latent relapse stages (hypnozoites) in the liver. Relapse episodes are characteristic and persistent even after treatment with blood schizontocides like chloroquine or quinine. Sporozoite induced infections with *P. cynomolgi* B in rhesus monkey provide excellent experimental model for chemotherapy of malaria particularly for establishing reliable test systems for screening of potential antimalarials for causal prophylactic and radical curative (anti-relapse) activity. The regular passage of the parasite through mosquito-monkey cycle would ensure the normal development of functional gametocytes which ensures high infectivity for the mosquito vector. On the other hand, the blood passaged strains of the malaria parasites frequently lose the power of producing gametocytes if maintained by blood passage for long time and such strains become non-infective for the vector. Sporozoite induced infections can also provide valuable challenge system for evaluation of irradiated sporozoite vaccines, assessment of vaccine potential of the synthetic CSP antigens and prophylactic efficacy of interferons (Maheshwari *et al.*1986; Puri *et al.* 1988) and immunomodulators etc.

Technology for Malaria Transmission

1. **Parasite:**

Plasmodium cynomolgi B procured in 1979 from late Professor W.E. Collins, CDC, Atlanta has been maintained in CDRI by successive blood induced passages or by cryopreservation. Since Nov 1982, the parasite had been regularly transmitted through *Anopheles stephensi* mosquitoes or cryopreserved from time to time.

The infection can be transmitted to naive rhesus monkeys (*Macaca mulatta*) by intravenous inoculation of either infected blood in acid citrate-dextrose (ACD) or sporozoites from infected mosquitoes.

2. **Maintenance of rhesus monkeys (*M. mulatta*)**

Rhesus monkeys (4-5 kg) used for antimalarial screening programme are procured from approved Government contractors, and kept under quarantine for four weeks. These monkeys are tuberculin tested before receiving them in the primate house and then after every 1 to 2 months. During quarantine period monkeys are chest x-rayed and examined for absence of blood protozoans and three blood smears at weekly intervals are preserved for record. Tuberculin negative monkeys free from any blood parasites are transferred to

experimental wing and kept in mosquito free rooms. They are supplied standard pellet diet, seasonal fruits, vegetables and water *ad libitum*. The monkeys are kept under 12 hrs photoperiodicity.

3. Recording of Parasitaemia

Both thick and thin blood films of infected rhesus were stained with the following modification of Giemsa staining.

Giemsa stain (Glaxo)	10 ml
Acetone	5 ml
Phosphate buffer M/15 (pH 7.2)	85 ml

The thick film is prepared from 2 drops of ear vein blood spread in 10 mm diameter circle and air dried. The thin blood film is fixed in methanol for 1 minute and slides are stained for 45 min in diluted Giemsa stain.

The blood smears of the rhesus monkeys infected intravenously with sporozoites are stained and examined for patency from day 8 onwards. The parasitaemia is initially recorded in terms of number of parasites/mm3 from thick blood smears, on the basis of number of parasites per 50 oil immersion fields which when multiplied by 20, gives parasitaemia/mm^3. When number of parasites/50 thick fields is more than 50, further recording is made from thin blood films by determining the number of parasites/100 WBC, and parasitaemia/mm^3 is calculated after recording number of WBC/mm^3 using haemocytometer. Finally when the number of parasites/100 WBC is more than 1000, further recording is made from thin films by counting the parasitized cells/10^4 RBC and parasitaemia/mm^3 is calculated after determining the number of RBC/mm^3 using haemocytometer (Puri and Dutta, 1988).

4. Preparation of Sporozoite Inoculum

Insectary

A large-scale rearing of *Anopheles stephensi* (NICD strain) for transmission studies has been set up with an average production of 2000-4000 pupae daily. Rhesus monkey has been found to be ideal for normal blood meal to mosquitoes for egg laying.

The larval stages are reared on powdered yeast and maintained at 25±1^oC. The pupation starts after 8-10 days and adults emerge 36 to 48 hr later. The adults are

maintained at 26±1°C with relative humidity 75 to 80% and they are fed on 5% multivitamin solution.

Infection of Mosquitoes

Two or three days, old mosquitoes are fed on infected rhesus monkey showing optimum gametocyte number of 2000-4000/mm^3. The monkey is anaesthetized with sodium-intraval (25 mg/kg, i.v.) and mosquitoes are allowed to have blood meal for 20-30 minutes. The fed mosquitoes are kept in insectary whereas the males and unfed females from each cage are discarded.

On day 7 after blood meal, 5-10 mosquitoes from each of the infected batch are dissected for determining the number of oocysts on the gut. The mean number and the size of the oocysts is recorded, which generally ranges between 20-80 in number and 40-50 µ in size.

Estimation of sporozoite Number in Infected Mosquitoes

On day 13 after blood meal (or one day prior to inoculation of monkeys), the sporozoite number is estimated in the mosquitoes from the batch which had earlier been found to show high oocyst number on day 7. The infected mosquitoes from the sample batch are anaesthetized and legs, head and abdomen are removed. The thoraces are grinded with a mixture of 0.5 ml saline and 0.5 ml normal rhesus serum (inactivated at 56°C, ½ h). With the help of a graduated capillary (or an eppendorf pipett), 5 µl of the sporozoite suspension is applied and spread within the etched circule of a FA slide. The slide is allowed to air dry, fixed in methanol (5 minutes) and stained with Giemsa stain for 40 minutes. The number of sporozoites/100 oil immersion fields is counted from duplicate slides and the mean value multiplied by the magnification factor of the microscope (x 10974), gives the sporozoite number per ml or sporozoite number/10 infected mosquitoes. From the estimation, the number of mosquitoes required to obtain 1 million sporozoites/monkey can be determined.

Harvesting of Sporozoites for Inoculation

On day 14, the required number of mosquitoes are grinded in a prechilled pestle mortar, and the sporozoite inoculum is diluted with rhesus serum-saline mixture. After grinding, the suspension is centrifuged at slow speed (2000 rpm for 1 min at 4°C). The

sediment containing mosquito debris is discarded and the supernatant is further diluted with serum/saline mixture to get the required inoculum. The whole process after anaesthetization of mosquitoes to the inoculation of monkeys should be completed within 45-60 min. Each monkey is inoculated via i/v route with 1 ml of the inoculums, and the exact number of sporozoites inoculated per monkey is determined from the sample inoculums.

5. Cyclic passage of sporozoite induced *P. cynomolgi* B infection

In order to maintain the rhesus prophylactic test in operational order it is necessary to keep the serial cyclic passage of the parasite which would involve the development of technology for mass infection of *A. stephensi* and harvesting of sporozoites for rhesus inoculation. Since 1982, the parasite has passed through ≥200 serial cyclic passages in rhesus. The dates and number of sporozoites inoculated in rhesus and the day of patency are recorded in Table 27. In the initial five passages (I-V) the patency was recorded from thin smears and it ranged from 10-12 days, while in the subsequent passages the patency was recorded from thick smears and it ranged from 8-9 days. In 2 monkeys the patency was observed on day 10 (one in passage XVI and the other in passage XXXVI). When lower sporozoite inoculums (2.3-5.0 x 10^4 sporozoites) was given in passage XXX and XXXV, the patency was observed between day 11 to 13. The analysis of the data on duration between successive passages showed a mean duration of 39.07 ±4.62 days (range 27-47 days) for each passage.

6. Course of Parasitaemia after Patency

The characteristics of primary and secondary asexual peak parasitaemia during sporozoite induced *Plasmodium cynomolgi* B infections in 40 rhesus monkeys, have been studied. The mean primary peak parasitaemia level of 224318.98 ±5887.41/mm^3 was reached in 8.38 ±2.27 days after patency. Subsequently the mean secondary peak parasitaemia level (44205.33 ±24325.64/mm3) was attained on 15.15 ±2.63 days after patency or 7.05± 1.72 days after primary peak. The course of gametocytaemia in infected monkeys followed the asexual parasitaemia and the mean peaks of gametocytaemia level were recorded on same days as that for the asexual parasitaemia.

Concluding Remarks

The technology for sporozoite transmission of *P.cynomolgi* B established at CDRI during the last 30 years has considerably increased the capability of this institution in the area of anti-malarial drug development. Although the rodent antimalarial screening models are operational at many laboratories in the world, not many new antimalarials have emerged because of the lack of primate antimalarial screening facilities.

The sporozoite transmission studies established at this institute have contributed to development of new causal prophylactic agents, gametocytocidal agents and anti-relapse agents.

The capability of producing large number of sporozoites will boost the vaccine development programme including the development and evaluation of new immunomodulators.

Since the first report on the discovery of the parasite by Garnham, this is the first documented report on the continuous maintenance of the parasite through mosquito transmission for 30 years.

ii) **Technology for large scale sporozoite production for monkey inoculation**

Colony bred Anopheles stephensi were fed on different days on the gametocyte carrying monkeys and the infectivity of mosquitoes as determined by oocyst count on day 8 post-feeding was recorded. Following the day of sporozoite inoculation, the mean prepatent period was 8.58±0.87 days, the primary asexual peak was attained on day 8.38±2.27 and the secondary peak on day 15.15±2.63 after patency. Infectivity rate was 100% in 144 batches of mosquitoes. The high oocyst count was obtained between day 12-18 after patency. Infective gametocyte peak coincided with the secondary asexual peak parasitaemia, with the result that maximum oocyst number was observed in batches fed on the day of secondary peak or one day prior to or one day after the secondary peak. Infectivity during the period of primary asexual peak was inconsistent. Yield of approximately 1x10^6 sporozoites can be obtained by processing 10 infected mosquitoes harbouring oocysts ranging between 15-40 per gut.

INTRODUCTION

Ever since the discovery of *Plasmodium cynomolgi bastianelli* (Garnham, 1959) this parasite has been extensively used for sporozoite induced infections. The time of feeding of the mosquitoes on gametocyte carrying monkey needs to be standardized in order to achieve maximum infectivity of mosquitoes. Since the availability of large number of sporozoites is an essential prerequisite for such studies, the data on correlation of infectivity of mosquitoes with parasitaemia/gametocytaemia would provide useful information on acquisition of well infected batches of mosquitoes. However, no published information is available on the relationship of the time of mosquito feeding to the maximum infectivity of *P. cynomolgi* B using *A. stephensi* as vector for laboratory transmission.

1. Maintenance of mosquitoes

Healthy colonies of *A. stephensi* (NICD strain) are being maintained in the insectorium under controlled conditions of temperature (26-28^{o}C) and relatively humidity (70-80%). The larval stages o the mosquitoes are fed on powdered yeast and adults are maintained on 5% commercial multivitamin syrup.

Infected mosquitoes are maintained in the insectary at 27±1^{o}C and dissections for oocyst count are made on day 7-8 after infective blood meal. The monkeys are anaesthetized for infection by intravenous inoculation of 25 mg/kg sodium intraval (May & Baker). The protocols for infection of mosquitoes, harvesting of sporozoites from infected mosquitoes, staining of blood smears, and the recording of parasitaemia have been described in detail in the preceding pages.

2. Quantitation of mosquito infectivity

The correlation between the infectivity of mosquitoes in relation to asexual parasitaemia and gametocytaemia levels, is presented in Tables 28-29. A total of 35 batches were fed on 14 monkeys between day -3 - +3 of primary peak (Table 28). The infectivity as shown by oocyst development was recorded in six out of seven batches of mosquitoes fed on day -1, 7 out of eleven batches fed on day 0, 4 out of 8 batches fed on day +1 and 2 out of six batches fed on day +2, thus showing the mosquitoes fed around

primary peak did not produce consistent infectivity. On the other hand, 100% infectivity was recorded in 144 batches of mosquitoes fed on 40 monkeys between day -3 to +3 of secondary asexual peak (Table 29). Comparison of the oocyst number in infected mosquitoes showed that maximum oocyst number was obtained in batches fed on day -1 or +1 of secondary peak, when the gametocyte number and ratio of male and female gametocytes was optimum for producing maximum oocyst infectivity. The relationship between the oocyst count and the gametocyte number indicated that infection to mosquitoes was not always proportional to gametocyte count alone since higher gametocyte number at the primary peak did not produce comparable infection in mosquitoes, whereas during the phase of secondary peak although the gametocyte number was lower, they were more infective and gave higher oocyst count. Mosquitoes infectivity results showed that the ideal time for infective blood meal was during the ascending as well as descending phases of the secondary peak particularly between day 12-18 after patency.

3. Quantitation of sporozoite yield

The sporogonic development on the mosquito midgut is complete by day 12 when majority of the oocysts rupture to release sporozoites in the haemocoel. These sporozoites migrate anteriorly and invade the salivary glands from where these are passed on into the vertebrate host during the act of biting. An estimate of the sporozoite yield is made on day 13 post-infective blood meal, by determining the sporozoite number in 10 randomly caught female mosquitoes from an infected group. The data in Table 30 present the sample estimate of the sporozoite yield and the corresponding day 7 oocyst number in different batches of infected mosquitoes. The results show that 10 mosquitoes from an infected batch with oocyst numbers ranging between 15-40 can yield an infective inocula of nearly $1x10^6$ sporozoites for initiating infection in the rhesus monkey.

B. Causal Prophylaxis Against *P. cynomolgi* B

(i) **Standardization of 3-day primate screening model for causal prophylactic activity against *Plasmodium cynomolgi* B**

Test system for large-scale screening of antimalarials for causal prophylactic activity against sporozoite induced infections with Plasmodium

cynomolgi B has been standardized at CDRI. A dose of 1.78 mg/kg primaquine (base) x 3 days (day -1, 0 and +1) has been found to be consistently curative. There was no escalation of primaquine curative dose when sporozoites from different cyclic passages were used for challenge in the prophylactic test.

This model would be useful for screening of potential causal prophylactic compounds. The primate causal prophylactic screen is superior to the standard rodent causal prophylactic screen since the predictive value of the rodent screen is low and the compound may not be effective against P. vivax or P. cynomolgi B.

INTRODUCTION

Ever since the second World War, efforts have been made to develop mainly 8 aminoquinolines for prophylactic efficacy against *Plasmodium vivax* which is responsible for a series of relapses in untreated sporozoite induced infections. Their prophylactic and radical curative efficacy against *P. vivax* had been documented by Covell *et al.* (1955). Researches carried out by Schmidt *et al.* (1966, 1982) had established the prophylactic efficacy of pamaquine, pentaquine, primaquine, and isopentaquine against simian malaria parasite (*P. cynomolgi s*trains M, B and Ro). Out of these, primaquine was found to be most active and least toxic when screened in 9 day treatment prophylactic test.

For development of causal prophylactic drugs, a shorter 3 day treatment schedule would have the advantage, that the potential compound would act only on the sporozoites or on very early tissue stages infecting hepatocytes, while the 9 day treatment schedule proposed by Schmidt *et al.*, (1966, 1982) would also cover the period of development of primary tissue schizonts. Nine day treatment regimen also has operational difficulties because of the prolonged drug administration. It was, therefore, proposed to develop a shorter 3- day treatment schedule and compare its efficacy with 9 day treatment schedule using primaquine as the standard reference drug.

7.4 Technology Established For Screening Of Potential Anti-Malarials For Causal Prophylactic Activity

Infection of Monkeys

Rhesus monkeys are inoculated intravenously with approximately $1x10^6$ *P. cynomolgi* sporozoites suspended in 1:1 mixture of normal monkey serum and saline. The detailed procedure is described under Chapter on the cyclic transmission of *P. cynomolgi* B. The untreated monkeys develop patent infection by day 8-10 as observed in Giemsa stained blood smears.

Standard reference drug

Primaquine is used as standard reference.

1.0 mg primaquine (base) = 1.76 mg primaquine diphosphate

Drug administration

Pure sample of primaquine diphosphate (SIGMA) has been used in the present study. The daily dose of primaquine base for each monkey was suspended in 10 ml of 0.3% methyl cellulose solution. The drug was administered orally via a catheter preferably between 9 A.M. to 11 A.M.

PROTOCOLS FOR PROPHYLACTIC TESTS

Nine day rhesus prophylactic test

Limited studies with 9 day prophylactic test developed by Schmidt *et al.* (1966, 1982) were also carried out. In 9 day prophylactic test, the drug treatment (primaquine) was given on day -1, day 0, and day +1 to day +7. The results presented in Table 31 show that 0.5 mg/kg primaquine dose (base) x 9 days was not protective. The next higher dose i.e. 1.0 mg/kg x 9 days was protective in 5/6 monkeys. Treatment with 2.0 mg/kg x 9 days protected all the four monkeys. Untreated five control monkeys became patent on day 8 or 9.

Three-day rhesus prophylactic test

A three day treatment model has been standardized for evaluation of causal prophylactic activity. Primaquine was administered in three doses (on day -1, day 0 and day +1 of sporozoite inoculation). Experimental monkeys were treated with primaquine (base) at 0.316 mg/kg, 0.62 mg/kg, 1.00 mg/kg, 1.30 mg/kg, 1.78 mg/kg, 3.16 mg/kg and

10.0 mg/kg x 3 day (Table 32). Our results showed that all the 6 monkeys treated at 0.316 mg/kg and 5 monkeys treated at 0.62 mg/kg became patent between day 11 to 18; seven out of 16 monkeys treated at 1.00 mg/kg became patent between day 15-33 while remaining nine monkeys were cured at this dose. Treatment of monkeys at higher dose levels i.e. 1.3 mg/kg (2 monkeys), 1.78 mg/kg (7 monkeys), 3.16 mg/kg (11 monkeys) and 10.0 mg/kg (4 monkeys) resulted in complete protection.

Since 1.3 mg/kg primaquine (base) was border-line protective dose, we have considered the next higher dose i.e. 1.78 mg/kg as valid which would ensure the reproducibility of the test. This dose (1.78 mg/kg x 3 day) has been revalidated for prophylactic activity several times during 45 successive passages in a total of 20 monkeys and all the monkeys were protected as shown by observations up to 70 days of follow-up.

DISCUSSION

Development of prophylactic agents for the control of P. vivax has received high priority in countries like India where vivax incidence is high. *P. cynomolgi* B discovered by Garnham (1959) is a simian parasite readily transmissible to man and resembles *P. vivax* in its biological characteristics (Coatney *et al.* 1971). Most of the work on development of simian model for causal prophylactic activity was carried out earlier by Schmidt *et al.* (1966) using 9 day treatment schedule and primaquine at 1 mg(base)/kg was reported to be effective against *P. cynomolgi* B infections. Infections with M strain of *P. cynomolgi* were prevented with 0.375 mg/kg dose while infections with RO strain, like strain B could be interrupted with 1.0 mg/kg dose (Schmidt *et al.* 1982). Our results with 3-day test show that 1.78 mg/kg primaquine (base) was consistently curative. This dose has been revalidated against sporozoite induced infection in 20 monkeys in 13 different cyclic passages. No shift in the sensitivity of sporozoite induced infections to primaquine was observed over a four year period. A comparison of the total primaquine dose administered in 9 day model and 3 day model shows that while in the former, 9 mg/kg total dose was required for complete prophylactic efficacy, in the latter similar response was achieved with 5.34 mg/kg total dose.

This three day treatment simian model has been used for evaluation of causal prophylactic activity of several new 8-aminoquinoline compounds under CDRI-WRAIR collaborative project.

Radical Curative Screening for Anti-relapse Activity against *P. cynomolgi* B

Experimental transmission of sporozoite induced P. cynomolgi B infection has been maintained in rhesus monkeys since long by serial cyclic passage, using A. stephensi as vector. The radical curative dose of primaquine in this model has been found to be 1 mg(base)/kg x 7 day (by oral route) which gives 100% curative rate. The sensitivity of the relapse stages from 17 different cyclic passages to this drug has remained unaltered. Chloroquine used as companion blood schizontocide with primaquine, was curative at 3 mg(base)/kg x 7 days. No escalation of primaquine/chloroquine curative doses was observed during the study.

The protocol reported in this communication would be useful for large-scale screening of new antirelapse compounds which would be potentially useful for radical curative treatment of vivax malaria.

INTRODUCTION

Even before the discovery of tissue phase of malaria by Shortt and Garnham (1948), relapse of *P. vivax* had been well known. Garnham (1977) proposed that relapses were caused by the activation of dormant tissue stages. The stages of *P. cynomolgi* in hepatocytes which were responsible for initiating relapses, were identified as hypnozoites by Krotoski *et al.* (1980). Though primaquine was known since long to cure the relapses of *P. vivax* (Edgcomb *et al.* 1950), the demonstration of its efficacy against *P. cynomolgi* B in simian model was considerably delayed and finally reported by Schmidt *et al.* (1966).

Although rodent and avian antimalarial screens for the tissue schizontocidal activity had been extensively used earlier (Davey, 1946; Singh *et al.,* 1962; Hill, 1975; Peters, 1980), the predictive value of these models to select compounds that would be active against *P. vivax* in man, had been reported to be very poor (Clark 1946; Schmidt, 1983).

The protocols established for anti-relapse screening by Schmidt *et al.* (1966, 1977, 1982a) were based on the use of vector species *A. freeborni* and *A. quadrimaculatus* for production of sporozoites for monkey inoculation. Since above vectors can not be imported in our country, methodology had to be developed for bulk sporozoite production using indigenous *A. stephensi* as vector. The protocol for radical curative test reported in this communication has been in operation at CDRI since 1988. In order to find out if there was any escalation of primaquine radical curative dose, sporozoite induced infections from 17 different cyclic passages were tested for primaquine sensitivity and the results are presented in this communication.

7.5 Technology Established for Screening Of Potential Antimalarials for Radical Curative Anti-Relapse Activity

Parasite

Sporozoite induced infection of *Plasmodium cynomolgi* B strain which shows typical relapse pattern after treatment with curative doses of chloroquine, has been used during the present study. The parasite was procured from late Dr. William E. Collins in 1979 and is being maintained by cyclic transmission through *A. stephensi* since 1982.

Host

Rhesus monkeys of either sex, weighing 3-5 kg, obtained from animal suppliers were quarantined for 3 weeks, and certified to be tuberculin negative and free from any natural blood protozoan infections. The animals were maintained on standard diet comprising of bread, fruits and pellet, and water *ad libitum* was made available.

Vector

Anopheles stephensi (NICD strain) is being maintained under controlled conditions of temperature (25-26^{o}C) and relative humidity (70-80%). These mosquitoes have been used for bulk sporozoite production for initiating infections in the rhesus monkeys. The details of large-scale rearing of *A. stephensi*, infection of mosquitoes, estimation of sporozoite number in the infected batches and harvesting of sporozoites have been reported in the preceding pages.

Drug administration

Pure samples of chloroquine diphosphate and primaquine diphosphate have been used as te reference blood schizontocidal and antirelapse drugs respectively. Doses of chloroquine and primaquine calculated to the drug base, (1 mg chloroquine base = 1.67 mg chloroquine diphosphate; 1 mg primaquine base = 1.76 mg primaquine diphosphate) were suspended in distilled water or in 0.3% methyl cellulose solution in distilled water and prepared on the day of treatment.

Drugs were administered separately in 10 ml volume via oral catheter followed by 5 ml water to flush the catheter. The treatment was administered once daily for seven consecutive days when asexual parasitaemia reached levels of approximately 5000/mm^3.

Parasitaemia record

Thick and thin blood smears prepared from ear-vein were stained with Giemsa stain and observed under oil immersion lens for the presence of parasites. The method of recording parasitaemia has been described earlier.

PROTOCOL FOR RADICAL CURATIVE TEST

Determination of chloroquine dose as blood schizontocide

For conducting the blood schizontocidal test, five groups of monkeys each were infected with $1x10^6$ parasitized RBC by i/v route and when the parasitaemia had reached 5,000-20,000/mm^3, each group (5 monkeys) was administered chloroquine dosages of 1.0, 3.0, 5.0, 7.0 and 10.0 mg/kg (base) for 7 days by oral route. Both thick and thin smears were examined daily to monitor the course of parasitaemia. Our results show that all the 5 monkeys at 1 mg/kg dose showed recrudescence, while those at 3.0, 5.0, 7.0 and 10.0 mg/kg were cured radically and showed no recrudescence till day 30. The 5 monkeys showing recrudescence of parasitaemia at 1.0 mg/kg were retreated with the next higher dose i.e. 3.0 mg/kg x 7 days. This dose was again found to be curative and no recrudescence was observed till 30 days.

Revalidation of curative dose of chloroquine

Three batches of 5 monkeys were given blood induced infection from a sporozoite infected monkey in serial sporozoite passage number II. When the parasitaemia had

reached approximately 5000/mm^3, they were administered chloroquine at 3.0, 5.0 and 7.0 mg/kg (base) x 7 days by oral route, and all the monkeys were followed for 30 days. All the three doses were again found to be curative. The dose validation was again carried out using parasites obtained from infected monkeys in the XXXVth serial sporozoite passage. Three monkeys each were treated with chloroquine at 1.0, 3.0 and 5.0 mg (base)/kg dose levels. Monkeys treated at lowest dose of 1.0 mg/kg showed recrudescence on days 11, 17 and 25, while monkeys at higher dose levels were protected.

Both initial tests and revalidation tests thus show that 3.0 mg/kg x 7 doses of chloroquine (base) has curative action on blood induced *P. cynomolgi* B. Although 3.0 mg/kg chloroquine has been found to be consistently curative, we prefer to use 5.0 mg/kg chloroquine as companion blood schizontocidal dose for radical curative tests.

Determination of primaquine radical curative dose

For the determination of the radical curative dose of primaquine, monkeys were treated orally at 0.180, 0.316, 0.56, 1.0, 1.3, 1.80 and 5.680 mg (base)/kg dose levels. The treatment was initiated when blood parasitaemia reached 5000/mm^3 and administered once daily for seven days. The blood smears were examined for 100 days after end of treatment for appearance of relapse infection. The results (Table 33) showed that monkeys treated at 0.180 mg/kg and 0.316 mg/kg relapsed while monkeys treated at higher doses were cured. The relapsing monkeys were retreated at 1.0 mg (base)/kg dose x 7 days and these monkeys were cured.

For the revalidation of radical curative dose, the treatment was given orally at 1.0 mg/kg dose to 8 monkeys. All the 8 treatment monkeys were cured of infection. The dose 1.0 mg/kg (base) is being used as the standard radical curative dose in our study. This dose of priamquine is combined with 5 mg/kg (base) chloroquine as the curative blood schizontocidal drug.

Sensitivity of primaquine curative dose in different cyclic passages

The sporozoite induced infection of *P. cynomolgi* has undergone 140 serial cyclic passages in rhesus monkeys during the last 18 years. The sensitivity of curative dose of primaquine was validated against primary infection or after 1st and 2nd relapse in a total of 32 monkeys in 17 different passages between XIth and XXXXVth serial passages and the dose of 1.00 mg(base)/kg primaquine was consistently found to be curative (Table 34).

8-aminoquinoline, particularly pamaquine, pentaquine, isopentaquine and primaquine have been reported to affect radical cure of sporozoite induced infections of both *P. cynomolgi* and P. vivax (Schmidt *et al.*, 1982b; Edgcomb *et al.* 1950; Alving *et al.* 1948; Coatney *et al.* 1950; Jones *et al.* 1948; Cooper *et al.*1953). The relapse stages of both these parasites do not respond to the conventional blood schizontocides like chloroquine (Jeffery, 1956; Schmidt *et al.*1977). The above studies show that sporozoite induced *P. cynomolgi* infection in rhesus is an ideal model for screening of potential antirelapse agents.

Simian malaria parasites *P. cynomolgi* B, which closely resembles human malaria *P. vivax* in its biological characteristics and relapse patterns, has been used for anti-relapse efficacy test.

Primaquine has been used as the reference anti-relapse drug and chloroquine has been invariably used as the companion blood schizontocide. Chloroquine was used as a companion blood schizontocide in curative dose to effectively eliminate all blood parasitaemia from patent monkeys. Generally, the monkeys become patent (i.e. slide positive blood smears) on day 8 depending upon the sporozoite inoculum. Once the monkey becomes patent, there is 100% evidence of establishment of sporozoite induced malaria infection in the rhesus monkey.

In our study 1 mg/kg dose of primaquine has been found to be consistently curative. No escalation of the curative dose has been observed over a period of 5 years when sporozoites from seventeen different cyclic passages were used for initiating the infection. Even when the monkeys were treated at primary patency or after relapse, the curative dose of primaquine remained unaltered.

This protocol would be useful for development of potential anti-relapse compounds.

7.6 Gametocytocidal/Sporontocidal Screening against *P. cynomolgi* B

In order to screen new antimalarials for gametocytocidal activity, Plasmodium cynomolgi B infection in rhesus monkey provides an ideal test system since mosquitoes (Anopheles stephensi) can be readily infected with this parasite after feeding on gametocyte carrying monkeys and the data can be quantitated by dissecting mosquito

guts on day 8 post-feeding for recording the reduction of mosquito infectivity rate and mean oocyst count on the gut.

Gametocytocidal test

Oral primaquine administration at 3.16 mg base/kg in a single dose to the gametocyte carrying rhesus monkey, is able to completely abolish the oocyst infectivity of a gut as shown by day 8-9 dissections of mosquitoes. Primaquine produces 100% sterilization of the guts within 24 hrs of drug treatment (Tripathi and Dutta, 1994).

MATERIALS AND METHODS

P. cynomolgi B strain which is human transmissible and maintained by serial cyclic passages through *A. stephensi* in this Institute has been used in the present study. Daily asexual parasiaemia and gametocytaemia was monitored in Giemsa stained blood films.

Quantitation of mosquito infectivity

In order to achieve high infectivity of mosquito (*Anopheles stephensi*) two to three day old colony bred, healthy mosquitoes were allowed to feed on *P. cynomolgi* B infected rhesus monkeys carrying gametocytes. The rhesus monkeys were anaesthetized with 25 mg/kg sodium interval (May & Baker) by i/v route. The unfed mosquitoes were removed and the fully engorged female mosquitoes were maintained under standard insectary conditions for 8-9 days. Each mosquito was then dissected and its gut was examined in 0.1% mercurochrome solution for recording the number of oocysts/gut.

Gametocytocidal test

Primaquine was used as a standard reference drug to establish the model for gametocytocidal activity. The feeding of the mosquitoes during the declining phase of the secondary asexual peak parasitaemia when there are maximum number of infective gametocytes in circulation, gives high infectivity rate and oocyst count/gut consistently for 3-4 days in control (untreated) infection in rhesus monkey.

Pre-treatment (control mosquito feeding)

Batches of mosquitoes are fed at appropriate time to obtain high infectivity/oocyst count. The control feeding is done 1 hour before drug (Primaquine) administration. The mosquito feeding is done after anaesthetizing the monkey with sodium intraval.

Drug Treatment

Single dose primaquine is given orally at 3.16 mg/kg (base) which is standard reference drug for establishing baseline data for gametocytocidal activity. The drug is given to the monkey by rubber catheter.

Post-treatment mosquito feeding

Fresh batches of mosquitoes are allowed to feed on primaquine treated monkeys 24 and 48 hrs. after the drug treatment and the effect of drug is monitored by examining the oocyst number on day 8 or 9.

Sporontocidal test

In the test system developed for sporontocidal screening, the reference drug primaquine was not fed directly to the infected mosquito but the drug was given orally to healthy rhesus monkey and 5 hrs after drug administration, 3-4 days old infected *A. stephensi* were fed on this drug treated monkey. The dose of primaquine administered to the monkey was 10 to 20 mg (base)/kg (single dose) by oral route. The oocyst count on the gut of individual mosquitoes was recorded on day 8 post-infection.

RESULTS

Gametocytocidal test

A single oral dose of primaquine (3.16 mg base/kg) was able to completely abolish the mosquito infectivity in two monkeys as shown by the feedings done at 24 hr or 48 hrs after primaquine treatment. Dissections of a large number of mosquitoes showed complete absence of oocyst development on the gut of the mosquitoes fed 24 hrs post-treatment or later. The results show 100% lethal gametocytocidal effect after single primaquine dose (3.16 mg/kg) (Table 35).

Sporontocidal test

In vitro sporontocidal effect of primaquine against *P. cynomolgi* B was studied (Table 36). Mosquitoes which were infected 3-4 days previously by feeding them on infected monkeys, were again allowed to feed on drug treated healthy monkey. Our results show that 10 mg and 20 mg base/kg of primaquine did not abolish infectivity of

mosquitoes and the oocyst count of all the batches fed on drug treated monkey was reasonably high. The infectivity rate of control batches of infected mosquitoes was practically similar to the batch of mosquitoes fed on primaquine treated monkeys.

The present report shows that 100% gametocytocidal activity of primaquine against *P. cynomolgi* B has been established in the simian model. A single dose of 3.16 mg/kg primaquine was able to sterilize gametocyte infectivity though the gametocytes were seen in circulation at 24 hrs after drug administration. The gametocytes persisting were non-infective.

Primaquine seems to have no sporontocidal effect on the development of oocysts even at 10-20 mg (base)/kg. None of the earlier investigators had done in vivo sporontocidal test involving administration of the drug to healthy monkey and feeding infected mosquitoes on the drug treated monkey. Since Fletcher *et al.* (1987) had reported a peak plasma level of primaquine in 5 hrs after oral administration of primaquine to rhesus monkey, we, therefore, fed the infected mosquitoes 5 hrs after primaquine administration to healthy monkey but observed no significant effect on oocyst development and sporozoite maturation in the salivary glands. Since oocyst development as well as sporozoite maturation was normal our conclusion is that the drug has no sporontocidal effect. Similar conclusion was also drawn by Terzian *et al.* (1968) and Terzian *et al.* (1970) who fed the drug primaquine directly to the infected mosquitoes for the sporontocidal test.

This model will be useful for screening potential antimalarials for gametocytocidal/sporontocidal activity (Tripathi and Dutta, 1994).

7.7 Assessment of Methaemoglobinaemia in beagle dogs for met-Hb toxicity

Beagle dog model has been standardized to evaluate changes in methaemoglobin levels as well as alterations in other haematological parameters (viz. haemoglobin, RBC count, WBC count, differential leucocyte count, mean corpuscular volume) and the liver function tests (viz. SGOT, SGPT, LDH and bilirubin) using primaquine as the reference 8-aminoquinoline drug. The results showed that primaquine treatment at 1.0 mg/kg and 3.0 mg/kg for 7 days raised methaemoglobin levels by 3.7 and 10.5 folds respectively over the corresponding day 0 value. No significant alterations in other haematological indices and liver function tests were observed.

Table 15: Antimalarial screening models developed at Central Drug Research Institute for screening of new antimalarial compounds.

1.	Blood-schizontocidal activity	*P.cynomolgi*/Rhesus monkey (Chloroquine 3 mg/kgx7 days) *P. knowlesi*/Rhesus monkey (Chloroquine 20 mg/kgx3 days) *P. coatneyi*/Rhesus monkey (Chloroquine 20 mg/kg x 3 days)	Dutta *et al.* (1984). *Bull. Wld. Hlth. Org.* **62,** 931-939 Dutta & Singh (1980). *Indian J. Med. Res.* **72**, 23-32 Dutta, Puri, Kamboj, Srivastava & Kamboj (1984). *Bull. Wld. Hlth. Org.* **62**, 931-939.
2.	Anti-relapse activity	*P. cynomolgi/Anopheles stephensi*/ Rhesus monkey (Primaquine 1 mg/kg + Chloroquine 5 mg/kgx7 days)	Dutta & Puri (1988). *Indian J. Parasit.* **12,** 109-115.
3.	Causal-prophylactic activity	*P. cynomolgi/A. stephensi*/Rhesus monkey (Primaquine 1.78 mg/kgx3 days)	Dutta & Puri (1988). *Indian J. Parasit.* **12,** 99-107.
4.	Gametocytocidal/sporontocidal activity	*P. cynomolgi/A. stephensi*/Rhesus monkey (Artemisinin 20 mg/kg single dose)	Dutta, Bajpai & Vishwakarma (1989). *Chemotherapy (Basel)* **35**, 200-207.

The experimental models have been useful to screen and identify new 8-aminoquinolines as primaquine substitute.

The primate screening protocols are also approved by World Health Organization (CC59-WHO collaborating centre at CDRI was approved)

Primate Malaria Antimalarial Screening Models at CDRI

Table 16: Determination of LD_{50} of Primaquine
Host: Swiss mice 25 g.

Dose mg/kg	No. of mice	No. survived / No. treated	% Survival
21.5	5	5/5	100%
46.4	5	5/5	100%
100.0	5	1/5	20%
215.0	5	0/5	0%

LD_{50} = 79.4 mg/kg

Technology for developing antimalarials

LD_{50} Primaquine in mice

Table 17: Determination of LD_{50} of Primaquine

Host : White rats (Druckeray strain) 40 g.

Dose mg/kg	No. of mice	No. survived / No. treated	% Survival
21.5	5	5/5	100%
46.4	5	5/5	100%
100.0	5	2/5	40%
215.0	5	0/5	0%

LD_{50} = 92.6 mg/kg
*Horn, H.J. (1956)
Technology for developing antimalarials
LD_{50} Primaquine in white rats

Table 18: Determination of LD_{50} of chloroquine

Host: Swiss mice 27 g.

Dose mg/kg	No. of mice	No. survived / No. treated	% survival
46.4	5	5/5	100%
100.0	5	5/5	100%
215.0	5	5/5	100%
464.0	5	1/5	20%
1000.0	5	0/5	0%

LD_{50} = 369 mg base/kg
Technology for developing antimalarials
LD_{50} Chloroquine in mice

Table 19: Determination of LD_{50} of chloroquine.

Host: White rats (Druckeray strain) 50 g.

Dose mg/kg	No. of mice	No. survived / No. treated	% survival
46.4	5	5/5	100%
100.0	5	5/5	100%
215.0	5	5/5	100%
464.0	5	2/5	40%
1000.0	5	0/5	0%

LD_{50} = 430 mg base/kg
Technology for developing antimalarials
LD_{50} Chloroquine in white rats

Table 20: Main features of blood induced *P. knowlesi* infection in different primate hosts studied at CDRI

Characteristics of infection	*Macaca mulatta*	*M.assamensis*	*M.radiata*	*Presbytis entellus*
Prepatent period (days)	2-3	2-4	2-3 days	3-5
Duration of patency (days)	5-8 (Av. 5.9)	3-5	50 days (chronic)	5-7
Death occurs on day	7-10 (fatal)	4-7 (fatal)	Non fatal	7-10 (fatal)
Peak parasitaemia before data	35-90%	18-25%	4.5-8.5% (in chronic infections)	35-90%
Mortality rate	26/26 (100%)	8/8 (100%)	1/11 (9.9%)*	9/9 (100%)
Course of infection	Synchronous	Synchronous	Synchronous	Synchronous

*With standard inocula ($1 \times 10^5 - 1 \times 10^6$)

P. knowlesi infection in different monkeys

Table 21: Course of parasitaemia in langur *P.entellus* and rhesus monkey *M. mulatta* inoculated iv with *P.knowlesi*

Animal No.	No. of Parasitized RBC inoculated	Percent parasitaemia on days (post infection)								
		2	3	4	5	6	7	8	9	10
Presbytis entellus										
1. L 393	1×10^6		0.03	0.5	8	50	D			
2. L 394	1×10^6		0.18	0.2	7.2	55	D			
3. L 395	1×10^6			0.01	0.1	0.3	1.2	4.5	35 90*	D
4. L 398	1×10^6			0.03	2.1	22	30	7.5	D	
5. L 399	1×10^6			0.01	1.2	6.5	10	80	D	
6. L 396	1×10^6				0.01	0.05	4.5	33	35	D
7. L 392	1×10^7			0.01	0.07	3.5	12	44	75	D
8. L393	1×10^7		0.01	0.04	5.6	20	60 85*	D		
9. L 397	1×10^7		0.02	0.06	7.8	22.4	58	D		
Macaca mulatta										
1. 390	1×10^6		0.04	2.5	3.8	16.4 60*	D			
2. 389	1×10^6		0.02	0.2	1.5	6.3	25	43	60	D
3. 374	1×10^6		0.02	0.3	4.5	30	35	D		
4. 312	1×10^6		0.01	0.7	1.0	8.0	46	D		
5. 314	1×10^6		0.02	0.3	9	30	45 85*	D		
6. 313	1×10^6		0.06	2.1	4	24	70	D		
7. 311	1×10^6		0.02	0.7	1.5	44	68			
8. 376	1×10^6		0.04	1.5	8	40	D			
9. 383	1×10^6		0.01	0.8	4.5	40.5				
10. 384	1×10^6	0.01	0.03	0.9	5	35.5 90*	D			

*Parasitaemia recorded at 20.00 h D= Died

Course of *P. knowlesi* parasitaemic from Dutta, Singh, and Saibaba (1981) *Ind. Jr. Med. Res*. **73**: (Suppl.) 63-66.

Table 22: Course of parasitaemia in rhesus monkey (*M. mulatta*) and Assamese monkey (*M. assamensis*) inoculated iv with *P. knowlesi*.

Animal No.	No. of Parasitized RBC inoculated	Percent parasitaemia on days (post infection)							
		2	3	4	5	6	7	8	9
M. mulatta									
1. P4	1×10^6	-	-	0.1	0.4	1.0	6.5	21.04	55.0
2. P73	1×10^6	-	-	0.05	0.25	4.90	40.0	Died	-
3. P78	1×10^6	-	-	0.3	3.0	10.0	65.0	94.0	Died
4. P30	1×10^6	-	0.7	4.0	20.0	55.0	Died		
5. P51	1×10^7	0.1	1.0	8.0	46.0	Died			
6. P5	1×10^7	-	0.7	1.5	44.3	68.7			
7. P44	1×10^7	0.2	0.7	8.0	60.0	Died			
8. P48	1×10^7	0.5	1.5	6.0	40.0	Died			
M. assamensis									
1. P113	1×10^6	-	0.08	0.2	20.0	Died			
2. P114	1×10^6	-	0.03	0.32	18.0	died			
3. P121	1×10^6	-	0.01	0.1	25.0	Died			
4. P129	1×10^6	-	-	0.01	0.05	-	2.8	5.4	24.8
5. P130	1×10^7	-	0.01	0.1	7.6	18.0 (50.0)* Died			38.0 Died
6. P94	1×10^7		0.5	18.0	Died				
7. P106	1×10^7	0.1	-	0.1	20.0	Died			
8. P107	1×10^7	-	2.0	11.0					
9. P110	1×10^7	0.2	13.0	25.5	Died				
10. P111	1×10^7	0.5	0.1	1.5	20.0	Died			
11. P112	1×10^7	- -	0.1	1.7	24.0	Died			

*Parasitaemia in these animals was recorded at 8 p.m.
In all other animals the parasitaemia was recorded at 11.0 a.m.
Course of *P. knowlesi* parasitaemia in Rhesus monkey & Assamese monkey from Dutta, Singh, and Saibaba (1981). *Ind. Jr. Med. Res*. **73**: suppl. 63-66

Table 23 : Treatment of *P. knowlesi* infection in rhesus monkey (*M. mulatta*) with chloroquine (base) 20 mg/kg for 3 days.

Infective inoculum	Drug admin. On days	No. Of Parasitized RBC's/10^4 on day								
		3	4	5	6	7	8	9	10	11
Intramuscular chloroquine treatment:										
1.0×10^4	7-9	-	12	80	240	615	400	10	-	-
1.0×10^4	7-9	-	10	90	310	1500	200	1	-	-
1.0×10^4	7-9	-	20	120	230	400	20	-	-	-
1.0×10^5	7-9	-	50	300	1000	1800	700	50	-	-
1.0×10^5	6-8	-	210	260	560	70	1	-	-	-
1.0×10^5	6-8	-	10	300	1300	200	1	-	-	-
1.0×10^5	6-8	-	20	560	6000	4000	460	30	5	-
1.0×10^6	6-8	-	10	140	1000	160	-	-	-	-
1.0×10^6	6-8	-	8	200	800	120	-	-	-	-
1.0×10^6	6-8	-	10	150	1500	200	-	-	-	-
1.0×10^6	6-8	-	5	120	800	140	-	-	-	-
1.0×10^7	5-7	15	175	870	300	10	-	-	-	-
1.0×10^7	5-7	20	300	1400	540	70	-	-	-	-
1.0×10^7	5-7	10	150	650	120	5	-	-	-	-
1.0×10^7	5-7	10	170	700	210	35	-	-	-	-
Oral chloroquine treatment:										
1.0×10^4	7-9	-	-	1	10	170	70	-	-	-
1.0×10^4	7-9	-	4	30	170	200	10	-	-	-
1.0×10^4	7-9	-	-	-	3	20	5	-	-	-
1.0×10^5	6-8	-	-	10	180	40	-	-	-	-
1.0×10^5	6-8	-	-	2	10	-	-	-	-	-
1.0×10^7	6-8	20	170	650	1150	80	-	-	-	-
1.0×10^7	6-8	-	10	40	750	450	40	-	-	-
1.0×10^7	6-8	-	72	150	250	150	-	-	-	-

P. knowlesi treatment with chloroquine from: Singh and Dutta (1981). *Ind. J. Med. Res*. **73**: Suppl. 23-28

Table 24: Treatment of *P. knowlesi* infection with mefloquine 20 mg/kg; 10 mg/kg and 10 mg/kg orally (Group I) and 40 mg/kg, 20 mg/kg and 20 mg/kg orally (Group II) on three consecutive days.

Monkey No.	Inoculum	Drug admin On days	No. of parasitized RBC'S/10^4 on day									
			2	3	4	5	6	7	8	9	10	11
Group I												
161	1.0 x 10^4	7-9	-	-	-	70	1200	6500	500	1000	1	-
164	1.0 x 10^4	7-9	-	-	-	40	500	5000	Died	-	-	-
165	1.0 x 10^4	7-9	-	-	-	40	1000	2500	1500	700	100	1
176	1.0 x 10^6	5-7	-	-	50	900	2000	100	10	-	-	-
177	1.0 x 10^6	5-7	-	-	20	300	200	5	-	-	-	-
178	1.0 x 10^6	5-7	-	-	30	500	400	10	-	-	-	-
179	1.0 x 10^6	5-7	-	-	200	1200	700	1	-	-	-	-
180	1.0 x 10^6	5-7	-	10	200	2000	2500	Died	-	-	-	-
181	1.0 x 10^6	5-7	-	10	200	2000	1400	3	-	-	-	-
183	Frozen	3-5	1	180	100	1	-	-	-	-	-	-
184	Frozen	3-5	1	200	50	1	-	-	-	-	-	-
185	Frozen	3-5	1	200	50	1	-	-	-	-	-	-
186	Frozen	3-5	1	150	100	1	-	-	-	-	-	-
Group II												
219	1.0 x 10^6	4-6		10	500	200	80	20	-	-	-	-
220	1.0 x 10^6	4-6		10	400	120	30	10	-	-	-	-
221	1.0 x 10^6		14	370	160	100	10	-	-	-	-	-
222	1.0 x 10^6	4-6		70	680	480	90	10	-	-	-	-

P. knowlesi treatment with Mefloquine from: Singh and Dutta (1981). *Ind. J. Med. Res*. **73**: Suppl. 23-28

Table 25: Results of the Blood Schizontocidal Activity of Chloroquine Against Blood Induced Infections of *P. cynomolgi* B in rhesus monkeys

Dose mg/(base)/kg	No. of monkey* Protected/treated	Results
1.0 x 7 days	0/8	Recrudescence on day 10, 10, 10, 11, 11, 11, 12, 25
3.0 x 7 days	18/18	Cured
5.0 x 7 days	13/13	Cured
7.0 x 7 days	10/10	Cured
10.0 x 7 days	5/5	Cured

*Observation period upto day 50 after end of treatment.
Route : Oral
Vehicle : Distilled water
From: Dutta and Puri (1988). *Ind. J. Parasitol*. **12**: 109-115.

Table 26: Revalidation of Chloroquine Curative Dose Against Trophozoite induced infections of *P. cynomolgi* B in rhesus monkeys.

Dose mg(base)/kg	No. of monkeys			Recrudescence days
	Treated	Recrudescence	Cured*(%)	
Revalidation in cyclic passage No. II				
3.00	5	Nil	5(100%)	-
5.00	5	Nil	5(100%)	-
7.00	5	Nil	5(100%)	-
Revalidation in cyclic passage No. XXXV				
1.00	3	3	Nil	11,17,25
3.00	3	Nil	3(100%)	-
5.00	3	Nil	3(100%)	-

*Observations till day 50 after last dose of drug.
From Dutta and Puri (1988) *Indian J. Parasit*. **12,**109-115

Table 27: Serial cyclic passage of sporozoite induced *P. cynomolgi* B in rhesus monkeys between 1982-1984.

Sporozoite passage No.	Date of inoculation	Monkey No	Sporozoite inoculums (i.v.)	Day of patency
I	06.11.82	1617	$1x10^6$	10*
II	17.12.82	1725	$1x10^6$	12*
III	02.02.83	1719	$1x10^6$	10*
	02.02.83	1731	$1x10^6$	11*
IV	07.03.83	1832	$1x10^6$	10*
V	09.04.83	1821	$1x10^6$	11*
	09.04.83	1044	$1x10^6$	11*
VI	27.09.83	2102	$0.68x10^6$	9
	27.09.83	2103	$0.68x10^6$	9
	07.10.83	2226	$0.68x10^6$	9
VII	08.11.83	2227	$0.78x10^6$	8
	12.11.83	2229	$0.91x10^6$	9
	16.11.83	2230	$0.74x10^6$	9
	22.11.83	2192	$1.0x10^6$	9
VIII	17.12.83	2187	$0.54x10^6$	9
	17.12.83	2338	$0.54x10^6$	9
	26.12.83	2356	$0.66x10^6$	9
	26.12.83	2357	$0.66x10^6$	9
	06.01.84	2371	$0.85x10^6$	8
IX	22.01.84	2327	$0.93x10^6$	9
	22.01.84	2370	$0.93x10^6$	9
	24.01.84	2231	$0.81x10^6$	9
	24.01.84	2232	$0.81x10^6$	9
	24.01.84	2396	$0.67x10^6$	9
X	20.02.84	2242	$0.64x10^6$	9
	05.03.84	2325	$0.75x10^6$	9
	05.03.84	2337	$0.75x10^6$	9

Serial Cyclic Passage of Sporozoite Induced *P. cynomolgi* B

Table 28: Correlation of the infectivity of *P. cynomolgi* B to *A. stephensi* mosquitoes at primary peak parasitaemia level in rhesus monkeys.

Infective blood meal on days in relation to primary peak	No. of batches fed	Batches positive/ negative for oocysts	Mean oocyst count in positive batches (±S.D.)	Mean (±S.D.) Parasitaemia/ mm^3	Mean (±S.D.) Gametocytaemia/ mm^3
-3	1	1/0	1.60	42805.00 ± 30786.37	276.50 ± 210.61
-2	1	0/1	-	95864.29 ± 1067.14	46647.10 ± 753.52
-1	7	6/1	25.73 ± 20.11	171240.57 ± 51795.33	2099.71 ± 1047.15
0	11	7/4	14.14 ± 15.21	240531.36 ± 50333.95	2637.57 ± 1184.96
+1	8	4/4	29.04 ± 46.08	132293.00 ± 71417.90	2637.57 ± 1224.27
+2	6	2/4	10.43 ± 0.81	40475.93 ± 31732.16	898.19 ± 559.25
+3	1	0/1	-	22476.14 ± 13324.14	551.71 ± 551.71

From: Puri, Kamboj and Dutta (1989). *Trop. Med. Parasitol*. **40:** 409-411.

Table 29: Correlation of the infectivity of *P. cynomolgi* B to *A. stephensi* mosquitoes at secondary peak parasitaemia level in rhesus monkey.

Infective blood meal on days in relation to Secondary peak	No. of batches fed	Batches positive/negative for oocysts	Mean oocyst count in positive batches (±SD)	Mean (± SD) parasitaemia/ mm^3	Mean (±SD) gametocytaemia/ mm^3
-3	6	6/0	18.43 ± 23.27	1871.80 ± 13896.56	633.18 ± 438.43
-2	11	10/1	27.42 ± 22.41	23741.00 ± 14602.97	1015.63 ± 841.99
-1	24	23/1	40.48 ± 20.60	31361.95 ± 18133.61	1190.43 ± 789.92
0	34	30/4	45.40 ± 23.93	44205.33 ± 24325.64	2181.95 ± 1407.99
+1	30	26/4	45.71 ± 27.50	27296.60 ± 17064.91	1580.05 ± 1224.45
+2	22	18/4	33.25 ± 20.88	17821.40 ± 13478.79	1319.28 ± 1368.40
+3	17	16/1	32.36 ± 14.11	10464.55 ± 6975.08	815.00 ± 533.88

From: Puri, Kamboj and Dutta (1989). *Trop. Med. Parasitol*. **40**: 409-411.

Table 30: Estimation of sporozoite yield from different batches of *P. cynomolgi* B infected *A. stephensi* mosquitoes.

Serial passage No.	Monkey No.	Oocyst count on day 7		Estimated sporozoite from 10 monsquitoes on day 13
		Mean±SD	Range	
6	2102	14.78±7.84	5-28	$0.62x10^6$
6	2226	13.63±4.41	8-22	$0.88x10^6$
7	2229	15.22±3.93	9-27	$0.89x10^6$
8	2338	51.33±9.50	38-65	$1.20x10^6$
9	2327	18.71±8.40	10-34	$0.76x10^6$
10	2242	35.25±9.56	24-56	$1.32x10^6$
12	2569	4.44±1.74	2-7	$0.39x10^6$
13	2642	21.00±7.62	13-34	$1.16x10^6$
14	2644	7.55±4.39	2-18	$0.46x10^6$
15	2775	29.63±15.65	12-64	$1.17x10^6$
16	2812	42.43±20.81	17-78	$1.32x10^6$
19	3093	21.33±7.57	11-30	$0.94x10^6$
20	3159	47.25±8.97	37-54	$1.25x10^6$
21	3199	68.00±20.56	40-100	$1.56x10^6$
22	3142	37.33±15.39	16-61	$1.15x10^6$
24	3456	59.00±14.43	35-76	$1.77x10^6$
25	3501	16.80±8.39	5-33	$0.67x10^6$

Table 31: Causal prophylactic activity of primaquine against sporozoite induced infection of *P. cynomolgi B* in 9 day treatment.

Dose mg (base)/ kg	Monkey No.	Sporozoite inoculum	Response to treatment	No. of monkeys Protected/Treated
0.5	2103	$0.68x10^6$	Patent on day 19	0/2
	2109	$0.68x10^6$	Patent on day 24	
1.0	2109	$0.68x10^6$	Patent on day 28	5/6
	2110	$0.68x10^6$	Cured	
	2281	$0.74x10^6$	Cured	
	2282	$0.74x10^6$	Cured	
	2283	$0.74x10^6$	Cured	
	2284	$0.62x10^6$	Cured	
2.0	2231	$0.91x10^6$	Cured	4/4
	2232	$0.91x10^6$	Cured	
	2339	$0.78x10^6$	Cured	
	2340	$0.78x10^6$	Cured	
Control	2102	$0.68x10^6$	Patent on day 9	0/5
	2103	$0.68x10^6$	Patent on day 9	
	2227	$0.68x10^6$	Patent on day 8	
	2229	$0.68x10^6$	Patent on day 9	
	2230	$0.68x10^6$	Patent on day 9	

Observations upto day 70 post-infection
From: Puri and Dutta (1988) *Ind. Jr. Parasitol*. **12**: 99-107.

Table 32 : Cuasal prophylactic activity of various primaquine doses against sporozoite induced infections of *P. cynomolgi* B in 3 day treatment schedule.

Dose Mg(base)/kg	No. of Monkeys	Sprozoite inoculum	Response to treatment			
			Patent		Protected	
			No.	Day of patency	No.	%
0.316	6	$1.0x10^6$	6	11,13,13,15,17,18	0	Nil
0.62	5	$1.0x10^6$	5	16,17,17,17,18	0	Nil
1.00	2	$0.66x10^6$	0		2	100%
	2	$0.81x10^6$	1	33	1	50%
	7	$1.0x10^6$	4	20,21,21,22	3	43%
	5	$1.1x10^6$	2	15,15	3	60%
1.3	2	$1.0x10^6$	0		2	100%
1.78	7	$0.66\text{-}1.1x10^6$	0		7	100%
3.16	11	$0.66\text{-}1.1x10^6$	0		11	100%
10.00	4	$0.66\text{-}1.0x10^6$	0		4	100%
Control	2	$0.66x10^6$	2	9,9		
	2	$0.81x10^6$	2	9,9		
	4	$1.0x10^6$	4	10,11,11,11		
	2	$1.1x10^6$	2	8,8		

Observation upto day 70 post-infection
From: Puri and Dutta (1988). *Ind. Jr. Parasitol*. **12:** 99-107.

Table 33: Radical curative activity of various doses of primaquine against established infections with sporozoites of *P. cynomolgi* B.

Dose x 7 day (mg base/kg)		No. of monkeys			Relapse on day
Primaquine	Chloroquine	Treated	Relapsed	Cured(%)***	
0.180	5 mg/kg	2	2	Nil	10,10
0.316	5 mg/kg	2	2	Nil	18,28
0.56	5 mg/kg	11	2	9(81.8%)	13,26
1.00	5 mg/kg	4*	0	4(100%)	-
1.00	5 mg/kg	8**	0	8(100%)	-
1.30	5 mg/kg	5	0	5(100%)	-
1.80	5 mg/kg	2	0	2(100%)	-
5.68	5 mg/kg	2	0	2(100%)	-
Nil	5 mg/kg	10	10	Nil	10,11,11,12, 12,13,14,16,17

*Treated with primaquine after first relapse (R1)
**Revalidation of 1.00 mg/kg dose
***Observations till day 100 after last dose of drug
From: Dutta and Puri (1988). *Ind. Jr. Parasitol*. 12: 109-115.

Table 34: Primaquine sensitivity of relapse stages of sporozoites induced *P. cynomolgi* B in different serial passages.

Dose mg(base)/ kg	Sporozoite Passage No.	No. of monkeys	Status of infection	Result*
1.00	XI	2	Primary	Cured
1.00	XI	2	R1	Cured
1.00	XIII	1	Primary	Cured
1.00	XVI	2	Primary	Cured
1.00	XXI	4	Primary	Cured
1.00	XXI	2	R1	Cured
1.00	XXII	1	Primary	Cured
1.00	XXV	1	Primary	Cured
1.00	XXVI	2	Primary	Cured
1.00	XXVIII	1	Primary	Cured
1.00	XXIX	1	Primary	Cured
1.00	XXIX	1	R2	Cured
1.00	XXXII	1	Primary	Cured
1.00	XXXIII	1	Primary	Cured
1.00	XXXIII	1	R1	Cured
1.00	XXXV	1	Primary	Cured
1.00	XXXVII	1	Primary	Cured
1.00	XXXX	1	Primary	Cured
1.00	XXXXI	3	R1	Cured
1.00	XXXXIV	1	Primary	Cured
1.00	XXXXIV	1	R1	Cured
1.00	XXXXV	1	Primary	Cured

Primaquine sensitivity of relapse stage of sporozoites induced *P. cynomolgi* B in different serial passages

Table 35: Gametocytocidal activity of primaquine diphosphate as shown by oocyst counts on gut of *A. stephensi* fed on infected monkey.

Monkey No. (infection)	Mosquito feeding	Asexual parasistaemia (%)	Gametocy-taemia (%)	Primaquine dose	Mosquito dissection No. infected/ No. exam-ined	Infect-ivity	Oocyst count (Mean ±SD)
1.4909 (sporo-zoite (infec-tion)	Pre-treatment 1. At -1h Post-treatment 2. At 24h 3. At 48h	1.5% .2% .07%	0.25% 0.09% 0.01%	- 3.16 mg/kgx1 -	13/16 Nil/25 Nil/9	81.25% Nil Nil	16.15 ±14.62 Nil Nil
2.4910 (sporo-zoite induced)	Pre-treatment 1. At -1h Post-treatment 2. At 24h 3. At 48h	2.0% 0.12% 0.1%	0.25% 0.004% 0.001%	- 3.16 mg/kgx1	9/9 Nil/56 Nil/30	100% Nil Nil	66.33 ±32.49 Nil Nil

Gametocytocidal activity of Primaquine.

Table 36: Sporozoite test with primaquine administered to healthy monkey, on which infected (*P. cynomolgi* B) mosquitoes (*A. stephensi*) were allowed to feed.

Age of mosquito infection	Primaquine treatment	Mosquito dissection No. infected/ No. examined	Infectivity (%)	Oocyst count (Mean±SD)
1-3 day	10mg/kg at -5h	22/26	84/62	45.32±23.19
Control	Untreated	20/24	83.33	53.6±24.29
2-4 day	10mg/kg at -5h	20/25	80.00	84.75±29.84
Control	Untreated	22/27	81.48	101.36±29.08

Note:

1. Drug was administered to healthy monkey 5 hours before mosquito feeding.
2. Results of feeding with 3.16 mg(base)/kg primaquine were similar to above.

CHAPTER-III

ANTIMALARIAL DRUG DISCOVERY AND DRUG DEVELOPMENT (CENTRAL DRUG RESEARCH INSTITUTE, LUCKNOW)

1. NEW BLOOD SCHIZONTOCIDES FOR CONTROL OF MULTI-DRUG RESISTANT MALARIA/UNCOMPLICATED/COMPLICATED AND CEREBRAL MALARIAS

1.1 Blood Schizontocidal Activity of Artemisinin (Qinghaosu) and a New Antimalarial α/β-Arteether

The potential antimalarial value of *Artemisia annua* L. a Chinese herb was first documented in Zhou Hou Bei Ji Fang (Handbook of Prescriptions for Emergency Treatments) written as early as 340 AD by Ge Hong of the Eastern Jin Dynasty. Qinghao preparations made from this plant have been used extensively in the Chinese traditional medicine for the control of malaria and this formulation is described in famous book Ben Cao Gang Mu (Compendium of Materia Medica) published in 1596 AD by Li Shizhen of the Ming Dynasty (Anon, 1979). The active antimalarial constituent of this plant isolated in 1971 was described as a new type of sesquiterpene lactone with a peroxy group and designated as artemisinin (Qinghaosu) (Annon, 1979). The antimalarial activity of artemisinin (a neutral portion of qinghaosu) against experimental rodent malaria infection was first observed in 1971 and oral administration of 1 g/kg/day x 3 doses was reported to clear blood parasitaemia in mice (Anon. 1982a). Since the first documented activity of artemisinin against rodent malaria in 1971, numerous investigators have studied the blood schizontocidal efficacy of artemisinin and its oil soluble derivative β-arteether and their results are summarized in Table 37.

The present communication reports the antimalarial activity of artemisinin and its derivative arteether, against blood induced *P. berghei* infection in Swiss mice.

RESULTS

Artemisinin was given by intramuscular route at doses ranging from 3.12 mg/kg to 100 mg/kg (x 4 days) in the form of aqueous suspension and the course of parasitaemia in *P. berghei* infected mice was monitored in different drug treated and control groups of mice (Table 38). Doses of 6.25, 12.5, 50 and 100 mg/kg showed only suppressive action on day 4 parasitaemia and by day 7 the level of parasitaemia in different groups had again increased in all the drug treated groups of mice.

Artemisinin (suspension in neutralized ground-nut oil) was also given by intramuscular route to the infected mice at doses ranging from 31.6, 100, 316 and 1000 mg/kgx7 days. The higher doses (316 and 1000 mg/kg) were partly toxic while the lower doses (31.6 and 100 mg/kg) were well-tolerated by the infected mice. The dose of 100 mg/kg was curative as shown by clearance of parasitaemia beyond 21 days of observation, while the lower doses (31.6 mg/kg) was partly curative. Clearance of parasitaemia by day 4 was incomplete with all the doses, but when the treatment was continued for 7 days, the parasitaemia was cleared (Table 39).

Arteether showed very high antimalarial activity even after a single 5 mg/kg dose and the parasitaemia was cleared within 24 hrs. When 5 mg/kg dose was given for 2 consecutive days, one out of eight mice showed recrudescence of low level of parasitaemia by day 21 post-infection. However, the cure rate with one or two doses of arteether (5 mg/kg) was 80% while 3 doses were fully curative and no recrudescence of parasitaemia was observed up to 28 days of observation (Table 40).

DISCUSSION

Most of the earlier data on the antimalarial activity of artemisinin had been generated by the Chinese scientists during the last 15 years. The ED_{50} of this drug against *P. berghei* (sensitive strain) had been reported to be 130.7 ± 21.2 to 176.6 mg/kg by oral route, and 0.77 mg/kg by intramuscular route, while its ED_{50} against chloroquine resistant strain was 439.8 ± 64.2 mg/kg (Anon, 1979, 1982c). The ED_{50} values recorded against sensitive and chloroquine resistant line were 2.15 and 6.20 mg/kg respectively (Anon. 1982b,c). Besides other Chinese workers had recorded the clearance of parasitaemia after oral doses ranging from 50-80 mg/kg, 50-100 mg/kg and 1 g/kg. Like-wise 40 mg/kg dose by subcutaneous route was reported to clear parasitaemia due to both sensitive and resistant strains. These reports do not show whether the parasitaemia had been totally cleared or not. A recent report by Lin *et al.* (1987) has shown that 160 mg/kg was curative against sensitive strain of *P. berghei* when the drug was given by subcutaneous route. Lower dose (40 mg/kg) was partly curative in 2 out of 5 mice. In the present study a dose of 100 mg/kg artemisinin for 7 days was found to be curative in 5 out of 8 mice as shown by clearance of parasitaemia beyond 21 days. At the lower dose (31.6 mg/kg)

there was partial cure and 3 out of 8 mice survived. The studies conducted so far show that although very low ED_{50} (0.77 mg/kg) and ED_{90} values (2.15-6.20 mg/kg) have been reported earlier for *P. berghei* (Anon. 1982b,c), the **curative dose of artemisinin for this parasite is very high and ranges from 100 mg/kgx7 day** to 160 mg/kgx3 day by i/m and subcutaneous routes, respectively.

The new ethyl ether derivative of dihydroartemisinin (**arteether**) has shown very high **curative efficacy at 5 mg/kg dose**. Even a single injection at 5 mg/kg in our study produced 80% curative effect against *P. berghei*. This is **potential future drug** for the **treatment of cerebral malaria**. It is highly active drug for **eradicating infection with multiple drug-resistant strain** of *P. yoelii nigeriensis* as well as *P. knowlesi* (Dutta *et al.* unpublished data). β-enantiomer of arteether had been reported earlier to be effective against a chloroquine resistant strain of *P. berghei* with an ED_{90} of 1.95 mg/kg (Anon. 1982b).

1.2 Causal Prophylactic and Anti-relapse Efficacy of Artemisinin and α/β-Arteether

The discovery of Qinghaosu (artemisinin) and its analogue arteether as a new class of antimalarials for treating drug-resistant cases of *P. falciparum* and cerebral malaria cases had aroused world-wide interest to explore these compounds for their antimalarial activities. Blood schizontocidal activity of artemisinin against drug-resistant parasites had been documented by Chinese workers (Anon. 1979, 1982a,b). The authors have reported curative activity of α/β-arteether (30:70 mixture of enantiomers) against *P. berghei* and *P. cynomolgi* B (Dutta, Bajpai and Vishwakarma, 1987, 1988).

The protocols reported by Schmidt *et al.* (1982) and modified by Dutta and Puri (1988) for blood schizontocidal and radical curative tests and the 3-day treatment schedule protocol reported by Puri and Dutta (1988) for sporozoite induced causal prophylactic test which is a modification of 9-day protocol proposed by Schmidt *et al.* (1982), were used in this study.

Artemisinin (Qinghaosu) and arteether (α/β 30:70 mixture of enantiomers were prepared by Central Institute of Medicinal and Aromatic Plants for this study. Both the drugs were given as intramuscular injection in sterile neutralized double refined ground-nut oil. Artemisinin was administered as suspension in 1 ml oil and arteether as solution in 1 ml oil. Chloroquine diphosphate and primaquine phosphate (Sigma) were used as

standard drugs for blood and tissue schizontocidal activity, respectively. The curative blood schizontocidal dose of chloroquine against *P. cynomolgi* B is 5 mg (base)/kgx7 days (Dutta and Puri, 1983) and that of primaquine is 1 mg (base)/kg x 7 days for radical curative (Dutta and Puri, 1988) and 1.78 mg (base)/kg x 3 days for causal prophylactic rest (Puri and Dutta, 1988).

RESULTS

Causal prophylactic activity

The data presented in Table 41 show that **both arteether and artemisinin had no prophylactic activity** against sporozoite induced *P. cynomolgi* B at 20 mg/kg x 3 day schedule.

The standard drug **primaquine at 1.78 mg (base)/kg x 3 days** was found to be curative in two monkeys as no patency was found up to 60 days of follow-up.

Anti-relapse activity

The standard drug **primaquine** at 1 mg (base)/kg x 7 days when administered concurrently with curative dose of blood schizontocide (chloroquine 5 mg (base)/kg x 7 days), was found to be effective as **anti-relapse agent against *P. cynomolgi* B** infections, and no patency was observed up to 100 days observation period (Table 42). Another control monkey treated with chloroquine alone (at 5 mg (base)/kg x 7 days) showed a relapse on day 16 after the last dose of chloroquine. However, **both artemisinin and arteether showed no anti-relapse activity** at 20 mg/kg x 7 day.

1.3 DRUG DISCOVERY OF AREETHER α/β (30:70)

New Blood Schizontocide/Gametocytocide – Transmission Blocking Drug

1. **Fast-acting blood schizontocide** for *P. falciparum*, *P. vivax* (Dutta *et al.* 1987/Patent 1990 CSIR-CDRI/CIMAP).
2. Active against chloroquine/Mefloquine/Quinine resistant/multidrug resistant complicated malarias (*P. falciparum/P. vivax*).
3. Active against uncomplicated/severe complicated malarias.

4. Powerful **injectible α/β-arteether** containing 150 mg drug, formulated in double refined, neutralized ground-nut oil for the control of comatose/cerebral malaria cases.
5. Safe intramuscular injection (3 x doses),having successfully **completed Phase I, II, III, post-marketing (IV) clinical trials.**
6. α/β-arteether exerts **strong gametocytocidal (transmission blocking action)** against *P. cynomolgi* B and *P. falciparum.*
7. α/β-arteether is effective against simian malaria ***P. knowlesi, P. cynomolgi* B, *P. fragile*) and zoonotic *P. knowlesi* (monkey) malaria** infections/epidemics in human subjects.
8. α/β-arteether pharmacokinetics showed that each injection dose is effective beyond 32 hrs, and antimalarial action of 3 dose (IM injections) extends beyond 120 hrs..
9. Both α and β annomers (30:70) in α/β-arteether exert **powerful synergistic antimalarial action** against MDR malaria/cures highly **multidrug resistant *Plasmodium yoelii nigeriensis* MDR strain** (in standard 4 dose IM treatment schedule in Swiss mice) developed at CDRI.

α/β-Arteether Major Activities

1. α/β-Arteether is a semi-synthetic compound derived from the Chinese plant "*Artemisia annua*". This compound has been **developed jointly by CSIR-Central Drug Research Institute, and CIMAP, Lucknow**. After extensive preclinical, clinical and toxicological safety studies, the compound has been well accepted as the **most effective emergency drug** against virulent MDR *P. falciparum* and chloroquine/mefloquine/quinine resistant malarias.
2. It rapidly kills asexual stage of MDR *P. falciparum* and considered most efficacious in the treatment of **severe complicated *P. falciparum* malaria**.
3. Indian experience has demonstrated that arteether provides **93-100% cure rate in uncomplicated** *P. falciparum* malaria and **91.5-100% cure rate in complicated** *P. falciparum* malaria.

4. The drug α/β-arteether is lipophilic, helps greater **accumulation in brain tissue** and is a drug of choice for the treatment of **dreaded cerebral malaria** caused by *P. falciparum/P. vivax.*
5. No drug related side effects. Drug is safe and ensures good patient compliance.
6. Discovery of **gametocytocidal action of artemisinin** (Qinghaosu) was first discovered by Dutta *et al.* (1989) indicating the transmission blocking activity of artemisinins. The **α/β-arteether also exerts gametocytocidal action** against simian parasites (Tripathi *et al.* 1990, 1996) and *P. falciparum* (Dutta *et al.* 2001 US Patent).
7. **National Drug Policy on Malaria – India** Directorate of National Vector Borne Disease Control Ministry of Health and Family Welfare Programme (Government of India, 2010)

   Arteether injection for **severe malaria** included in malaria control.
8. **Malaria WHO Report: RBM** – The Malaria Problem in South-East Asia Region Success Stories in Malaria Control from the SEA Region

   α/β-Arteether : α/β-arteether (30:70 ratio) is racemic mixture of ethyl derivative of artemisinin developed at the Central Drug Research Institute, Lucknow, India. Multi-centric studies in over 500 patients have confirmed the efficacy of drug in uncomplicated and complicated falciparum malaria with recrudescence rate of about 8%. The drug is produced by Themis Chemicals, Mumbai,India. It is available throughout the country and has been **introduced in the treatment of severe malaria by the NAMP.**
9. **CDS/RBM Report by Dr. Andrea Bosman**: Roll Back Malaria dated 18 March, 2002 on α/β-arteether : α/β-arteether was **registered as E-MAL®** in India for the treatment of acute uncomplicated **multi-drug resistant *falciparum* malaria** and for **severe malaria in January 1997**. The drug released for **marketing in April 1997**. The external packaging and the package insert clearly indicate that the drug is for **hospital use only**.

α/β-arteether: Capability of Production (1997-2001) : Production of α/β-arteether is by a plant which is **WHO approved for GMP** and conforms to international standards. Themis reports that it has a **100,000 ampoule/month** capacity for production and, since

registration 1997, 1,752,233 adult treatment doses have been sold in India (129 891 in 1997, 262 117 in 1998, 356 160 in 1999 and 1,004,065 in 2000 and 2001).

Although WHO guidelines on the specific use of α/β-arteether do not exist, the Company's recommended 3-day dosage regimen does not **conform with WHO recommendations that state that monotherapy with regimens of artemisinin or its derivatives of less than 7 days should not be used because of the unacceptable high levels of recrudescence. No comparative studies of artemotil and α/β-arteether. α/β-arteether is probably cheaper to produce as it does not require the chemical step to produce the pure β form from the chiral mixture.**

Authors Comment: Roll Back Malaria/WHO/TDR/Any International Agency may kindly conduct three clinical trials to clarify the curative action of 3 dose α/β-arteether (IM) and Artemotil (3 doses).

Criteria Used For Selection of α/β-Arteether (30:70) Intramuscular Injection

- **No *in vitro* screening test was used for selecting α/β-arteether**.
- Rodent Malaria Parasite – *Plasmodium yoelii nigeriensis* MDR selected at CDRI was used for **Drug Discovery of Antimalarial α/β-Arteether and several other blood schizontocides in the Primary In vivo Screening Protocol using rodent model (Random bred Swiss mice) at CDRI.**
- *P. yoelii nigeriensis* MDR is 100% fatal in Swiss mice 18-22 g).
- This strain was procured from the London School of Tropical Medicine & Hygiene (showing innate resistance to only chloroquine). This strain was subsequently exposed to a large variety of standard drugs and antibiotics listed below and has been designated as **MDR *P. yoelii nigeriensis* CDRI strain**, which was developed by the author and cryopreserved from time to time and also passaged in Swiss mice. This strain has been **used at CDRI for drug discovery** of new antimalarials (fast-acting blood schizontocide) including α/β-Arteether.
    1. *P. yoelii nigeriensis* has also been used as primary strain for selection of α/β-Arteether (Dutta *et al.* 1989b).
    2. *P. knowlesi* WI simian malaria (Bajpai *et al.* 1989a).
    3. *P. cynomolgi* B simian malaria (Dutta *et al.* 1988, Tripathi *et al.* 1991)

4. *P. fragile* (Tripathi *et al.* 1996)
5. *P. coatneyi* (Dutta *et al.* 2003)
6. *P. falciparum* (Dutta *et al.* 1990) and **U.S. patent 2001**

P. yoelii nigeriensis MDR developed at CDRI is resistant to various doses of following antimalarial drugs and antibiotics:
Chloroquine, Amodiaquine, Mefloquine, Mepacrine, Quinine, Quinidine, Halofantrine, Pyrimethamine, WR238605, Tetracycline, Demeclocycline, Doxycycline, Erythromycin, Ciprofloxacin, Norfloxacin, Chloroquine + Tetracycline, Amodiaquine + Tetracycline, Mefloquine + Tetracycline, Mepacrine + Tetracycline, Quinine + Tetracycline, Quinine + Oxytetracycline, Quinine + Doxycycline, Quinine + Erythromycin, Ciprofloxacine + Chloroquine, Norfloxacine + Chloroquine, Quinidine + Tetracycline

Development of α/β-arteether

Since 1986, efforts were therefore, directed to develop jointly by CDRI-CIMAP, Lucknow, α/β-artheer, (a 30:70 mixture of enantiomers) as a fast-acting blood schizontocide specifically for the control of drug-resistant malaria and as a potential compound for the control of **cerebral malaria cases**. α/β-Artheer, was patented jointly by CIMAP-CDRI in 1990 (Vishwakarma R A, Thakur R S, Dutta G P and Bajpai R– **Indian Patent No. 173947/1070 DEL 90**) as a novel blood schizontocide. The major antimalarial activities of α/β-artheer, and clinical profile of the drug have been briefly reviewed by Dutta *et al.* (1994), Dutta and Tripathi (1996), Asthana *et al.* (1994,1997), Dutta *et al.* (1998), Kamboj and Dutta (1998), Jain *et al.* (2000) and Dutta and Tripathi (2003).

Antimalarial studies

Blood Schizontocidal Activity of α/β-artheer - α/β-Artheer, has shown fast-acting blood schizontocidal action against the following experimental malaria models:

P. berghei (sensitive strain): Curative dose is 5 mg/kg x 3 doses (Dutta *et al.* 1987, Bajpai *et al.* 1988) (Table 43).

P. yoelii nigeriensis MDR (lethal multi-resistant strain) showing complete resistance to chloroquine, mefloquine and quinine (Dutta & Pandey 1986) and Dutta *et*

al. (2000) patent. Curative dose is 5 mg/kg x 3 doses in Swiss mice (Dutta *et al.* 1989b) (Table 44).

P. knowlesi WI – a cerebral malaria model: Curative dose is 12 mg/kg x 5 doses (Bajpai *et al.* 1989b) (Table 45).

P. cynomolgi B – a human transmissible *P. vivax* like model: Curative dose of α/β-artheer, is 5 mg/kg x 3 doses (Dutta *et al.* 1989a, Tripathi *et al.* 1991) (Table 46).

***Plasmodium fragile*: Efficacy of Arteether (α/β) against Cerebral Malaria Model**

Cerebral malaria is a serious complication of *Plasmodium falciparum* infection. Cerebral malaria-associated pathologic manifestations include deep vascular schizogony and sequestration of schizonts in the cerebral microvessels leading to occlusion of blood capillaries, impaired blood flow, anoxia, and consequent organ failure (Warrell *et al.* 1988).Recent studies have established that the sequestration is due to the presence of a variety of host adhesion molecules such as CD-36 (Barnwell *et al.* 1989), thrombospondin (TSP) (Roberts *et al.* 1985), E-selectin (Roberts *et al.* 1993), intracellular adhesion molecule-1 (ICAM-1) (Berndt *et al.* 1989; Aikawa *et al.* 1990), endothelial leukocyte adhesion molecule-1 (ELAM-1), vascular cell adhesion molecule-1 (VCAM-1) (Ockenhouse *et al.* 1992), and chondroitin sulphate A (CSA) (Rogerson and Brown 1997) which are believed to serve as ligands for corresponding receptors present on the knobs of infected RBCs. Both *P. fragile* and *P. coatneyi* infections in rhesus monkey show characteristic cytoadherence of the schizont-infected RBCs to the endothelial cells in the cerebral capillaries which is mediated by endothelial cell surface receptors, namely CD36, TSP, and ICAM-1 (Aikawa *et al.* 1992; Fujioka *et al.* 1994), similar to those reported in human cerebral malaria. Because of the identical cerebral complications produced by ***P. fragile*** and *P. falciparum,* Nguyen-Dinh *et al.* (1988), Fujioka *et al.* (1994), and Mendis *et al.* (1997) suggested that the former could be a useful **primate model for human cerebral malaria**. Since *P. fragile* shares several characteristics with *P. falciparum* infection, the model was used in the present study for evaluation of blood schizontocidal activity of arteether.

In this study, we used male and female rhesus monkeys (*Macaca mulatta*), weighing approximately 4 kg, maintained in the Primate House of the Central Drug Research Institute. Monkeys free from *Mycobacteria tuberculosis* and blood protozoan

infections were used. *P. fragile* shows high virulence for rhesus monkey and attains a 12.5 to 25.0% level of parasitaemia with inoculum of 1×10^5 parasitized RBCs. The mortality rate of *Macaca mulatta* with this infection has been reported to be 75% (Puri and Dutta 1994). The peak parasitaemia reached was around the 16th day of inoculation. In the present study, each rhesus monkey was inoculated with 1×10^6 infected RBCs and the parasitaemia was recorded daily be examining Giemsa-stained blood smears. The drug was given to the monkeys when the infection became patent. α/β-Arteether (30:70 mixture of enantiomers) was dissolved in neutralized ground-nut oil (Arachis oil) and was administered at four dose levels (2.5,5.0, 7.5, and 10.0 mg/kg doses) for 3 consecutive days by the intramuscular route. Blood smears from each monkey were obtained for 60 days after the end of the treatment and the recrudescence, if any, was recorded. Monkeys which remained negative for 60 days were considered cured.

Results of the blood schizontocidal activity of arteether, against *P. fragile* rhesus monkey, are summarized in Table 47. The compound was tested by im route at four dose levels viz. 2.5, 5.0, 7.5, and 10.0 mg/kg × 3 days. The lowest dose of 2.5 mg/kg was noncurative, as shown by recrudescence in all three monkeys between days 12 and 16. The 5.0- mg/kg dose gave a 50% cure rate, as shown by clearance of parasitaemia in four of eight monkeys up to 60 days of observation. The remaining four animals showed recrudescence between days 16 and 35. At the 7.5-mg/kg dose, the cure rate was 75%, as shown by a complete cure in six of eight monkeys, and the two monkeys which were not cured showed delayed recrudescence on days 31 and 38. At the highest dose, i.e., **10 mg/kg × 3 days, the cure rate was 100%** and none of the treated monkeys at this dose showed any recrudescence.

Our studies with arteether show that progression of the disease toward the development of cerebral complications can be successfully controlled by administration of arteether. In comparison to *P. fragile,* arteether has earlier been reported to produce a 100% curative effect against *P. cynomolgi* B at 5.0 mg/kg × 3 doses and against *P. knowlesi* at 12.0 mg/kg × 5 doses (Dutta *et al.* 1989; Bajpai *et al* 1989b*).* Judging from the clearance of parasitaemia, it can be concluded that the drug is a **very fast-acting blood schizontocide for control of cerebral malaria and is safer** on the basis of LD_{50} than previously used artemisinin derivatives (Dutta and Tripathi, 1996).

Gametocytocidal Activity

P. cynomolgi B – *Anopheles stephensi* – Rhesus model: α/β-Arteether shows **high gametocytocidal activity** at 10 mg/kg dose (Tripathi *et al.* 1990, 1996) (Table 48).

Dutta *et al.* (2000) also reported the gametocytocidal action of α/β-arteether against *P. falciparum* cases treated with 3 doses of IM arteether which completely cleared the gametocytes within 48 hour of treatment (Table 49).

Causal Prophylactic and Anti-relapse Activity - Arteether has **no causal prophylactic or antirelapse activity** (Bajpai *et al.* 1989b).

Pharmacological Studies

α/β-arteether had been found to be devoid of any significant pharmacological effect on CNS, CVS or urinary system. No drug related adverse pharmacological effect was reported (Kar *et al.* 1989). **LD_{50} of drug is 1267.33 mg/kg** in Swiss mice (i.m.) and it is **safest** among the artemisinin derivatives (Dutta & Tripathi 1996, Kar *et al.* 1989).

Sub-acute Toxicity Studies

Systemic toxicity studies on arteether were undertaken by Sethi *et al.* (1988) in two hosts, namely rats and monkeys. Experimental groups of rats were injected with arteether at 3 dose-levels (low, high and toxic), i.e. 2.5, 5.0 and 10.0 mg/kg body weight x 3 consecutive days per week for a period of 4 weeks. The monkeys were given 7.5, 15.0 and 30 mg/kg doses for 3 consecutive days per week for a period of 4 weeks. Detailed **subacute toxicity tests on arteether treated rats and monkeys** showed no significant change in body weight, haematological, bio-chemical, histopathological parameters of the test animals. Arteether has been found to be safe in regulatory subacute 1 month toxicity studies and the compound was recommended for clinical trials in humans (Sethi *et al.* 1988).

Teratogenicity Studies

Teratogenicity studies in rats and rabbits showed that α/β-artheer, has no teratogenic potential and is a **safe drug** (Sinha & Sethi 1994).

Clinical Trials with α/β-arteether

On the basis of **Dossier (1988) on arteether α/β-submitted to the Drugs Controller General, Govt. of India**, permission was granted to conduct Phase I clinical trials at CDRI.

Phase I : Single Dose Tolerance Study

Phase I single dose tolerance study with α/β-arteether (30:70) was undertaken at the Central Drug Research Institute, Lucknow. Thirty healthy human volunteers who consented to undergo the trial as per approved protocol completed the study. The trial was double blind placebo controlled and non-crossover. The dose of arteether injection ranged from 20 to 300 mg; 6 volunteers received placebo injection, while 4 each were given 20, 50, 100,150, 200 and 300mg arteether in a gradually escalated manner (Asthana *et al.* 1994).

In all cases, single injection of arteether/placebo (oil) was tolerated well and no tenderness, swelling or discomfort was experienced at the site of injection. Recording of clinical parameters and detailed haematological and biochemical investigations, ECG and urinalysis did not reveal any abnormality (Asthana *et al.* 1994).

Phase I : Multiple Dose Tolerance Study

After establishing the safety of **single doses of arteether ranging from 20 to 300 mg in healthy human volunteers**, multiple dose tolerance study was undertaken at CDRI Clinical Pharmacology Unit, Seth G.S. Medical College, Mumbai. Twenty healthy human volunteers who executed written informed consent completed the study as per approved protocol. Initially, the first group of 10 volunteers was given arteether (100 mg) injection (intramuscular) once a day on three consecutive days. Subsequently, the second group of 10 volunteers was given arteether (100 mg) injection (Intramuscular) once a day on three consecutive days. Subsequently, the second group of 10 healthy human subjects was treated with 150 mg of arteether by intramuscular injection on 3 consecutive days. All the 20 volunteers who completed multiple dose tolerance study were males. Each volunteer was subjected to a detailed history-taking, clinical examination and laboratory investigations before the administration of the first dose of arteether injection with the aim of having initial baseline pre-therapy values of each parameter. No untoward

reaction, experiences or drug-induced abnormalities could be detected in clinical and laboratory parameters and the variation observed in organ function tests was within normal limits, except a transient rise in transaminase levels (SGOT/SGPT). Thus, it can be concluded that areether therapy with 100 and 150 mg doses given as intramuscular injections once a day on 3 consecutive days, are **well tolerated and safe** (Asthana *et al.* 1994).

Phase I Clinical Studies on α/β Arteether in Healthy Human Volunteers

1. α/β-Areether is a fast-acting antimalarial (blood schizontocide).
2. It is curative against MDR plasmodia.
3. It is potential intramuscular injectible drug for **cerebral malaria/complicated *Plasmodium falciparum*.**
4. It is also curative blood schizontocide for ***P. vivax*.**
5. α/β-Areether has successfully cleared Phase I single and multiple dose safety and tolerance studies in healthy volunteers.

RESULTS

Phase I single dose tolerance study was completed in CDRI on 30 male healthy volunteers – 6 volunteers received placebo injections while 4 each received 20, 50, 100, 150, 200 and 300 mg α/β-Areether. Placebo as well as α/β-Areether was tolerated well and no tenderness, swelling or discomfort was experienced at the site of injection. All the laboratory investigations including haematological, biochemical, electrocardiographic and urinalysis parameters remained within normal limits in the post-drug phase.

The multiple dose tolerance study was completed at the Clinical Pharmacology Unit, Seth G.S. Medical College,Bombay. The injections were well tolerated in all volunteers with no untoward phenomenon at local injection site. All the vital parameters and investigations including haematology, biochemistry, ECG and urinalysis remained within normal limits in the post drug evaluation in all volunteers except a mild rise in transaminase levels, i.e. SGOT and SGPT in two volunteers who have been administered 150 mg arteether by im route on 3 consecutive days. These cases were carefully monitored for next 4 weeks with repeated clinical examination and liver function tests. In

both these volunteers the transaminase (SGOT/SGPT) reverted back to normal limits in the 3rd week. It has been reported that phase I testing of arteether possibly with β, began in 1992 and till date there are no clinical efficacy or toxicity data reported. The present day study for the first time confirms the **safety and tolerability of α/β-Areether injections** in healthy Indian subjects after single dose (20-300 mg) and multiple doses of arteether (100 and 150 mg) given on 3 consecutive days. The results indicate that α/β-Areether administration by intramuscular injection is safe, well tolerated and does not produce any adverse effect. Data of phase I clinical studies with α/β-Areether have been **accepted by the Drugs Controller (India)** and permission to undertake phase II clinical trials in patients of *P. falciparum* malaria has been granted. The trial was carried out at Ispat General Hospital, Rourkela (Orissa).

Asthana O P, Gaur S P S, Srivastava J S, Ghatak A, Gupta K C and Srimal R C 1994 Proc. Tropical Diseases: Molecular Biology & Control Strategies. P 318-324, Eds. S. Kumar, A.K. Sen, G.P. Dutta and R.N. Sharma. CSIR Publ. & Inform. Directorate, New Delhi.

Personal data of volunteers participating in arteether clinical trial

Type of study	Age year (means±SD)	Height cm (means±SD)	Weight kg (means±SD)
Single dose study (n=30)	19.78±3.88	158.66±4.82	44.57±5.89
Multiple dose study (n=20)	35.2±10.46	160.7±5.75	55.37±5.89

Phase II and III Clinical Trials with α/β-Artheer

The clinical data on phase II/III multicentric studies on arteether carried out against uncomplicated/complicated severe *P. falciparum* infections has been partly published by clinical groups (Table 50). In a preliminary study on the clinical efficacy of arteether in patients with uncomplicated *P. falciparum* infections, a dose of 150 mg daily

x 3 days (im), was reported to produce a cure rate of 85% (Asthana *et al.* 1996). The detailed report on first clinical trial (Phase-II) with α/β-artheer among 51 uncomplicated *P. falciparum* cases has been published by Mishra *et al.* (1995). The dose of 150 mg daily for 3 consecutive days by im injection, has shown very high curative activity in field trials. Complete parasite clearance was noted in 43% cases at 24 hr, in 80% cases at 48 hr and in 98% cases at 72 hr. Fever clearance time was 52.04 hr ± 27.09. Drug was well-tolerated, and 86% cure rate was recorded.

Thirty uncomplicated falciparum malaria cases completed Phase III clinical trials of the drug α/β-artheer in a malaria endemic tea garden of District Dibrugarh, Assam. Arteether was given intramuscularly in once a day dose of 150 mg for three consecutive days. The **cure rate was 100 percent** with mean fever and parasite clearance time of 42.4±17.5 and 37.6±13.6 hr, respectively. Recrudescence/reinfection rate was 6.7 percent (Mohapatra *et al.* 1996). Mohanty *et al.* (1997) have also reported the efficacy of the drug in severe *falciparum* malaria. Fifty patients with severe *falciparum* malaria were given intramuscular arteether, 150 mg, once daily on 3 consecutive days. The median fever clearance time was 72 hr (range 12-120 hr) and the median parasite clearance time was 2 d (range 1-4 day). Rapid **recovery from coma was observed in cerebral malaria** cases after a median of 18 h (range 6-72 hour). Two patients died and both had cerebral malaria and haemolytic jaundice. Recrudescence within 28 days was observed in 7 patients (Table 50).

Another phase-III clinical trial was conducted by Valecha *et al.* (1997) in 50 acute uncomplicated falciparum malaria cases from MRC, Delhi (Table 50). Their mean parasitaemia on day 0 was 0.65%. A standard dose of 150 mg i/m for three consecutive days resulted in mean parasite and fever clearance times of 19.94±6.87 and 37.81±21.67 hours respectively. Within 48h, 70% of the cases became afebrile and the peripheral blood smear was negative in 100% of the cases. The drug was well-tolerated. Three cases (6%) had recrudescence within 28 days. It was concluded that α/β-artheer is a safe effective and rapidly acting blood schizontocide. Post-marketing surveillance of arteether for the control and management of uncomplicated, severe complicated and cerebral malaria cases was carried out to monitor drug response. Arteether α/β has been **cleared by Drugs Controller Govt. of India, for hospital use in severe malaria cases**. The

drug is not so far approved for treatment of malaria in pregnant women due to its possible teratogenic action. Clinical trials to obtain permission for use of arteether in paediatric group are in progress (O.P. Asthana-unpublished data). Singh *et al.* 1998 reported a cure rate of 93.3% among 46 uncomplicated cases treated from Jabalpur and Mandla (Table 50).

The results of pooled data of Phase-III multicentric clinical trials with α/β-artheer (150 mg im x 3 doses) have been summarized by Dev *et al.* (1998) and Asthana *et al.* (2001a,b). Dev *et al.* (1998) had summarized the clinical trial (Phase-III) data from Assam based on 41 febrile malaria patients having moderate to acute *P. falciparum* parasitaemia (26 cases were categorized as uncomplicated and 14 cases as complicated *P. falciparum*). All these cases were treated with α/β-artheer and **100% cure rate** was reported in this study. The authors conclude that α/β-artheer also showed **gametocytocidal action** in two cases while in third case the gametocyte count persisted though, the viability of persisting gametocytes was not tested. In another report, 267 patients of uncomplicated *P. falciparum* from different centres were treated with arteether and the **cure rate recorded was 97%** (Asthana *et al.* 2001a). The third report summarized results of 211 complicated *P. falciparum* cases from different clinical centres who were treated with arteether and overall **cure rate was 93%** and mortality ranged between 4-8.5% at different centres (Asthana *et al.* 2001b). Out of 211 cases treated the mortality was recorded in 14 cases, out of which 10 cases died **prior to completing 3 dose therapy.**

Phase IV Clinical Trials with α/β-Artheer

The data on **post marketing surveillance** of α/β-artheer (30:70) has also been published by Asthana *et al.* (2002). In this study, 300 *P. falciparum* cases from States of Bihar, Gujarat, Madhya Pradesh, Maharashtra, Rajasthan and Uttar Pradesh, who were admitted to hospitals, nursing homes/clinics, were treated with 150 mg arteether injection (im) daily for three consecutive days, and the results showed a **cure rate of 98%.** This study on arteether further confirmed its safety and very high efficacy of the drug. According to a recent report 1.8 million malaria patients had been treated with α/β-artheer till June 2002 (Ramachandran 2002).

The **major advantage of α/β-arteether** injection **over β-arteether, artemether or artesunate** would be short 3 dose injection schedule with high efficacy against multidrug resistant *P. falciparum*/*P. vivax* infections etc. Since arteether monotherapy is very effective by itself, its combinations with mefloquine or sulpha-pyrimethamine synergistic combinations would be planned to further potentiate its curative effects and reduce malaria mortality.

ARTEMOTIL (β-ARTEETHER STATUS)

β arteether (Artemotil) injectible im formulation in **sesame oil (vehicle)** developed through the **collaborative efforts between WHO/TDR, WRAIR and the Dutch company Artecef BV**, had been approved on 22 May 2000 by Dutch registration authority for severe *P. falciparum* treatment (TDR News No.63, Oct. 2000, p-13). β arteether (Artemotil) 150 and 50 mg injections in sesame oil are proposed for hospital use for 3 day treatment of severe *P. falciparum* cases among children and adults. Cumulative dose of 11.2 mg/kg (**approximately 672 mg total in 3 days**) is recommended. Majority of drug β arteether is claimed to be metabolized to antimalarial metabolite dihydroartemisinin (Artenimol). However TDR News No. 63 Oct. 2000, p. 13 figure 2, does not show any significant plasma level of artenimol during 120 hr of treatment. It is surprising that β arteether injection (as sesame oil formulation) actually used for phase-II and III clinical trials was a **longer 5-day dose schedule** (3.2 mg/kg) on 1st day, followed by 1.6 mg/kg daily from day 2 to 5; total dose approximately 576 mg per 60 kg). Clinical trial data based on **5 day β arteether (in sesame oil formulation**) showed **lower cure rates (81-89%)** among uncomplicated *P. falciparum* cases (Looareesuwan *et al.* 2002). With 5-day β arteether regimen the cure rate **among cerebral malaria patients ranged between 73.2 - 79%** (Moyou-Somo *et al.* 2001, Thuma *et al.* 2000). Although no drug related toxicity/neurotoxicity was observed in patients, the safety of sesame oil formulation of β arteether is still debated and is a matter of serious concern (Li *et al.* 2002).

Pharmacokinetics Studies

The pharmacokinetic data on artemisinin derivatives summarized below show that arteether has obvious advantages over other derivatives.

A pharmacokinetic study on β arteether had been carried out in dogs after the i.m. administration of a 25 mg/kg dose of arteether dissolved in sterile sesame oil and benzyl alcohol (vehicle). Benakis *et al.* (1991) clearly established that β arteether had an elimination half life ($T_{½}$ β) of 27.95 ± 11.93 hr, which is considerably longer than that of dihydroartemisinin (2 hr), artesunate (45 min), artemether (6.5 hr) and artemisinin (1.6 hr). White (1994) had also reported that in human subjects, the elimination half-life of artemether was 11.1-13.2 hr after 10 mg/kg dose, artemisinin (oral or suppository) has 4.0-4.5 hr at the same dose, artesunate (i.v.) has 0.5-1.4 hr at 2.0-4.4 mg/kg dose and a half-life for arteeether at 3.6 mg/kg dose was reported to be longest (23.1 hr). In single and multiple dose studies with β arteether, the elimination half-life of the drug has been reported by Kager *et al.* (1994) to be 23.1 hr after a single 3.6 mg/kg dose, and after a multiple 5 dose schedule, the half-life was found to be 69.30 hr. Kager *et al.* (1994) finally concluded that β arteether may have the longest half-life of all the artemisinin derivatives studied so far.

The clinical trial carried out by this Institute involving 51 *P. falciparum* cases treated with 3 doses (150 mg each) of **α/β-arteether** (Mishra *et al.* 1995) showed extended **parasiticidal action of drug up to 96 hr** (i.e., 48 hr beyond the last arteether injection), which may be related to its **long half-life**. Moreover, a **3 dose schedule of α/β-arteether gave a parasitaemia clearance rate similar to that obtained by a 5-6 dose artemether or artesunate schedule** which can be explained on the basis of the **longer half-life of arteether (more than 24 hr)** in comparison to other derivatives such as **artemether** (11.1-13.2 hr), **artesunate** (0.5-1.4 hr) and **dihydroartemisinin** (2 hr). **Major metabolite of artetheer, artemether, artesunic acid/artesunate** *in vivo* **is dihydroartemisinin (DHA)** which is responsible for antimalarial activity of these derivatives (de Vries & Dien 1996, WHO 2000).

Comparison of Arteether with other Artemisinin Derivatives

The toxicity of arteether had been studied by several workers. At this institute, 7.5-30 mg/kg dose of α/β-arteether (3 injections per week for 4 weeks) given to the rhesus monkey and 2.5-10 mg/kg doses given to rats were **declared safe in regulatory toxicity trials** (Sethi *et al.* 1988), which justified human clinical trials with arteether. Subsequent single dose and multiple dose phase 1 safety/tolerance clinical studies in

human volunteers also had established the clinical safety of 100 and 150 mg doses given as i.m. injection once a day on 3 consecutive days in 20 volunteers and no undesirable side-effects related to the drug or oil vehicle were observed (Asthana *et al.* 1994).

Long term toxicity studies on β-arteether by Davidson (1994) in rats given 3 mg/kg/day dose for 28 days was reported to be safe. Brewer *et al.* (1994) also reported that 5-10 mg/kg dose of β-arteether given for 28 days to rats and dogs was safe and without any neurological effects. Recent neurotoxicological studies with arteether in rhesus monkeys (Petras *et al.* 1997) and with artemether and artesunate in MDR *P. falciparum* cases further confirmed the clinical safety of these drugs when used at moderate recommended doses. Looareesuwan *et al.* (1997) also reported no evidence of neurotoxicity in earlier clinical trials of aresunate and artemether involving more than 2500 patients treated in Bangkok hospital for tropical diseases. In more recent study by Van Vugt *et al.* 2000 and **WHO (2000), to-date no evidence of any neurotoxicity** in humans has been found. Since the LD_{50} of arteether is 1267.33 mg/kg in mice (Dutta & Tripathi 1996), the drug has a **higher safety margin than artemether and artesunate** with **LD_{50} doses of 263 and 475 mg/kg (i.m.), respectively** (Anon 1982a).

WHO (1994) report had given high priority for completion of preclinical/toxicological/clinical evaluation of β-arteether and artemether as potential antimalarials to be developed as life-saving drugs. **β-arteether (i.m.)** has been recently registered by WHO for clinical use in endemic areas. Hoever, the **safety of artemether is still debatable.** According to Brossi *et al.* (1988), β-arteether would be relatively safer compared to artemether because the latter can produce methanol after cleavage in biological system which could generate methanol toxicity. **Compared to arteether (LD_{50} 1267.33 mg/kg)**, the **artemether is 4-5 times more toxic due to its lower LD_{50} (263 mg/kg) dose in mice**. Clinically accepted treatment schedule for **artemether is 600 mg in 5 days**, while **arteether can be given as 450 mg total human dose in a 3 day** schedule to get the comparable or even better cure rate. Moreover, the arteether is more lipophilic and would have the possible advantage of its greater accumulation in brain tissue (Brossi *et al.* 1988). Our antimalarial studies against *P. cynomolgi* B clearly show that both β-arteether and α/β-arteether are equally curative (at 5 mg/kg x 3 doses), nevertheless we have preferred to develop α/β-arteether (30:70 mixture) because of its

ability to cure multi-resistant *P. yoelii nigeriensis* infection, ability to **cure *P. knowlesi*** infection – a cerebral malaria model, ***P. fragile* infection – a model comparable to *P. falciparum*,** and **because of its gametocytocidal potential** which can be **exploited to interrupt malaria transmission.** The drug has also successfully cleared regulatory animal pharmacology and sub-acute toxicity protocols acceptable by Drugs Controller General, India. The drug α/β-arteether has been found safe in clinical trials and has given consistent high cure rate in multicentric clinical efficacy trials carried out in different parts of the country.

Clinical Trials of α/β-Arteether

Mishra *et al.* (1995) had carried out first clinical efficacy study of alpha,beta-arteether in 51 patients suffering from *Plasmodium falciparum*.

Dose: 150 mg intramuscular x 3 consecutive days

Complete parasite clearance = 80% patients at 48 h

= 98% patients at 72 h

Mean PCT : 2 days (1-4 days)

FCT = 65% of the patients within 48 h

81% of the patients by 72 h

Mean FCT = 52.04±27.09 h

After 4 weeks of follow-up, seven patients were readmitted with *P. falciparum*. The authors could not decide whether these were due to reinfection or recrudescence.

Trans. R. Soc. Trop. Med. Hyg. 1995, 89, 299-301.

Valecha *et al.* (1997) Int. J. Clin. Pharmacol. Res. **17**, 11-15.

Valecha *et al.* (1997) had conducted phase III clinical trials with α/β-arteether among 50 patients suffering from acute uncomplicated *P. falciparum* malaria from **Delhi area**, during September to November 1995. The patients (age 27.2 yr average) were hospitalized with mean parasitaemia of 0.65%. Patients were treated with α/β-arteether (150 mg daily injection for 3 days).

Mean PCT = 19.94±6.87 h

FCT = 37.81±21.67 h

70% patients became afebrile within 48h.

Peripheral blood smears were negative for *P. falciparum* in 100% cases.

Recrudescence of *P. falciparum* was recorded in three cases (6%) within 28 days.

Conclusion: α/β-arteether is a safe, effective and rapidly acting antimalarial (Table 50).

Asthana *et al.* (2001b) J. Assoc. Physicians India, Dec. 49, 1155-60

Asthana *et al.* (2001b) carried out multicentric clinical trials for safety and efficacy evaluation of α/β-arteether in 211 complicated cases of P. falciparum malaria at four clinical centres (including **Bhilai, Guwahati, Jamshedpur and Rourkela** in India. In this trial fever clearance time (FCT) ranged between 24-168 h, parasite clearance time (PCT) 24-120 h, and overall mortality ranged between 4-8.5%. Out of 211 patients, only 14 patients expired during the study (10 of these patients expired within the first two days i.e. before completing three day arteether therapy. Overall cure rate among complicated *P. falciparum* cases was 93%. Tolerability to α/β-arteether was good and no adverse effects were reported. The conclusion of the study showed that α/β-arteether is a **fast-acting blood schizontocide for the control of drug resistant/complicated *P. falciparum* cases.**

Singh *et al.* (1998) Southeast Asian J. Trop. Med. Public Health, June 29, 225-227.

Singh *et al.* (1998) conducted the clinical trials on 46 patients suffering from uncomplicated *P. falciparum* malaria in **Jabalpur** and **Mandla districts**, who were treated with 150 mg α/β-arteether injection daily for three consecutive days. The patients showed rapid clearance of fever in all cases. The recrudescence of parasitaemia was reported in 6.7% within 28 days. The author reported that α/β-arteether is **fast acting blood schizontocide,** as well as **useful gametocytocidal drug for treatment of *P. falciparum*** (Table 50)**.**

Dev *et al.* (1998) conducted phase III multicentric clinical trials of α/β-arteether against complicated and uncomplicated *P. falciparum* cases from different malaria endemic pockets in India including **Assam** to study the efficacy as fast-acting blood schizontocide and its effect on sexual stages of *P. falciparum*. A total of 41 febrile patients (27 males + 14 females) having moderate to acute *P. falciparum* cases were included in trial. 26 uncomplicated and 14 complicated cases were treated with 150 mg

arteether injection daily x 3 days. The tribal patients included Karbi, Boro, Rabha, Lalung and tea tribes (age group 14-50 yrs). The results of clinical trials in **Tribal malaria** cases showed that 34 patients (83%) had cleared asexual parasites within 24 hours, six patients (15%) took 48 hours, **cure rate achieved was 100% in the North-East Tribal population.** Gametocytes numbers varied from 8 to 6400 per cubic mm of blood. Two of these *P. falciparum* cases **cleared gametocytes by day 14**. This study **shows gametocytocidal effect in two out of three *P. falciparum* cases**. The injectible α/β-arteether was found to be safe, well tolerated, highly effective schizontocidal, fast-acting convenient drug.

According to the investigators, α/β-arteether holds good promise for the management of acute *P. falciparum* cases. The intramuscular preparation ensures compliance in ethnic tribes for effective complicated *P. falciparum* treatment. The authors recorded similar observations in **tea tribal area of upper Assam (Mohapatra *et al.* 1996).**

The authors also quoted earlier trials (Mohapatra *et al.* 1996) in which α/β-arteether was used successfully. A total of 478 *P. falciparum* cases **(comprising of 267 uncomplicated plus 211 complicated/severe cases)** were successfully treated with α/β-arteether injectible. This antimalarial drug has been approved by Drugs Controller General of India for treatment of multidrug resistant *P. falciparum* cerebral malaria. The authors also quote that 22 clinical trials from 6 countries had been reviewed to compare the results with Indian Experience **(Asthana *et al.* 1997).**

Shukla *et al.* (2002) have reported the efficacy of α/β-arteether (30:70 mixture of enantiomers) in most severe 21 cases of complicated/comatose *Plasmodium falciparum* who were admitted to **Itarsi** (Govt. Civil Hospital). The patients were brought Hospital in a **state of unrousable coma** with moderate to high grade of fever, vomiting and convulsions in few cases followed by unconsciousness. A **diagnosis of cerebral malaria** was based on a brief history of fever, vomiting, convulsions, followed by unrousable coma, near normal cerebrospinal fluid picture, severe and profound anaemia and demonstration of asexual *P. falciparum* parasites in peripheral blood smears. In view of **prevailing R1 and R2 resistance to chloroquine in the tribal area**, α/β-arteether (an

ethyl ether of dihydroartemisinin) at a dose of 2.5 mg/kg body weight was administered by deep intramuscular injection once daily for 3 consecutive days.

Mohanty *et al.* (2000) have focused their extensive clinical study on the treatment options for treatment of cerebral malaria at **Ispat General Hospital, Rourkela** which is tertiary care centre for **complicated *P. falciparum* malaria cases from Western Orissa** and adjoining districts of **Madhya Pradesh, Bihar and West Bengal**, where malaria transmission is reported. During January 1995 to December 1997, the patients suspected to suffer from cerebral malaria were the diagnosis of *P. falciparum* is confirmed. Patients **fulfilling the WHO criteria of cerebral malaria** were given emergency therapy, and the confirmed cerebral malaria cases were hospitalized and treated with **new artemisninin based drug (α/β-arteether injection, 150 mg) on three consecutive days as specific treatment for cerebral malaria.** Besides, a parallel group of cerebral malaria cases were administered quinine (10 mg/kg body weight in 5% dextrose or dextrose saline over 4 hours every 8 hourly and then orally 8 hourly to complete 7 days quinine therapy (Mohanty *et al.* 2002).

The clinical details including neurological status, Glasgow Coma Score, fundus findings etc. were recorded. Patients recovery was regularly monitored for coma score or deterioration, fever clearance time, parasite clearance time, survival rate and mortality during the hospital admission. The results of clinical presentation of cerebral malaria cases presented in Table… show the clinical findings of 29 patients in α/β-arteether and 22 patients in quinine treatment group. The data on the outcome of two treatments α/β-arteether/quinine are summarized in Table 51. **Cerebral malaria patients treated** with **α/β-arteether showed quicker coma resolution** and **time to become conscious** was **24.33±14.33 hours** while **quinine treated cases took 38.87±22.45 hours to become conscious.** Fever clearance time and parasite clearance time in both arteether and quinine groups was practically similar. In addition the duration of fever or coma before hospitalization in both the groups was comparable. The **authors also reported that nine patients had developed features** of decerebration in the study; **five patients in α/β-arteether group and four of these patients recovered** and one of them died. In the **quinine group, out of 4 patients** treated with **quinine** had signs of **decerebration and all these four patients succumbed in spite of quinine therapy.**

In addition multiple complications were also observed in both treatment groups (see Table 52). Major complications were observed in quinine treated group (68.2% cases), whereas nearly 65.5% of arteether group reported the complication.

The peripheral parasitaemia records showed that by 48 hours of treatment, 82.7% patients in arteether group and 86.4% patients in quinine group had cleared the parasitaemia.

Mohanty *et al.* (2000) finally concluded that α/β-arteether (3 day intramuscular therapy) of **cerebral malaria** cases showed **faster coma recovery (p<0.002)** and **fewer deaths (6.67%) in arteether** treated cases while **mortality was higher (27.27%, <0.02) in seven day quinine treated cases**. Finally, no serious side-effects were observed in hospitalized arteether treated group.

Summary of Clinical Studies on α/β-Arteether for Treatment of Uncomplicated *P. falciparum* Malaria Conducted in India

A comprehensive review on multicentric Phase III clinical trials on new fast-acting antimalarial α/β-arteether efficacy in clinical cases of uncomplicated *Plasmodium falciparum* as reported by **seven clinical study groups all over India** has been published by **O.P. Asthana**, J.S. Srivastava, V.P. Kamboj, Neena Valecha, V.P. Sharma, S. Gupta, T.K. Pande, K.A. Vishwanathan, K.M. Mahapatra, N.C. Nayak, P.K. Mahapatra, J. Mahanta, V.K. Srivastava, Vasdev, N. Singh, M.M. Shukla,A.B. Balsara, S.K. Mishra, S.K. Satpathy, S. Mohanty and B. Dash (2001). The clinical trials on α/β-arteether had been independently conducted on two hundred and sixty seven *P. falciparum* (uncomplicated malaria) were conducted at the following malaria research centres in India which included:

1. CSIR-Central Drug Research Institute, **Lucknow**
2. Malaria Research Centre, **New Delhi**
3. Lady Hardinge Medical College, **New Delhi**
4. Jawahar Lal Nehru Hospital, **Bhilai**
5. Central Reserve Police Force, Base Hospital, **Guwahati**
6. Regional Malaria Research Centre (ICMR), Dibrugarh, **Assam**
7. Malaria Research Centre, Sonapur, **Assam**
8. Malaria Research Centre/Government Medical College, **Jabalpur**

9. Tata Main Hospital, **Jamshedpur**
10. Ispat General Hospital, **Rourkela**

The drug **α/β-arteether was first published** by Dutta, Bajpai and Vishwakarma (1987) as arteether and the role of α/β-arteether 30:70 anomers in control of rodent malaria was reported by **Dutta *et al.* (1998). Patent on α/β-arteether 30:70 was filed in 1990 (Indian Patent).** The drug was jointly produced in **1987 by CSIR-Central Drug Research Institute and Central Institute of Medicinal and Aromatic Plants, Lucknow (India).** The work on the production of arteether from *Artemisia annua* was initiated under the Direction of **Prof. Akhatar Hussain** (Former Director, CIMAP, Lucknow) under the guidance of Dr. S.P. Popli (Emeritus Scientist). Preclinical development studies and Phase I clinical studies were carried out at the CSIR-Central Drug Research Institute, Lucknow.

Asthana *et al.* (2001) have summarized the data on α/β-arteether cure rates in ***P. falciparum* uncomplicated cases** ranged from **93-100% among seven clinical trial centres in India** (Table 53) which are acceptable as very high cure rates as per WHO standards. The fever clearance and parasite clearance time following α/β-arteether three day therapy, is presented in Table 54. The parasite clearance was very fast following arteether 3 dose IM treatment (total dose 450 mg in 3 injections). The pre- and post-treatment haematological and biochemical findings are presented in Table 55.

This **open multicentric trial were carried out at seven centres** in different parts of India known for **high endemicity of *P. falciparum* malaria**, **confirmed the efficacy (97%) of three day schedule of arteether**. The present study also showed lower parasite reappearance rate (3%) than the earlier phase II study showing parasite reappearance of 14%. No adverse drug reactions were reported during the phase III multicentric trial. Arteether has been **cleared for marketing in India** with recommendations from Drugs Controller General (India) to conduct post marketing surveillance (Phase IV study) (This data has already been published).

1.3 ADVANTAGES OF NEW CDRI FORMULATION OF β-ARTEETHER [Prepared in Neutralized, Double Refined Arachis (groundnut/pea-nut oil)]

1. **Intramuscular injection of β-arteether formulation in neutral groundnut oil does not cause toxic or high levels of drug accumulation** in the plasma after triple dose IM injection.
2. IM formulation of β-arteether allows **rapid drug absorption** from the muscle site to provide effective plasma drug level for control of multidrug resistant malaria infection.
3. β-arteether formulated in groundnut oil, shows **adequate plasma clearance** after 3-5 dose IM injection schedule, which does not cause any neurotoxicity because there is no delayed drug absorption from the IM site.
4. IM formulation of β-arteether in double refined neutral oil **does not cause cumulative toxicity reported with sesame oil formulation of β-arteether** which showed **7.5 fold accumulated drug levels in plasma** causing delayed toxicity.
5. β-Arteether in neutral ground-nut (peanut) oil shows nearly complete absorption from muscle following three IM doses to provide **very effective blood levels and drug bioavailability as well as DHA metabolite levels, sufficient to produce curative action against highly MDR *P. yoelii nigeriensis*** infection in Swiss mice.
6. β-Arteether in neutral groundnut oil formulation is **devoid of any anorectic toxicity** and neurotoxicity because its absorption and drug metabolism is faster.
7. β-Arteether in **neutral groundnut oil formulation** did not cause delayed cumulative toxicity/mortality, as had been **reported with sesame oil formulation of β-arteether (i.e. Artemotil).**
8. Neutral groundnut oil formulation of β-arteether has improved safety over cremophore/sesame oil/1:9 dimethylacetamide (DMAC)/sesame oil formulations.
9. In acute phase infections and pregnancy, the first 3 doses of the β-arteether need to be given at 12 hourly intervals followed by two additional doses at 24 h interval, to control high level of parasitaemia/acute phase infection, which can be life-threatening, (total 5 dose schedule at 0, 12, 24, 48, 72 hours).

10. The IM formulation provides uniform drug absorption as shown by consistent cure rates at curative dose.
11. Pharmacokinetic data provided by earlier investigators show that **arteether reaches peak plasma level at 6 h** and has short **elimination half-life (7.1-13.2 h),** and complete elimination of the drug and its **antimalarial metabolite (dihydroartemisinin) from the body in 16-18 h after IM/oral dosing** is documented (Zhao *et al.* 1988, 1989; Karbwang *et al.* 1998). It is obvious that rapid drug clearance of available formulations in around 16 h in clinical cases as reported earlier, would result in recrudescence unless higher dose of drug is administered.

The investigators had developed "**double refined-neutralized groundnut oil" (Dutta *et al.* 1987 Indian J. Parasitol. 11(2), 253-257; Dutta *et al.* 1989b Pharmacol. Res.; Tripathi *et al.* 1991 Am. J. Trop. Med. Hyg.) primarily as a vehicle/solvent in which artemisinin/arteether α/β, as well as α or β arteether could be dissolved, prior to injecting** these drugs in animal models since these drugs could not be dissolved in water. It was **not visualized at that time** that the **solvent neutralized Arachis oil (groundnut oil) could contribute to improved antimalarial drug action, drug disposition or contribute to the drug efficacy, reduction of toxicity**, favourably **influence pharmacokinetic**, or **stability** of the drug etc. or improve the shelf-life of these drugs and thus contribute to **novelty of neutralized oil vehicle**.

The investigators in a preliminary report on preparation of "neutralized groundnut oil" vehicle (Dutta *et al.* 1987 Indian J. Parasitol. 11(2), 253-257) which was used to prepare artemisinin injection (in oil) and a dose of 100 mg/kg x 7 days IM) cured 5/8 mice (62.5%) infected with sensitive *P. berghei*. Besides α/β-arteether 30:70 mixture of anomers in oil, was able to cure sensitive *P. berghei* at 5 mg/kgx3 IM doses. Further studies showed that α/β-arteether 30:70 (soluble in groundnut oil) was curative against **highly multidrug resistant strain of *P. yoelii nigeriensis,* at 5 mg/kg x 3 IM doses** (Dutta *et al.* 1989b Pharmacol. Res. 21, 415-419). Since artemisinin and α/β arteether (30:70) are insoluble in water, therefore, groundnut oil vehicle was used to dissolve these drugs for preparing **intramuscular injections of α-arteether, β-arteether and α/β-arteether (Tripathi *et al.* 1991).**

Major advantage of β-arteether IM formulation in neutral groundnut oil is that this formulation provides improved and complete drug absorption from the IM site, drug safety/devoid of animal toxicity, and the drug does not accumulate in plasma when given as three consecutive doses.

1.4 ARTEMOTIL (β-ARTEETHER)

Comparison of β-Arteether (Artemotil) Injectible IM Formulation in Sesame Oil (Vehicle) Developed by WHO/TDR

β-arteether (Artemotil) injectible im formulation in sesame oil (vehicle) developed through the collaborative efforts between WHO/TDR, WRAIR and the Dutch company Artecef BV, had been approved on 22 May 2000 by Dutch registration authority for severe P. *falciparum* treatment (TDR News No. 63, Oct. 2000, p.- 13). **β-Arteether (Artemotil)** 150 and 50 mg injections in sesame oil are proposed for hospital **use for 3 day treatment of severe P. *falciparum* cases** among adults and children. Cumulative dose of 11.2 mg/ kg (approximately **672 mg total dose in 3 days)** is recommended for P. falciparum IM treatment. Majority of drug β-arteether is claimed to be metabolised to antimalarial metabolite dihydroartemisinin (Artenimol). However TDR News No. 63 Oct 2000 p.13 figure 2, does not show any significant plasma level of artenimol during 120 hr of treatment.

Clinical antimalarial efficacy of intramuscular Artemotil vs α/β-arteether

P. falciparum: clinical antimalarial efficacy trial data following treatment of P. falciparum cases with β-Artemotil (β-arteether formulation in sesame oil, **5 dose injection)** vs α/β-arteether (30:70) (formulation in neutral groundnut oil, **3 dose injection).** Clinical trials in which α/β 30:70 arteether formulation in neutral groundnut oil was used, was reported to cure P. falciparum with **450 mg total dose over 3-day of treatment (Mahapatra et al. 1996; Valecha et al. 1997)**, as described below:

1.	**β Arteether trial** Dose: 576 mg total dose (in 5 days) P. falciparum cure rate 81-83% Looareesuwan et al. (2002)	**α/β Arteether Trial*** Dose: 450 mg total dose (in 3 days) P. falciparum cure rate 100% Mahapatra et al. (1996)
2.	**β Arteether trial** Dose: 576 mg total dose (in 5 days) P. falciparum cure rate 73.2-85% Moyou-Somo et al. (2001), Thuma et al. (2000)	**α/β Arteether Trial*** Dose: 450 mg total dose (in 3 days) P. falciparum, cure rate 95% Valecha et al. (1997)

*Data on cure rate with **3 dose α/β Arteether** was better as compared to **Artemotil 5 dose injection**

It is surprising that **β-arteether** (**Artemotil**) injection (as sesame oil formulation) actually used for phase-II and III clinical trials was a **longer 5-day dose** schedule (3.2 mg/kg) on 1st day, followed by 1.6 mg/kg daily from day 2 to 5; total dose approximately 576 mg per 60 kg). Clinical trial data based on **5 day β-arteether** (in sesame oil formulation) showed **lower cure rates (81-83%)** among uncomplicated P. *falciparum* cases (Looareesuwan et al. 2002). **With 5-day β-arteether** regimen the cure rate among **cerebral malaria patients ranged between 73.2-85%** (Moyou-Somo et al. 2001, Thuma et al. 2000). Although no drug related toxicity/neurotoxicity was observed in patients, the **safety of sesame oil formulation of β-arteether is still debated** and is a matter of serious concern **(Li et al. 2002).**

Pharmacokinetics Studies

The pharmacokinetic data on artemisinin derivatives summarized below show that arteether has obvious advantages over other derivatives.

A pharmacokinetic study on β-Arteether had been carried out in dogs after the i.m. administration of a 25 mg/kg dose of arteether dissolved in sterile sesame oil and benzyl alcohol (vehicle). Benakis et al. (1991) had clearly established that β-Arteether had an elimination half-life ($T_{1/2}$ β) of 27.95 ± 11.93 hr, which is considerably longer than that of dihydroartemisinin (2 hr), artesunate (45 min), artemether (6.5 hr) and

artemisinin (1.6 hr). White (1994) had also reported that in human subjects, the elimination half-life of artemether was 11.1 - 13.2 hr after 10 mg/kg dose, artemisinin (oral or suppository) has 4.0-4.5 hr at the same dose, artesunate (i.v.) has 0.5-1.4 hr at 2.0-4.4 mg/kg dose and a half-life for arteether at 3.6 mg/kg dose was reported to be longest (23.1 hr). In single and multiple dose studies with β-Arteether, the elimination half-life of the drug has been reported by Kager et al. (1994) to be 23.1 hr after a single 3.6 mg/kg dose, and after a multiple 5 dose schedule, the half-life was found to be 69.30 hr. Kager et al. (1994) finally concluded that β-Arteether may have the longest half-life of all the artemisinin derivatives studied so far.

The clinical trial carried out by this Institute involving 51 P. *falciparum* cases treated with 3 doses (150 mg each) of α/β-arteether (Mishra et al. 1995) showed **extended parasiticidal action of drug up to 96 hr** (i. e., 48 hr beyond the last arteether injection), which may be related to its long half-life. Morever, a **3 dose schedule of α/β-arteether** gave a parasitaemia clearance rate similar to that obtained by a **5-6 dose artemether or artesunate** schedule which can be explained on the basis of the longer half-life of arteether (more than 24 hr) in comparison to other derivatives such as artemether (11.1-13.2 hr), artesunate (0.5-1.4 hr) and dihydroartemisinin (2 hr). **Major metabolite of arteether, artemether, artesunic acid/artesunate *in vivo* is dihydroartemisinin (DHA)** which is responsible for antimalarial activity of these derivatives (de Vries & Dien 1996, WHO 2000).

β-ARTEMOTIL

(Sesame oil injectible formulation of β-Arteether)

(Betamotil/Artecef[R] Artecef BV Maarsen, The Netherlands, β-Artanomer developed by WHO marketed)

β-Arteether Injectible

According to Pareek *et al.* (2006) *Am. J. Trop. Med. Hyg*. **75**,139-140:

1. Drug **α/β-arteether (30:70)** mixture of epimers patented (1990) by the Central Drug Research Institute is named as **E-mal (150 mg α/β-arteether** formulated in sterile 2 ml double refined neutralized Arachis oil (ground nut oil) was first marketed in 1997 by M/s Themis Medicare Ltd., Vapi, Gujarat, India. This is **first intramuscular arteether drug approved by Drugs Controller General of India** for treatment of **severe/complicated/cerebral malaria cases** which are hospitalized and used as **emergency medicine** (3 intramuscular injections are administered on three consecutive days). The drug is specifically **used in chloroquine/ quinine/ mefloquine resistant, complicated malaria cases which do not respond to quinine (7 x day) therapy.** According to **Pareek *et al.* (2006) the α/β-arteether, available in India**, was used in the study which was **designated as Rapither** AB; M/s Ipca Laboratories Ltd, Mumbai, India.
2. The drug β-Arteether (IM injections) **made outside India** designated as – **ARTECEF** is marketed in The Netherlands (Artecef BV, Maarsen). **Artecef formulation made in Sesame oil** has been registered. Drug β-arteether made **outside India is also named as Artemotil** and registered by Karger, P.A. 2003, Ned Tijdschr Geneeskd, 147,291-295. The **Artemotil** – formulation is also made in **sesame oil**.
3. According to Pareek *et al.* (2006), the drug β-arteether available in India and (designated by **IPCA as Betamotil injection**) was used in their clinical study. The oil used for preparing the formulation (**Betamotil**), **was not disclosed by Pareek,** A, Nandy, A, Kochar D, Patel K H, Mishra S K and Mathur P C 2006 *Am. J. Trop. Med. Hyg*. **75**, 139-142. Whether **Betamotil** was formulated in

sesame oil or in **neutral refined Arachis oil** (groundnut oil) **was not indicated in the publication.**

Pareek *et al.* (2006) conducted the clinical trials to monitor efficacy and safety of β-arteether (Betamotil) and α/β-arteether (Rapither). Several earlier studies on the clinical efficacy of α/β-arteether (Asthana *et al.* 2001; Singh *et al.* 1998) and efficacy of β-arteether vs quinine (Moyou-Somo *et al.* 2001) and trial of artemotil (β-arteether) in cases of cerebral malaria (Thuma *et al.* 2000), had been published. The authors further carried out the safety and efficacy of β-arteether (Betamotil) and α/β-arteether (Rapither) in Kolkata Hospital, Bikaner, Baroda, Rourkela and Gwalior because of the endemicity of *P. falciparum* in these areas. A total of 136 adult *P. falciparum* cases participated in the trial. **Malaria cases with severe complications including cerebral malaria or renal failure were excluded from the trial.** Following three intramuscular injections with β-arteether and α/β-arteether, the patients were followed for clearance of parasitaemia. A total of 70 cases treated with β-arteether showed a cure rate of 97.1%, while 68 cases treated with α/β-arteether also showed 97.1% cure rate. One patient in β-arteether group showed recrudescence while one patient treated with α/β-arteether died.

Pareek *et al.* (2006) have **argued whether three day treatment** schedule with β-arteether or α/β-arteether (injection) would be sufficient for complete cure of malaria or not. The authors propose that new trials to assess the justification of three day intramuscular treatment with β-arteether would be planned since **WHO recommends at least seven dose monotherapy with artemisinins alone** to achieve complete cure and also to protect the drug against development of resistance to artemisinins. The β-arteether formulations (if prepared in sesame oil as in Artemotil/Artecef) had been reported earlier to **exhibit lower order of activity in clinical trials (Moyou-Somo *et al.* 2001 and Thuma *et al.* 2000). Pharmacokinetic studies** with α/β-arteether (E.mal) carried out at CDRI (Sabari *et al.* 2005) had suggested a possible **synergistic antimalarial action of α and β (30:70 mixture) of anomers.** α/β-arteether formulated in refined/neutralized Arachis oil shows a **high plasma peak of α-arteether** within 2 h of injection, whereas the β-arteether component shows prolonged high plasma peak from 4 hour to 28 hours following intramuscular injection.

Artemotil (β-arteether)

Arteether β is the β-anomer of the ethyl ether of dihydroartemisinin. The compound synthesized originally in China by Li *et al.* (1981, Acta Pharmaceutica Sinica 16, 429-439) from artemisinin (isolated from plant *Artemisia annua*) was converted to dihydroartemisinin (artenimol = DHA) which was subjected to etherification in boron trifluoride etherate, and final step involved was the separation of α and β anomers (Brossi *et al.* 1988, J. Med. Chem. 31, 645-650). The UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases, and the Steering Committee on CHEMAL, decided in 1985 to develop a derivative of artemisinin, namely β-arteether (injectible) as fast-acting blood schizontocide for severe complicated *Plasmodium falciparum* infections.

Brossi *et al.* (1988, J. Med. Chem. 31, 645-650) had established *in vitro* antimalarial activity of β-arteether against *Plasmodium falciparum.* They also reported high antimalarial activity in terms of ED_{90} values of β-arteether formulation (prepared by dissolving the compound suspended by sonication with 10 ml of oil and 0.1% solution of Tween 80) and administered to mice by subcutaneous injection for 4 consecutive days, starting 3 h after infection of mice with battery of drug resistant lines of *P. berghei*. The *in vivo* activity of β-arteether was 2-4 times greater than that of QHS when ED_{90} values were compared by subcutaneous route. For further preclinical toxicity studies, Brossi *et al.* (1988) had proposed to develop a suitable **sesame oil formulation** of β-arteether (designated also as arteether) for intramuscular injection.

Since arteether is strongly lipophilic and practically insoluble in water, therefore, **sesame oil had been used extensively as a vehicle** for dissolving β-arteether for preclinical studies (Brossi *et al.* 1988 J. Med. Chem. 31, 645-650) and clinical efficacy studies were conducted by several workers following drug administration by intramuscular injections (Looareesuwan *et al.* (2002, Br. J. Clin. Pharmacol. 53, 492-500), Moyou-Somo *et al.* (2001, Am. J. Trop. Med. Hyg. 64, 229-232), Thuma *et al.* (2000, Am. J. Trop. Med. Hyg. 62, 524-529), Davidson Jr. (1994, Trans. Roy. Soc. Trop. Med. Hyg. 88, Suppl. 1, 51-52) and Karger *et al.* (1994, Trans. Roy. Soc. Trop. Med. Hyg. 88, Suppl. 1, 52-54).

The WHO (1985) had decided to develop β-arteether as fast acting blood schizontocide because of its lipophilic character, its accumulation in brain tissue in cerebral malaria cases. β-arteether was prepared in **sesame oil vehicle** and its evaluation in Swiss mice infected with *P. berghei* was carried out by **subcutaneous route** as a single dose and its 50% curative dose (CD_{50}) was reported to be 55 mg/kg (32-78 mg/kg). The ED_{50} in *Aotus* monkey infected with chloroquine resistant *P. falciparum* was 11.8 mg/kg (6.5 – 21.3 mg/kg) while its curative dose in *Aotus* was 48 mg/kg (total dose administered in 3 equal IM doses) (Shmuklarsky *et al.* 1993. Am. J. Trop. Med. Hyg. 48, 377-384). The major activity of the drug β-arteether was reported to be due to its metabolite-dihydroartemisinin (DHA) (Melendez *et al.* 1991. J. Pharm. Sci. 80, 132-138).

In a recent study, β-arteether sesame oil formulation given intramuscularly in a daily dose for three consecutive days (treatment commencing on day 3 of infection) was reported to show 50% curative dose (CD_{50}) = 34.1 (12.3-66.9) mg/kg in female mice infected with *P. berghei* (Li *et al.* 2002, Am. J. Trop. Med. Hyg. 66, 516-525), while a **new formulation of β-arteether prepared by dissoliving drug in Cremophore EL (a polyethylene oxide modified castor oil used as a solubilizing agent)developed by Li *et al.* (2002) was considered superior** as the CD_{50} dose of β-arteether-Cremophore EL was much lower (=14.2 (6.8-21.5) mg/kg) indicating **improved antimalarial activity** against *P. berghei.* This clearly established that β-arteether-Cremophore EL formulation containing 1:2 Cremophore/saline solution, showed significantly higher efficacy of drug than the sesame oil formulation when three daily multiple injections were given to mice. The safety of Cremophore EL proposed as vehicle (for β-arteetber IM injection) is still debatable. Cremophore EL itself was reported to be toxic and produced vasodilation, labored breathing, and hypotension when given i.v. (WHO Patent 99-12570, 1999). Besides, i.v. bolus administration of Cremophor EL had been found to produce hypersensitivity reactions (Weiss *et al.* 1990, J. Clin. Oncol. 8, 1263-1268; Rowinsky *et al.* 1993, Semin. Oncol. 20, Suppl. 3, 1-15).

Pharmacokinetic/Pharmacodynamic Comparison of both α/β-Arteether (30:70 isomers) and Artemotil

1. Single IM α/β (30:70 isomers) 150 mg (in 2 ml) was injected IM.
2. The extent of metabolism of arteether to DHA was negligibly low (≈5%) to have any therapeutic significance.
3. Maximum plasma conc. of α-arteether 4.77 ± 1.21 h, β-arteether 6.96±1.62 h.
4. Elimination half-lives of α : 13.24±1.08 h

   β : 30.17±2.4 h
5. Volume of distribution (Vz/F) of β-isomer was 3 times higher than α-isomer.
6. Four strains of Pf showed similar MIC for both α and β-isomers. Highest MIC of 8 ng/ml was selected for estimating Pk/PD descriptors.
7. α and β isomers of arteether appeared to complement each other pharmacokinetically:

   α-isomer providing rapid and higher plasma levels resulting in immediate reduction in % parasitaemia, while β-isomer with its longer biological half-life, MRT and sustained plasma levels, maintaining activity for longer periods.
8. Metabolic conversion of arteether to DHA was minimal so as to have any therapeutic or toxic significance.

Artemotil (β-arteether) formulated in sesame oil has several disadvantages

1. The total β-arteether dose recommended for full treatment course is 576 mg whereas the recommended 3-day dose would require 480 mg β-arteether which would reduce the potential toxicity.
2. The published reports show that day 0 dose of β-arteether ranges from 3.2 mg/kg-6.4 mg/kg while the dose from day 1 to day 4 is generally low (1.6 mg/kg/day x 4 days) which is responsible for partial cure rates ranging from 23% to 85% with total course dose of 576 mg. Even highest dose of 768 mg (in 5 day schedule) produced only 48% cure among several *P. falciparum* cases. It is obvious that 3.2 mg/kg (double dose) on 1st day followed by low dose β-arteether 1.6 mg/kg for four consecutive days, was only partially curative.
3. Five day extended IM β-arteether therapy would pose problems for compliance.

ARTEMOTIL

Looareesuwan *et al.* (2002) had earlier conducted extensive clinical evaluation of intramuscular **Artemotil** (β-arteether). The authors reported that in 1985, the World Health Organization (WHO) Steering Committee for Scientific Working Group on the Chemotherapy of Malaria had decided to evaluate artemisinin derivative – **Artemotil** (previously named **β-arteether**). **Artemotil was considered superior to sodium artesunate as it would be much more stable than Artesunate, its biochemical breakdown would not give methanol, as does artemether,** and it had the advantage of completely dissolving in oil for parenteral administration. Looareesuwan *et al.* (2002) also recorded that the Chinese investigators had already developed the process for the synthesis of Artemotil and also had confirmed its antimalarial activity (Gu *et al.* 1980; China Cooperative Research Group on Qinghaosu, 1982). WHO Group wanted to register the Artemotil in a European country for world-wide use in malaria endemic countries world-wide and it could be made available at low cost for public use in developing countries through NGOs for malaria control. **Preclinical development** was jointly conducted by **WHO** and coordinated by **Walter Reed Army Institute of Research** (WRAIR, Washington) (Davidson, Jr. 1994; Shmuklarsky *et al.* 1993; Brossi *et al.* 1988). Looareesuwan *et al.* (2002) reported that for clinical development, a **collaborative project was initiated in the year 1991 between WHO and Dutch Company ARTECEF BV** (Maarssen, The Netherlands) and the drug β-arteether was registered and the drug received marketing approval by the Dutch Company under the name of **ARTECEF® 50** or **Artecef 150®** containing 50 and 150 mg artemotil/ml respectively; **the drug was formulated in sesame oil for intramuscular injection**. Artemotil had been developed as an IM formulation (in Sesame oil) for the treatment of severe **(cerebral malaria in children and adolescents). Artecef® is registered** for emergency treatment of severe malaria and covered by European Registration and sold at low cost.

Pharmacokinetics of intramuscular (IM) Artemotil (formulated in Sesame oil) was studied by Looareesuwan *et al.* (2002) and the **plasma concentration profiles of artemotil indicated an irregular and variable rate of absorption** from the muscle after im injection. Moreover a rather **late onset of parasite clearance from Thai patients**

with acute uncomplicated *P. falciparum* malaria, was reported to be associated with **delayed absorption of drug and/or very low initial artemotil plasma concentrations**. Pharmacokinetic and pharmacodynamic evaluation in acute *P. falciparum* cases suggested a close relationship between the rate of parasite clearance and exposure to artemotil during the first 2 days of initiating treatment. The authors concluded that for **treatment of acute *P. falciparum*, artemotil (in sesame oil) exhibited a slow rate of absorption as compared to another drug artemether (formulated in pea-nut oil)**. The investigators **(Looreeesuwan *et al.* 2002) finally concluded that artemotil (in sesame oil) is more slowly absorbed from the im injection site** as compared to im injection of artemether (a pea-nut oil formulation supplied by injection artemether, **Kunming Factory, P.R. China**).

In a recent Cochrane Collaboration Review on intramuscular arteether for treating severe malaria, Afolabi and Okoromah (2009) have discussed the issue that there are **two forms of arteether**. They have reported that arteether is an ethyl ether derivative of artemisinin, soluble in oil, with a **long elimination half-life (greater than 20 hours**, which is more stable than the other artemisinin compounds **(WHO, 2001**). **Afolabi and Okoromah (2009)** further reported that **Artemotil (previously known as β-arteether) and α/β-arteether (E.Mal)** produced by Central Drug Research Institute, Lucknow are two available formulations.

Artemotil according to above reviewers was **registered in the Netherlands in May, 2000 (Lugt, 2002)** and its use was restricted to children and adolescents under the age of 16 because of its **possible effect on heart** (as shown in some **abnormal changes in ECG** in some of the artemotil clinical trials (WHO, 2002).

α/β-arteether was registered in India in January, 1997 for use in both children and adults with severe malaria (WHO, 2002).

The only differences in their formulations as reported by Reviewers are that the artemotil contains the beta form of arteether and is **dissolved in sesame oil** (Lugt, 2002; WHO, 2002), whereas the α/β-arteether (30:70) contains both α and β forms which are **dissolved in ground-nut oil (Mohanty, 1997 and Dutta *et al.* 1987, 1990).** Afolabi and Okoromah (2009) also reported that arteether was more effective than other artemisinin

derivatives because of its **oil solubility, longer half-life** and **increased chemical stability.**

In another study on the efficacy and safety of **α/β-arteether (30:70 combination** of epimers) which had been jointly developed by the **CSIR-CDRI and CIMAP Laboratories** and was registered in 1997 as E.mal (Themis Medicare Ltd., Vapi, Gujarat) available in India, had been **designated by Pareek *et al.* (2006) as RAPITHER (produced by M/s IPCA Laboratories Ltd., Mumbai).** The antimalarial activity of α/β-arteether was compared by Pareek *et al.* (2006) for clinical efficacy with β-arteether which was registered as **Artecef®** by Artcef BV, Maarsen in Netherland.

Pareek *et al.* (2006) compared the clinical efficacy of both β-arteether as well as α/β-arteether. The drugs were administered IM (150 mg dose once a day for three consecutive days in hospitalized patients (70 *P. falciparum* cases were treated with β-arteether, and 68 patients were treated with α/β-arteether group). The **cure rates were 97.14% for β-arteether and 97.01% for α/β-arteether treatment**. The authors concluded that both arteether formulations were safe and efficacious. Both the arteether formulations were effective in rapidly clearing parasitaemia. Finally, the authors observed one case of recrudescence with β-arteether and one case treated with α/β-arteether died during the trial on second day after receiving 2 doses of α/β-arteether. This study was supported by IPCA Laboratories Ltd. (Mumbai).

1.5.1 DISCOVERY OF DIHYDROARTEMISININ (ARTENIMOL/DIHYDRO-QUINGHAOSU)

(A Broad Spectrum Antimalarial Drug and as Antimalarial Metabolite)

Discovery of antimalarial potential of dihydroartemisinin was **first reported in the International Patent Application filed on 12th March, 1999 by Council of Scientific & Industrial Research, New Delhi 110 001 through CSIR-Central Drug Research Institute, Lucknow and Central Institute of Medicinal and Aromatic Plants, Lucknow**, the complete specifications of this patent was published on 13th September, 2000 (under European Patent Application (EP 1 034 794 A1) and also in U.S. Patent No. **To date sixteen countries have granted DHA patent (Dutta *et al.* 0205NF1998) (Table 56).**

1. Dihydroartemisinin (DHA)-an artemisinin derivative is a unique chemical entity which acts both as a **potential antimalarial drug**, **and also acts as powerful antimalarial metabolite produced by** various artemisinin derivatives (such as **α/β-arteether, β-arteether (Artemotil), Artesunate, Artemether, Artelinate** etc.). Treatment with **all these artemisinin derivatives** used for malaria therapy, **get metabolized into DHA** in the body and **produce dual antimalarial activity by transformation into metabolite DHA.**
2. DHA shows **unique antimalarial profile** as shown by a fast acting blood schizontocidal activity against **highly multidrug resistant** rodent malaria parasite. (***Plasmodium yoelii nigeriensis***).
3. DHA completely protects mice against 100 per cent fatal infections with *P. yoelii nigeriensis* and can be used to provide **100 per cent protection against fatal malaria infections.**
4. DHA is unique as it can provide complete protection against uncomplicated, severe complicated/cerebral malaria.
5. DHA is unique as it preferentially **acumilates 200 to 300 times higher concentration in the infected RBC.**
6. DHA formulation developed with neutralized groundnut oil has been reported to be valuable for **intramuscular injection, intrarectal** administration (as rectal **suppository)** and has been reported to be safe when administered even by

orogatric route (oral administration). Orally administered DHA formulation does not produce any adverse gastric effects.

7. DHA administration by intramuscular, intrarectal and oral route produces fast acting curative blood schizontocidal action against highly multidrug resistant malaria infection/fatal *P. yoelii nigeriensis* infection in Swiss mice as well as curative against drug resistant ***P. falciparum*** **and** ***P. vivax***.
8. DHA also exerts **transmission blocking/gametocytocidal action, without producing methaemoglobinaemia.**
9. DHA produce **dual action as drug as well as metabolite** produced by other artemisinin derivatives (e.g. arteunate, arteether, artemether and artelinate etc.). Metabolite DHA as claimed in National/International Patent Documents granted in 1999/2000 was based on the earlier **EMR-Emeritus Scientists Project Report of the author conducted at the Central Drug Research Institute, Lucknow** and submitted to Council of Scientific & Industrial Research, New Delhi in 1998. **Emeritus Scientist (CSIR) Research Project No. 21(9297)/94) EMR-II** (4 years 1.4.1994-31.3.1998) (EMR II Project Report **submitted on 31 March, 1998) priority date of patents IPRs filed on DHA is 12 Feb. 1998).**

Chemistry, formulation and antimalarial evaluation of dihydroartemisinin (DHA)

a) Preparation of dihydroartemisinin from artemisinin

Artemisinin (10 g) in 250 ml of methanol, was cooled in an ice bath to 0°C. To the stirred solution was added 4 g of $NaBH_4$ over a period of 30 min. The reaction mixture was stirred for 1 h after complete addition of $NaBH_4$. The crushed ice was added to the reaction mixture which produced a precipitate immediately. The white precipitate was filtered and washed with cold water. The precipitate was dissolved in CH_2Cl_2 (250 ml) and dried over anhydrous sodium sulphate. The solvent was evaporated under vacuum and residue was recrystallized with ethylacetate-hexane (1:3) solvent which yielded (9.1 gm) of pure crystalline substance of mp 151-153°C. The dihydroartemisinin MF: $C_{15}H_{24}O_5$; MW 284) has been found to be a hemiacetal and exist as a mixture of α and β anomers whose ratio is solvent dependent. When dihydroartemisinin is crystalline, the hydroxyl at β-position but when in solution, a mixture of epimers is formed. The ratio of α and β isomers in the ^{13}CNMR of dihydroartemisinin was found to be (1:1) in $CDCl_3$

and (2:1) in CD_3OD solvent (Pathak *et al.* Ind. J. Chem. **34B**, 992-93). The material was characterized by spectral methods (IR, ^1H NMR, ^{13}C NMR & mass) and authentic sample.

b) Formulation of dihydroatemisinin (DHA)

Dihydroartemisinin (DHA) 200 mg was taken in **sterile refined neutralized** (10 ml) **groundnut oil** and dissolving by mild heating at 80-90°C and cooling the solution at room temperature. The formulation was stored at room temperature till use. It has been found to be stable at room temperature for several months.

c) Antimalarial efficacy

Test Parasite: Multi-drug resistant *Plasmodium yoelii nigeriensis* (rodent malaria strain). The *P. yoelii nigeriensis* is multi-drug resistant strain which is **resistant to high oral doses** of chloroquine (128 mg/kgx4), amodiaquine (128 mg/kgx4), mepacrine (128 mg/kgx4), mefloquine (128 mg/kgx4), quinine (400 mg/kgx4) and halofantrine (32 mg/kgx4). This parasite produces 100% mortality in 20-25 g Swiss mice and it is highly virulent for mice. It is ideal parasite for antimalarial evaluation as it produces acute infection with a spectrum of wide range of multi-drug resistance similar to the spectrum of resistance reported in the field isolates of *P. falciparum.*

(i) Antimalarial (Blood Schizontocidal) activity of dihydroartemisinin formulation by rectal route:

DHA formulation was evaluated *in vivo* in Swiss mice (20 g). the mice were infected intraperitoneally with parasite (multi-resistant strain of *P. yoelii nigeriensis*) and the infected mice were treated for 5-7 consecutive days (Day 0 onwards) with each mouse dose (0.2-0.3 ml oil by rectal route) of a DHA formulation prepared in neutralized ground nut oil. For higher doses (15 and 20 mg/kg) the drug was given in divided doses by rectal route. The tail blood smears of the mice were examined periodically after Giemsa staining up to 30 days post-infections. DHA formulation given by **rectal route has shown curative effect against multi-drug resistant rodent infection and severe/virulent/lethal malaria infection** as shown by complete absence of parasitaemia till 30 days of observation (Table 57).

(ii) Antimalarial (Blood Schizontocidal) activity of dihydroartemisinin formulation by intramuscular route

DHA formulation was evaluated *in vivo* in Swiss mice (20±1 g) as well as baby mice (12±1 g). The mice were infected intraperitoneally with parasite (multi-resistant strain of *P. yoelii nigeriensis*) and the infected mice were treated intramuscularly for 5 consecutive days (Day 0, +1, +2, +3, +4) with each mouse dose (0.2 ml oil injection) of a DHA formulation. The tail blood smears of the mice were examined periodically after Giemsa staining up to 30 days post-infection. DHA formulation has shown **curative effect against multi-drug resistant rodent infection** as shown by complete absence of parasitaemia till 30 days of observation (Table 58).

The DHA formulation given **by im route** is able to completely **control multi-drug resistant *P. yoelii nigeriensis* malaria** which is highly fatal for Swiss mice. The drug DHA is also effective in controlling severe/virulent/lethal malaria infection in 20-25 g adult mice as well as in baby mice (12±1 g).

(iii) Antimalarial (blood schizontocidal) activity of dihydroartemisinin formulation by oral route

DHA formulation was evaluated *in vivo* in Swiss mice (20-25 g). The mice were infected intreaperitoneally with parasite (multi-drug resistant strain of *P. yoelii nigeriensis* and the infected mice were treated for 5 consecutive days (day 0 onwards) with each mouse dose of 0.25 ml (in oil) by oral feeding. The **DHA formulation is prepared in neutralized sterile ground nut oil**. The tail blood smears of treated mice were examined periodically after Giemsa for recording % parasitaemia. DHA formulation given by oral route has shown curative effect against multi-resistant plasmodium which is resistant to high doses of chloroquine, mefloquine, quinine, amodiaquine, mepacrine and halofantrine etc. The DHA oral formulation at 30 mg/kg dose x 5 days has shown **curative efficacy of 100% against MDR strain** of *P. yoelii nigeriensis* (Table 59).

PRIOR ART AND BACKGROUND STUDIES ON DIHYDROARTEMISININ

Dihydroartemisinin (DHA) is the simplest semisynthetic derivative of artemisinin, the principle antimalarial constituent of medicinal plant *Artemisia annua* (Warburton, D.

(1984) Handbook of Experimental Pharmacoogy, MaCmillan NY pp. 471-495; Hoffman, S.L. (1986). Clin. Trop. Med. Communicable Dis. 1, 171-274). It has considerable activity *in vivo* and *in vitro* and is 3.8 to 5 times more potent than artemisinin (de Vries, P. and Dien, T.K. *et al.* (1996). Drugs 52, 818-836; China Cooperative Research Group on Qinghaosu, (1982b). J. Trad. Chin. Med. 2, 17-24); Gu, H.M. *et al.* (1984) Trans. Roy. Soc. Trop. Med. Hyg. 78, 265-270). *In vitro* bioassay against drug-resistant *P. falciparum* cultures (W-2 Indo-China and D-6 Sierre Lone strains) showed that **direct antimalarial activity of DHA is superior to that of artemether and arteether β (Lin, A.J. *et al.* (1987). J. Med. Chem. 30, 2147-2150).** However, because of its poor solubility in water, DHA had only been formulated as an oral preparation (Tablet) (Li, Q.G. *et al.* (1998) J. Pharm. Pharmacol. **50**, 173-182). **DHA has been chiefly used for making semisynthetic** derivatives such as **artemether** (AM) **and arteether** (AE) which are soluble in oil, and the water soluble drug **artesunate** (AS) (Luo,X. *et al.* (1984) Helv. Chem. Acta 67, 1515-1522; Lin, A.J. *et al.* (1987) J. Med. Chem. 30, 2147-2150; Brossi, A. *et al.* (1988) J. Med. Chem. 31, 645-650).

Zhao and Song (1993 Yao Hsuch Pao, 28, 342-346) compared the pharmacokinetics of oral dihydroartemisinin (DHA) and qinghaosu (QHS) Tablets in human volunteers and reported **very high bioavailability of DHA (oral tablets)** as compared to QHS (oral) and serum level after QHS were only 1.62-10.8% that of oral DHA preparation. For oral administration DHA was reported to be far superior blood schizontocide compared to QHS. **Qi-Gui Li *et al.* (1998)** J. Pharm. Pharmacol. 50, 173-182) compared the **oral bioactivity of dihydroartemisinin (DHA)** with **artemether, arteether and artesunic** acid in rats and concluded that **maximum plasma concentration was produced by oral DHA (769±218)** in comparison to **artemether (381±113), arteether (324±10) and artesunic acid (208±25 ng ml^{-1}).** Further **area under plasma concentration time curve (ngh ml^{-1}) was highest with oral DHA (615±56)** as compared to artemether (306±52), arteether (298±68) and artesunic acid (217±34). **Elimination half life (h)** was longer for **DHA (4.94±0.73)** as compared to artemether (2.04±0.18), arteether (1.79±0.47) and artesunic acid (1.34±26 H). **DHA was reported to be active metabolite of other artemisinin derivatives** such as **Artemether** (**AM**), **Arteether** (**AE**), **Artesunate** (**AS**) and **Artelinic acid** (**AL**).

Benakis *et al.* (1996) administered (60 mg dose representing half of daily dose) oral dihydroartemisinin tablets to six uncomplicated falciparum malaria cases and the peak plasma levels of dihydroartemisinin (0.26-0.71 µg/ml) were recorded between 1-2 hrs in four subjects. Thereafter, plasma level dropped and there was complete excretion by 6 hrs. **Elimination half-life of DHA was 0.71-1.45 h**, drug was well tolerated in 2 subjects while 4 subjects experienced mild headache, nausea or vomiting. One subject showed gastrointestinal hemorrhage and stupor 30 min after drug (tablet) administration. **Benakis *et al.* (1996** J. Trop. Med. Hyg. 24, Suppl. 1,7-11) suggested that the development of a **sustained release form of DHA could overcome the adverse drug reaction of tablet form of drug.**

Benakis *et al.* (1997) also reported that **following oral artesunate administration, the maximum dihydroartemisinin plasma level** produced was much higher 0.57±0.18µg/ml compared to that of parent compound artesunate which recorded peak level of 0.12±0.11 µg/ml. It may be emphasized, that **DHA level was 5-fold higher compared to that of artesunate** and that **DHA is the main antimalarial principle following artesunate administration**. Further it was pointed out that for **artesunate type** of antimalarials, **dose administration (every 3 hourly**) would be necessary **because of a very short half-life** of the drug. However, Bethell *et al.* (1997 Trans. R. Soc. Trop. Med. Hyg. 91, 195-198) had observed wide variation in peak plasma concentration of DHA (664 ng of DHA/ml, 95/Cl 387-9410, range 79-1394) in **Vietnamese *P. falciparum* cases** who were administered **oral artesunate.**

The water **soluble derivative of DHA**, namely the **artesunate acid** has been found to be very effective against malaria *in vitro* and has got **low toxicity *in vivo*** and *in vitro* (Yang, O. *et al.* (1982) J. Trad. Chin. Med. 3, 99-103; Lin, A.J. *et al.* (1987) J. Med. Chem. 30, 2147-2150). Although artesunate (AS) is 3.2 time more potent than artemisinin and is less toxic than artemether, it has **limited stability in solution (Zhou, Z.M. *et al.* (1987)** J. Chromato. 414, 77-90; Panisko, M.d. and Keystone, J.S. (1990) Drugs 39, 160-169) and dose must be **prepared immediately before administration**. Pharmacokinetic data obtained in man, rabbit, rat and dog suggested that **artesunic acid is** distributed and **hydrolysed to DHA by plasma esterase with an elimination half-life of 2-4 min in rabbits and 27 min in dogs (Zhao, K.C. *et al.* (1986)** Acta Pharm. Sin.

21, 736-739). Due to its rapid and extensive conversion to DHA, **artesunic acid could be considered a prodrug of DHA (Titulaer, H.A.C. *et al.* (1991)** J. Pharm. 69, 83-90). The **pharmacodynamic activity of artesunate is due to the metabolite (DHA) rather than to the administered product.**

Karbwang J. *et al.* (1998) (Ann. Trop. Med. and Parasitol. 92, 31-36) studied the pharmacokinetics of **artemether (oral) and its conversion to DHA in *P. falciparum* cases and concluded that DHA was believed to be the main determinant of the successful treatment** suggesting that **antimalarial activity corresponded to DHA level in plasma**. Plasma level of DHA metabolite was nearly three-fold higher in patients with sensitive *P. falciparum* infection. The mean ratio of inhibitory activities of artemether vs DHA against *P. falciparum* isolates in Thailand was 1:2.9 within 6-12 hrs, **the plasma artemether levels decreased fast, while DHA level was still higher**. However, both artemether and artesunate are susceptible to **breakdown by humidity, light and acidic conditions** at room temperature. An aqueous **solution of sodium artesunate** at pH 7-8 **hydrolyses within 1 h to DHA**.

DHA is a major metabolite of artemether, arteether and artesunic acid/artesunte *in vivo* (de Vriens, P.J. and Dien, T.K. (1996) Drugs 52, 818-836; Chi, **H.T. *et al.* (1991)** Biol. Mass Spectrum 20, 609-628; **Zhou, Z.M. *et al.* (1987)** J. Chromatog. 414, 77-90; **Li, Q.G. (1998)** J. Pharm. Pharmacol. 50, 173-182). The **high rate of malaria recrudescence associated** with other artemisinin derivatives has been ascribed at least partly **to short plasma half-life**.

The **pharmacodynamic activity of artemether** in healthy adults was reported to be **due to the metabolite DHA rather than administered prodrug**. The main **metabolite DHA** is approximately **three times as active as the parent compound in terms of antimalarial activity against *P. falciparum* (Teja-Isavadharm, P. *et al.* (1996)** Br. J. Clin. Pharmacol. 42,599-604). The contribution of other unidentified metabolites to antimalarial activity was reported to be negligible (Lee, I.S. and Heefford, C.D. (1990) Pharmacol. Ther. 48, 345).

In vivo conversion of arteether (AE), artemether (AM) and artesunate (AS) to DHA in rats was compared via all the three routes (oral, im and rectal) of administration, and the **conversion rate was faster with AS followed by AE and AM**. The high

conversion of AS to DHA can be explained by the fact that AS and DHA show same *in vitro* antimalarial potency against chloroquine sensitive and chloroquine resistant strains of *P. berghei.* Although the DHA level obtained after dosing with AM, AE and AS were less than 2.6% those after im dosing with DHA, the **high antimalarial activity of DHA probably contributes significantly to overall antimalarial activity** of these drugs *in vivo* **(Li, Q.G. *et al.* (1998)** J. Pharm. Pharmacol. 50, 173-182).

The bio-availability of artemether in healthy subjects given drug by intramuscular and intra-rectal routes showed that plasma profile of its active metabolite dihydroartemisinin following **intra-rectal (ir) administration suggested that this route should be assessed as an alternative to intramuscular route in the rural tropics (Teja-Isavadharm *et al.* (1996)** Br. J. Clin. Pharmacol. 42, 599-604). The conversion of artesunate to it active metabolite dihydroartemisinin appears **unique in antimalarial pharmacology because of its rapid clearance rate (Benthell *et al.* (1998)** Br. J. Clin. Pharmacol. 45, 123-129).

The im **bio-availability of AM and AE were very low**, indicating **incomplete absorption of thee drugs at least during first 8 h** after im administration. The bio-availability of DHA on the other hand, was midway between other oil soluble drugs and water soluble drugs. The relatively **slow and incomplete bio-availability of both IM and oral preparation of artemether** is of some concern as **this is a front line treatment** for **severe and complicated falciparum malaria**. The im **absorption of artemether in children with severe malaria (Cerebral malaria (CM) accompanied with respiratory distress** was reported to be **erratic and five CM cases did not show plasma DHA levels** in one study **(Murphy, S.A. *et al.* (1997)** Trans. Roy. Soc. Trop. Med. Hyg. 91, 332-334). In view of the **impaired absorption of artemether in children**, there is an urgent need to develop an effective antimalarial for children. **Oral artemether** has been reported to undergo **inadequate drug absorption in *P. falciparum* cases which resulted in recrudescence (Bangchang, K.N.A. *et al.* (1994)** J. Chem. Pharmac. 37, 249-253). Clinical investigators, therefore, advocate the **replacement of artemether with iv artesunate** preparation for **treatment of severe malaria in children (Murphy, S.A. *et al.* (1997)** Trans. Roy. Soc. Trop. Med. Hyg. 91, 332-334).

The **plasma level of DHA by im route were sustained over 30 hr, and parasitidal effect was maintained for much longer time period compared to oral or intra-rectal route** (DHA activity by im route, AUC 3445 nml^{-1} compared to oral route 3855 $nmol^{-1}$ h but **activity due to DHA was prolonged beyond 30 h** while the **oral dose provided effective level up to 10 h (Teja-Isavadharm, P. *et al.* (1996).** Br. J. Clin. Pharmacol. 42, 599-604).

The earlier work on **artemisinin, arteether, artelinate, artesunate administered by im formulations and arteether and DHA administered by oral route have shown good gametocytocidal activity both in animal model and against *P. falciparum*.** These drugs have **potential for the interruption of malaria transmission (Dutta, G.P. *et al.* (1989)** Chemotherapy (Basal) 35, 2000-2007; **Tripathi, R. *et al.* (1990)** Amer. J. Trop. Med. Hyg. 43, 571-575; ibid (1995) 54, 652-654).

Cost is a critical factor in determining use of antimalarial drugs. Artemisinin derived drugs are now available commercially in a few countries but they are restricted because of high cost, e.g., **oral artesunate currently cost about $ 5-6 per treatment** as compared to **$ 1.85 for mefloquine** and **70C for drugs such as chloroquine.** Injectable Artesunate, artemether and arteether cost even more compared to quinine injections at less than $ 2.0 per treatment.

There is undoubtedly a real **need for an effective new antimalarial drugs** based on artemisinin or its semisynthetic derivatives with improved absorption, bio-availability, high plasma drug concentration for longer duration of time and cost effectiveness for the treatment of uncomplicated/**multidrug resistant and severe complicated/cerebral malaria**.

The major objective of the present invention will be to develop rectal and oral formulations of dihydroartemisinin + piperaquine combinations for the control of wide spectrum of malaria.

Another objective will be to develop improved safe formulation which should be less expensive, for uncomplicated malaria as well as for emergency treatment of MDR complicated/cerebral malaria.

DETAILED DESCRIPTION OF DIHYDROARTEMISININ

DHA is superior to the original molecule, artemisinin in terms of efficacy. **Dihydroartemisinin is simplest one step, high yield and economic semi-synthetic derivative of artemisinin**. Dihydroartemisinin is also known to be an **active metabolite of artemether, arteether, artesunic acid and artelinic acid** which is partly responsible for the antimalarial activity of these drugs **(Li, Q.G. *et al.* (1998)** J. Pharm. Pharmacol. 50, 173-182).

The antimalarial profile of the compound given by **oral route** has been confirmed by **high activity of dihydroartemisinin formulation**. The **oral route of administration** is important for **treatment in villages as well as in rural and urban areas**. The **oral** formulation gives **100% cure rate against MDR *P. yoelii nigeriensis* malaria** and the efficiency is **better than QHS (artemisinin) and its other derivatives** used by earlier workers which are effective up to 50-90% at exceptionally high doses. Comparable antimalarial effects can be achieved with DHA at safe and low dose whereas high doses of **QHS up to 5.0 g dose gives cure rate 55%. Artemether up to 700 mg gives cure rate 90% and artesunate up to 750 mg gives cure rate 70-90%. Safety of DHA formulation is better than artemether and artesunate.**

The route of administration is important for the emergency treatment of severe complicated/cerebral malaria (Shen *et al.* (1989). Antimalarial drug development in China 31-45; Hein, T. and White N.J. (1993) Lancet 34, 603-608). The **DHA rectal formulation** is simple to administer, easy to store and its administration does not require any special equipment in order to achieve higher efficacy. The repeated rectal administration of the **drug every 4-6 hrs initially** can control severe complicated infections without causing any toxicity.

An **intramuscular DHA** formulation would be effective in controlling **severe complicated *P. falciparum* infections**. The im formulation would not need an intravenous infusion apparatus. **Intramuscular DHA is ready to use, unlike intravenous artesunte** which is available in the form of an **ampoule of lophilisate and an ampoule of solvent** which must be **mixed before use**. The **prepared solution of artesunate is not very stable** on storage and injection must be given rapidly. Moreover, the risk of accidental overdose is certainly less in im than with an iv preparation.

According to **Li *et al.* (1998)** J. Pharm. Pharmacol. 50, 173-182), the maximum **plasma concentration by im route attained in rats with DHA is very high** in comparison to **arteether** as well as **artemether (DHA 1579±443; AM 692±234 and AE 160±12.4).** From the pharmacokinetic point of view the **half-life of DHA is longer than that of other** artemisinin derivatives (DHA 4.44±0.27 h vs AE 3.62±0.9 h vs AM 1.78±0.80 h). When the cytotoxicity of artemisinin and dihydroartemisinin were compared with ethers and esters of dihydroartemisinin, it could be seen that **dihydroartemisinin was significantly less cytotoxic than artemether, arteether, artelinic acid and sodium artelinate (Woerdenbag *et al.* (1993)** J. Nat. Prod. 56, 848-856). The **absorption (%) of DHA within 8 h is 85.4±12.1 which is much higher than AM (54.2±25.9) and AE (34.0±9.6).**

In contrast to above artemisinin derivatives, **dihydroartemisinin (DHA)** seems to be a good antimalarial candidate as it has the **highest plasma concentration, the highest binding capacity in RBC, the longest elimination half-life and the lowest toxicity**. The **absorption of DHA in 8 h is very high** in comparison to other drugs. **DHA is a major metabolite of all these drugs** and **antimalarial activity of these drugs is due to major metabolites DHA.** DHA showed the **low rate of recrudescence** in **comparison to artemether and arteether.** Therefore the **dihydroartemisinin** has been synthesized and **stable formulation** with **enhanced bio-availability** was prepared for the control and emergent treatment of **uncomplicated/severe complicated/cerebral malaria and multidrug-resistant malaria infections.**

The invention further provides a formulation which has **more than 80% absorption** of compound **within 8 h**, which is much higher than other artemisinin derivatives (artemether, areether).

In another embodiment of the invention, the dihydroartemisinin used in the preparation appears in **mixture of α and β tautomers** with the ratio of α and β in the solvent chloroform (1:1) and methanol (2:1).

The invention further provides a formulation which is **fast acting, having blood schizontocidal activity** and useful for the treatment of uncomplicated/severe complicated/cerebral and multidrug-resistant malaria infections.

The invention further provides a formulation which is **effective against multidrug-resistant** *Plasmodium yoelli nigeriensis* parasite which is **resistant to high oral dose** of chloroquine, amodiaquine, mapacrine, mefloquine, quinine and halofantrine.

The invention further provides a formulation which is the **safe substitute of primaquine** and **useful for interrupting transmission of *P. falciparum*** and other malarial infections.

The invention further provides a formulation which can be administered through **oral, intra-rectal and intramuscular routes and produce the gametocidal effect**.

The invention further provides a formulation which is **effective against blood asexual stage as well as sexual stage** of the both ***P. falciparum* and *P. vivax*** parasites.

1.5.2 NEW ARTEMISININ BASED DRUG COMBINATION APPROVED BY WORLD HEALTH ORGANIZATION 2010

DIHYDROARTEMISININ + PIPERAQUINE ACT COMBINATION FOR UNCOMPLICATED MULTIDRUG-RESISTANT MALARIA

Myint *et al.* (2007) have reviewed fourteen clinical trials data on artemisinin-based drug combinations (ACT's) published between 2002-2006 for the control of uncomplicated multidrug-resistant *P. falciparum/P. vivax* malarias from Cambodia, Thailand, China, Vietnam, Myanmar, Lao PDR and Rwanda, in which the antimalarial efficacy of **latest ACT drug combination** namely (1) **Dihydroartemisinin (DHA) – piperaquine (PIP)** had been compared with the other ACT combinations, (2) DHA + PIP + Trimethoprime (TMP), (3) Mefloquine + Artesunate (MAS3); (4) DHA + PIP + TMP + Primaquine, (5) DHA + PIP + Artesunate, (6) DHA + PIP + DHA, (7) Artesunate + Amodiaquine (AMO), (8) Sulfadoxine-Pyrimethamine (SP) +Amodiaquine (AMO) (Table 60). A total of **2636 patients** (included in **22 treatment arms** were exposed to the latest ACT dihydroartemisinin + piperaquine (**DHA + PIP**) which have been systematically analyzed, the valuable data carefully arranged and **efficacy of 3 or 4 dose schedules** compared with the various ACT's in different malaria trials. Special care was taken by the reviewers to record and summarize the **PCR genotyping** to **distinguish the recrudescence from reinfections** in seven clinical trial studies. The comparator drug was 3-day artesunate-mefloquine (MAS3) combination in eight clinical trials studies. The authors conclude that dihydroartemisinin-piperaquine **(DHA – PIP) three dose schedule**

was excellent in the treatment of multidrug-resistant *P. falciparum* with overall 28 day cure rates of ~97-98% (n=2636) in trials conducted in **China, Cambodia, Myanmar, Laos PDR, Thailand and Vietnam** (Table 60). The authors also conclude that the efficacy of DHA-PIP was as good as mefloquine + artesunate (3 dose). **DHA-PIP was better than artesunate + amodiaquine,** and sulfadoxine-pyrimethamine + amodiaquine in trials conducted in Rwanda. The authors suggest that the dose of DHA might not be optimal. In one of the trials the lowest efficacy of DHA-PIP reported was 88.6% (80.3-93.7) at 28 days of observation, during high seasonal transmission in Rwanda trial. **DHA-PIP was also found to suppress many cases of *P. vivax* malaria**, although the combination has been able to suppress the erythrocytic infections due to *P.vivax* mono-infection. It has also been clearly emphasized that the DHA + PIP has **no radical curative activity against relapsing malaria**. Besides the **authors concluded that DHA-PIP was reported to be safe in all 14 clinical trials**. This ACT was well-tolerated and **effective in the *P. falciparum* treatment of children** as well as **adults** in various trials included in the review although abdominal pain/discomfort and occasional diarrhoea was recorded in few cases. Occasional headache, dizziness and nausea and rarely vomiting was reported in a few cases. Further the authors concluded no clinically significant effects on myocardial electrophysiology at the therapeutic doses of DHA + PIP administered for malaria treatment. Although **five deaths were reported in the 14 published articles, they were not related to DHA + PIP specific treatment.** The reviewers finally conclude that DHA – PIP could be useful as a **fixed three dose ACT regimen** for treatment of **MDR *P. falciparum*. The DHA – PIP drug tablets used earlier** in these trials were **not manufactured according to prescribed WHO specifications for International GMP standards.** Since **GMP standard DHA-PIP co-formulation are now available**, it is hoped that this ACT will be found to be more efficient for MDR *P. falciparum* malarias in future.

Smithus *et al.* (2006) reported that as judged by PCR-confirmed parasitological failure, **DHA-PIP is a highly efficacious in South-East Asia and inexpensive treatment of MDR falciparum malaria**, besides it is well-tolerated by all age groups. DHA-PIP 3-day treatment, was reported to be a **good alternative to artesunate-mefloquine** combination for treatment of uncomplicated *P. falciparum* in Wetern

Myanmar (Burma). G**ametocytaemia**: In this study gametocyte level at day 18 post-treatment was higher (10%) in DHA-PIP group as compared to artesunate-mefloquine group (2%). Follow-up studies on DHA-PIP treated patients showed **gametocyte clearance by 35-42 day,** while the parallel group treated with AS-MFQ showed gametocyte clearance by day 21. The authors concluded that **dihydroartemisinin-piperaquine would be good alternative for artesunate-mefloquine** because it is co-formulated, less expensive and had shown fewer side-effects. It was also suggested that **dihydroartemisinin dose needs to be increased to achieve better gametocytocidal effect** in 3-day DHA-PIP combination for future malaria management programme (Table 61).

Karema *et al.* (2006) evaluated the safety and efficacy of dihydroartemisinin (DHA) – piperaquine (PQP) ACT **combination (Artekin co-formulated tablet)** for the treatment of uncomplicated *P. falciparum* malaria in Rwandan children. The treatment of children (n=252) included daily oral dose of DHA (1.6-3.1 mg/kg) and PQP (12.8-24.6 mg/kg) x 3 days and 28 days cure rate was found to be **90.4%,** and the **PCR corrected data showed overall cure rate with DHA/PQP was found to be 95.2%.** This new ACT combination used to treat uncomplicated *P. falciparum* in **children** was reported to be highly efficacious (**cure rates at 28 day ranging from 99.0% in Mashesha and Kicukiro to 87.05 in Rukara area.** DHA-PQP was reported earlier also to be **highly efficacious** in the areas of MDR *P. falciparum* **(Ashley *et al.* 2004, Dennis *et al.* 2002**, **Tran *et al.* 2004).** DHA-PQP was reported to be inexpensive in this study (**costing US$ 1.5-3.0 for adult** treatment) (Table 61).

Thanh *et al.* (2009) studied the efficacy of dihydroartemisinin (DHA) and piperaquine against MDR *P. falciparum* in **Central Vietnam**. Forty two day follow-up on 49 cases in this study showed **42 day cure rates adjusted for reinfection by PCR genotyping showed 100% cure rate in this study (cure rate 49/49)**. For DHA-piperaquine combination trials, there are several earlier reports of its high efficacy reported from clinical trials conducted earlier in South-East Asia and Oceania where MDR *P. falciparum* malaria was present **(Denis *et al.* 2002; Karunajeewa *et al.* 2004b; Ashley *et al.* 2005; Myint *et al.* 2007; Ratcliff *et al.* 2007).** In the previous study in Vietnam, a total dose of **6.8 mg/kg DHA, and 54 mg/kg piperaquine** produced **56-day**

cure rate of 98.7% in children and adult patients suffering from **uncomplicated *P. falciparum*** could be cured (Tran *et al.* 2004). The authors also reported that DHA-piperaquine showed **superior post-treatment prophylactic activity** compared to **artesunate-amodiaquine** combination (Table 61).

Dihydroartemisinin + Piperaquine Trials

According to Gargano *et al.* (2012) dihydroartemisinin-piperaquine (DHA-PQP) represents a new extremely promising fixed combination for treatment of uncomplicated *P. falciparum* and the asexual stages of *P. vivax* malaria. **Clinical studies with DHA-PQP had shown cure rates consistently above 95%**, in most of the trials, with the only exception being a study in **Papua New Guinea**, which reported a high rate of treatment failures. The authors have suggested that such treatment failure could be related to **cross resistance mechanism between piperaquine and other quinolines,** or even due to a **reduced susceptibility of parasites to artemisinin derivatives**. In authors opinion the studies published so far seemed to exclude both of these possibilities. The authors conclude that there is sufficient evidence to establish the safety and efficacy of the dihydroartemisinin-piperaquine combination therapy for malaria.

Keating (2012) has recently reviewed the dihydroartemisinin-piperaquine clinical efficacy data in uncomplicated *P. falciparum* malaria, and reported that **Eurartesim®** is the only **dihydroartemisinin/piperaquine that meets the international good manufacturing practice standards**. The author has published extensive data on **pharmacological** properties of dihydroartemisinin and piperaquine, the therapeutic efficacy and **safety** of dihydroartemisinin/piperaquine used extensively for the treatment of *P. falciparum* malaria. The two open-label, multicentric trials that **Eurartesim®** formulation of **DHA/Pipq was not inferior to artesunate plus mefloquine in children and adults in Asia** and also was **non-inferior to artemether/lumefantrine or artemether/piperaquine in children in Africa**, in terms of PCR-corrected cure-rates. Patients treated with DHA-Pipq were **less likely than artesunate-mefloquine combination to experience re-infection.** The **gametocyte carriage** was greater in patients receiving DHA-Pipq than in these patients receiving comparator. The DHA-Pipq treated patients are less likely to get nausea, vomiting or dizziness. In **conclusion**,

DHA/Pipq therapy can be considered **valuable first line treatment for uncomplicated *P. falciparum* malaria.**

Valecha *et al.* (2010) have carried out exhaustive clinical (**Phase III**) trials on dihydroartemisinin-piperaquine (ACT) a promising novel antimalarial drug effective againt the multidrug-resistant *P. falciparum* malaria in **Asian countries** which selected patients from **six centres in Thailand**, **two centres in Laos and three centres in India** (**Guwahati, Goa and Mangalore** from June 2005-Feb. 2007) which included a range of **malaria transmission conditions, multidrug-resistant areas in Thailand carrying both *P. falciparum* and *P. vivax*** infections, including **high levels of chloroquine resistance** in **Laos**, and the **three India sites covering** areas of **perennial transmission/chloroquine resistance (32%** in **Assam,** 2002), 54% in **Goa** (2007) and 67% in **Mangalore** (2005) which are covered with **artesunate + sulfadoxine/pyrimethamine** combination drugs used as standard malaria control regimen in these states.

In all these trial sites the major objective of this study was to show **non-inferiority** of dihydroatemisinin/piperaquine **(DHA + PQP) regimen vs artesunate-mefloquine** (AS + MQ) in the **Asian Region**. This Asian clinical trial was an open-label, randomized, non-inferiority, **63-day follow-up** clinical study conducted in **Thailand, Laos and India.** Patients diagnosed with *P. falciparum* or mixed with *P. vivax* infection in age group 3 months-65 years. A total of 769 cases were enrolled for trial, with an allocation ratio of 2:1 patients assigned to a standard fixed dose (DHA + PQP) combination tablets (**adult dosage 40 mg DHA +160 mg PQP, and children dose 20 mg + 320 mg** n=769 patients; or a loose combination of AS + MQ (AS 50 mg + MQ 250 mg administered to 381 patients. The cumulative doses (over the 3 day treatment were about **6.75 mg/kg DHA and 54 mg/kg of PQP** and about **12 mg/kg of AS and 25 mg/kg of MQ.** The primary endpoint was day **-63 polymerase chain reaction (PCR) genotype–corrected cure rate.**

The data on cure rate obtained in the trial after 3 day treatment course showed **87.9% cure for DHA/PQP** and **86.6%for AS +MQ** treatment in the intention to treat. **The final overall calculated cure with DHA/PQP was 98.7%** and with **As + MQ was 97.0%. New infections** encountered on day 63 measured as **persons-gametocyte-weeks**

was significantly **higher in DHA+PQP** treated patients (10-15% vs 4.88% for AS + MQ treatment group.

Valecha *et al.* (2010) finally concluded that **new ACT comprising of dihydroartemisinin + piperaquine was considered safe, as it can play an important role as the first-line antimalarial therapy for uncomplicated falciparum malaria**. In the study, dihydroartemisinin+piperaquine (Eurartesim^{TM}, Sigma-Tau, Italy administered as 3 dose schedule (day 0, 1 and 2) was found to be **curative for *P. falciparum* uncomplicated cases.**

Gargano *et al.* (2012) conducted clinical efficacy and safety trials between 2006-2007, on antimalarial dihydroartemisinin-piperaquine and artesunate-mefloquine combinations amongst 150 uncomplicated *P. falciparum* infected Indian malaria patients who were part of the **Phase III multicentric study published by Valecha *et al.* (2010).** Out of 154 patients screened, 101 were treated with dihydroartemisin-piperaquine (DP) and 49 cases were assigned to artemisinin + mefloquine (A+M) treatment. All the patients were followed up for 63 days as part of an open-label, non-inferiority, randomized multicentric trial in Asia. **Both ACTs (DP and A+M) were judged to be highly efficacious in Indian trials**. Three centres with distinct background regarding parasite transmission, resistance and seasonality were finally selected: (1) **Guwahati** (in Assam region) with perennial malaria transmission (30% resistance to chloroquine was recorded); from which 46 (DP) and 22 (A+M) patients were enrolled, (2) **Goa**, with perennial transmission, peak in June-September (CQ resistance about 50%, where 19 DP and 9 (A+M) patients were enrolled; (3) **Mangalore** with seasonal transmission area during post-monsoon months (with CQ resistance 70%) where 36 DP and 18 A+M patients were enrolled.

At the time of enrollment, the DP and A+M groups showed higher geometric mean parasitaemia in DP group compared to A+M group. Out of 150 enrolled cases, 84% (126) completed 63 day evaluation.

In the final analysis of PCR-corrected cure rate at day 63 the response of patients to **DP (dihydroartemisinin + piperaquine) was found to be similar to A+M (Artesunate + Mefloquine) treatment schedule.** For the ITT (intention to treat) group,

the cure rates for DP was 80.2% and that of A+M group was 83.7% and for the PP population, the **cure rates were 98.8% (DP) and 100% (A+M).**

These **clinical trials in India** concluded that the **fixed dose dihydroartemisinin-piperaquine** emerged as a **highly efficacious treatment for *P. falciparum* malaria in India**. The authors report that combination of DP provided some protection against new infections post-treatment. **The major conclusion of this Indian study** suggested that **dihydroartemisinin-piperaquine can play an important role in controlling and eliminating malaria in India.**

1.5.3 ARTEMISININ-BASED DRUG COMBINATIONS (WHO SPONSORED) AND NON-ACT COMBINATIONS UNDER CLINICAL TRIALS: REVIEW BY SINCLAIR *ET AL.*

Sinclair *et al.* (2009) have carried out an extensive data analysis of the results of clinical malaria drug trials in which the World Health Organization recommended efficacy of artemisinin based drug combinations were used for treatment and control of uncomplicated *Plasmodium falciparum* malaria. Global malaria clinical trials data search during August 2008 to March 2009 identified 517 trials. Out of these full published data of 85 trials were examined initially but data on 50 clinical trials in which artemisinin-based combination therapy (ACT's) prescribed by WHO clinical trial group had been strictly used, were finally reviewed for clinical efficacy in uncomplicated *P. falciparum* malaria control. The data from trials in which WHO approved five ACT's were clinically used included:

1. ACT: Dihydroartemisinin plus Piperaquine Combination (DHA + P).
2. ACT: Artesunate plus mefloquine (AS + MQ).
3. ACT: Artemether plus lumefantrine (6 doses) (AL6).
4. ACT: Artesunate plus amodiaquine (AS + AQ).
5. ACT: Artesunate plus sulfadoxine-pyrimethamine (AS + SP).
6. Non-ACT combination: Amodiaquine plus Sulfadoxine-pyrimethamine (AQ + SP).

Sinclair, Zani, Donegan, Olliaro and Garner (2009) compiled a comprehensive review of clinical trials on the treatment of uncomplicated *P. falciparum* malaria through

the use of various artemisinin-based (ACTs) combinations sponsored and approved by the World Health Organization with a sharp focus on the curative efficacy in clinical trials conducted by International Public Health Agencies. The **clinical efficacy of five artemisinin based ACT's namely**, 1. **Dihydroartemisinin – Piperaquine (DHA-Pipq),** 2. **Artesunate – Mefloquine (AS-MFQ),** 3. **Artemether-Lumefantrine (ARTEM-Lumefantrine)**, 4. **Artesunate-Amodiaquine (AS-AMOD),** 5. **Artesunate-Sulfadoxine/Pyrimethamine (AS-SD/Py),** and **one non-ACT combination** 6. **Amodiaquine-Sulfadoxine/Pyrimethamine (AMOD-SD/Py)**. Reviewers identified 517 clinical trials against malaria, and finally selected data **from fifty trials among uncomplicated *P. falciparum* cases**, for the compilation of the current review which covered published data up to 2009.

The major conclusion regarding the performance of **new ACT-Dihydroartemisinin-Piperaquine** in the treatment of **uncomplicated *P. falciparum* trials** was that **this ACT achieved the WHO standard <5% total failure (i.e. more than 95% cure rate) in 15 clinical trials out of 17 trials in which DHA-Pipq was used**. Further **DHA-Pipq ACT was as effective as Artesunate-Mefloquine in eight clinical trials in African Continent**. Besides **DHA-Pipq appeared to be more effective than widely used ACT-Artesunate-Lumefantrine** combination in four clinical trials. Finally, the **latest ACT : DHA-Pipq ACT was adjudged better than : AS + MFQ** as shown by 63 day follow-up after treatment, than Artem + Lumefantrine at 42 day follow-up, than AS + Amod at 28 day follow-up, than ACT AS + SD/Py at 42 day follow-up and finally better than Amod + SD/Py combination at 28 follow-up in clinical trials against uncomplicated *P. falciparum.* The author (Sinclair *et al.* 2009) conclude that there is now growing weight of evidence available to justify the use of Dihydroartemisinin-Piperaquine as a first line treatment option for uncomplicated *P. falciparum* malaria.

Sinclair *et al.* (2009) also analysed the effect of ACT – DHA + Pipq and AS – Mefloquine on the **clearance of *P. falciparum* gametocytaemia**, and reported that Sinclair, Gogtay, Brand and Olliaro (2011) have carried out an extensive review on the efficacy of Artemisinin-based combinations in the treatment of uncomplicated *P. vivax* malaria. In view of emerging chloroquine resistant *P. vivax* in Asia, South America and some parts of Oceania, the authors have reviewed the clinical trials data on control of

various Artemisinin-based combination therapies. The authors reviewed four clinical trials (1185 participants) in areas where chloroquine is still effective in treating *P. vivax* blood stage parasitaemia, and suggested that Artemisinin derivatives are equally effective as blood schizontocide as chloroquine in controlling recurrent fever and asexual parasitaemia. According to the investigators (Sinclair *et al.* 2011), **Dihydroartemisinin-Piperaquine is one of the most studied ACT for treatment of *P. vivax*** malaria and they have concluded that **in high transmission area, the ACT Dihydroartemisinin-Piperaquine is superior to other three artemisinin derivatives (ACT's)** 1. Artemether-Lumefantrine, 2. Artesunate-Amodiaquine and 3. Artesunte + Sulfadoxine/Pyrimethamine combination, as shown in *P. vivax* clinical trials. According to these reviewers, the DHA-Piperaquine can provide longer post-treatment prophylaxis between 28-42 days, because of longer half-life of piperaquine in this ACT. Both DHA-Piperaquine are blood schizontocides and are known to lack anti-relapse activity against the hypnozoite stage (relapsing stage) in the liver. In order to achieve radical cure of *P. vivax,* **primaquine or bulaquine treatment for 7-14 days** are generally recommended.

PERU Trials

Clinical trials for efficacy and safety of **Dihydroartemisinin/Piperaquine (Artekin®)** for treatment of uncomplicated *P. falciparum* malaria were conducted in PERU by **Institute of Tropical Medicine, Belgium (2010)** and DHA-PPQ (x 3 dose) was reported to be **highly effective antimalarial drug** in Peruvian Amazon region. No early treatment failure was reported and this combination was **cheaper and more affordable than Mefloquine-Artesunate** (3 day) treatment **(Grande *et al.* 2007, PLoS ONE, 2007 Oct. 31,2 (10) e1101).**

Further clinical trials on **dihydroartemisinin-piperaquine** have been in progress under the **sponsorships of London School of Hygiene and Tropical Medicine (2011); University of Oxford (2012); Kwame Nkrumah University of Science and Technology (2012) and Kilimanjaro Christian Medical Centre, Tanzania.**

1.5.4 DIHYDROARTEMISININ-PIPERAQUINE (ACT) FOR TREATMENT OF MULTIDRUG-RESISTANT *P. FALCIPARUM* MALARIA IN PREGNANCY

Rijken *et al.* (2008) reported the importance of serious complications and severity of multidrug-resistant *P. falciparum* in the pregnant women. In the prior art, the authors report the available treatments for pregnancy which include **Quinine, Quinine plus Clindamycin, Mefloquie and Artemether-Lumefantrine are not satisfactory for MDR, complicated *P. falciparum*** in pregnant women.

Rijken *et al.* (2008) selected women with recurrence of *P. falciparum* after failure of 7-day treatments with quinine (30 mg/kg/day, + Clindamycin (15 mg/kg/day), artesunte (2 mg/kg/day) or after treatment with both quinine and Clindamycin etc. These authors finally administered 3 day treatment with **Dihydroartemisinin (40 mg) + Piperaquie (320 mg per tablet) (Holley Pharm PRC) per day.** The final dose administered was **6.5 mg DHA/kg** and **51.2 mg PPQ/kg** daily for 3 consecutive days. In this retrospective study, 50 Karen pregnant women were enrolled with **recurrent *P. falciparum*** infections who were diagnosed and treated with **DHA-PPQ regimen** between June 2006 and January 2007. These women had a total of 192 episodes of *P. falciparum* infection during their pregnancy. No women was reported to deteriorate following DHA-PPQ 3 day treatment. The authors reported that 50 women were treated with DHA + PPQ. Five women left the area before the end of pregnancy, and 45 women delivered live children. One child at delivery showed **Patau syndrome (trisomy 13)** died 6 hours after birth. All the remaining children were normal at 1 month of age. The authors reported that **oral DHA-PPQ therapy was successful for the treatment of highly drug resistant *P. falciparum* infection in pregnant women**. There was no serious adverse effect of DHA-PPQ treatment. None of the pregnant women developed vomiting with DHA-PPQ treatment. The **PCR adjusted cure rate by Kaplan Meier analysis at day 63 was 92.2% and no evidence of toxicity for mother or the fetus.**

Gametocyte carriage rates post-treatment were also **low** and similar to rates from **non-pregnant patients treated with DHA-PPQ** (Ashley *et al.* 2005).

DHA+PPQ has been judged as an effective, short-course, relatively **inexpensive**, fixed artemisinin combination has never been available to pregnant women on the Thai-Burmese border and **DHA-PPQ currently looks to be the most promising candidate** ACT in pregnancy for the treatment of **MDR uncomplicated *P. falciparum* malaria.**

Tjitra *et al.* (2008) carried out a prospective study in Indonesia on **multidrug-resistant *P. vivax*** malaria patients and reported that severe disease was recorded in 22% (2,634) in patients diagnosed with malaria, with the risk greater among *P. vivax* (23% (675/2,937) infections compared to *P. falciparum* infections in 20% percent (1,570/7,817) cases. The mixed infections accounted for 31% (389/1,273 cases). Severe anaemia (hemoglobin <5 g/dl) was found in *P. vivax* cases and **severe disease in *P. falciparum*, 78 patients developed respiratory distress and 42 patients presented with coma. Mortality** was recorded in 2%malaria cases (**total mortality 242**), out of this *P. vivax* case mortality was 1.6% (46/2,916), *P. falciparum* mortality was 2.2% (167/7,722) and mixed infections caused 2.3% (29/1,260) mortality. The authors reported that nearly **half of the Indonesia's population of 250 million live in malaria endemic areas** and nearly 15 million people seek treatment for clinical malaria each year. The Editor's summary of the above article recorded that in this study **one in 50 patients with malaria died.** The risk of death was the same for patients infected with *P. falciparum, P. vivax* or both parasites. The authors also report that in May 2005, the protocols for drug treatment of severe malaria were revised temporarily to **intravenous artesunate** (Dondorp *et al.* 2005) and since March 2006 **was the first-line treatment of uncomplicated *P. falciparum* and *P. vivax* malaria was changed to dihydroartemisinin-piperaquine (Ratcliff *et al.* 2007).**

In another meta-analysis on the safety and efficacy of dihydroartemisinin-piperaquine for the treatment of uncomplicated *P. falciparum* in malaria endemic countries, was compared with other artemisinin based combination therapies (Naing *et al.* 2012). Analysis of 26 randomised controlled trials showed that dihydroartemisinin-piperaquine (DP), artesunate-mefloquine (MAS_3) and artemether-lumefantrine (AL) treatments were **equally effective for reducing the risk of recurrent parasitaemia** in uncomplicated *P. falciparum* cases. Further the PCR confirmed that DP treatment showed 99.5% cure as compared to MAS3 (97.7%) treatment. **Comparable efficacy** was also recorded for **DP (95.6%) vs AL (94.3%).** The major conclusion of this meta-analysis was that the ACT-DP was not inferior to other current used ACTs such as MAS3 (Mefloquine + Artesunate) or AL (Artemether-Lumefantrine). Further DP has been reported to show potential to become a first-line treatment for uncomplicated malaria.

1.6 α and β ARTELINATE

(Discovery of Fast-Acting Blood Schizontocide and Gametocytocide)

1. α and β Artelinate – a **fast acting blood schizontocide** for the control of multidrug resistant malarias. (β-Artelinate was supplied to the author by late Professor D.L. Klayman, WRAIR, Washington, D.C.).
2. A water-soluble artemisinin derivative for intravenous injection for emergency treatment of **comatose/cerebral malaria** *P. falciparum/P. vivax* cases (Tripathi, Dutta and Klayman, 1992; Tripathi, Vishwakarma and Dutta, 2008).
3. Fast-acting antimalarial can prevent mortality in severe multidrug resistant malaria cases.
4. A safe blood schizontocide with LD_{50} in range 670-1000 mg/kg.
5. Effective against highly virulent *P. knowlesi* infection in Rhesus monkey (Vishwakarma *et al.* 1989, 1992).
6. Artelinate **shows high gametocytocidal action** and can **block malaria transmission** (Tripathi, Puri and Dutta, 1996).
7. Safe drug as shown by pre-clinical studies in primate malaria model.

Artelinic acid β was synthesized by Lin, Klayman and Mihous (1988-U.S. Patent 4,791,135) and its first water soluble formulation in sodium bicarbonate 5% solution was tested for antimalarial activity in mice and the results based on this study were published by Lin *et al.* (1987) who showed that it was a promising water soluble and stable antimalarial derivative of artemisinin, which showed curative blood schizontocidal activity at 40 mg/kg x 3 days against *Plasmodium berghei* in Swiss mice when the compound dissolved in 5% sodium bicarbonate was administered subcutaneously in mice and the compound was **safe up to 640 mg/kg base**. In comparison to artelinic acid, the **artesunic acid** which is also water soluble showed weak antimalarial activity; its **curative dose being 640 mg/kg x 3 days**. ***In vitro*** studies have confirmed the antimalarial action of artelinic acid against drug resistant *P. falciparum* isolates (Lin *et al.* 1987; Basco and Le Bras (1993) and van Vianen *et al.* (1990).

Transdermal absorption of artelinic acid and sodium β-artelinate from transdermal patch has also been reported to cure *P. berghei*_infection at 480 mg/kg total dose administered in 3 days treatment (Lin *et al.* 1994).

In all of the earlier studies on artelinate, the rodent malaria model was used to establish the antimalarial profile of β sodium artelinate, while α sodium artelinate had not been examined for its antimalarial potential. Using *P. knowlesi* - rhesus monkey infection, a rigorous animal model for selection of candidate antimalarials for drug development, the authors had very briefly published the first report on the antimalarial potential/curative dose data of both α and β artelinate in abstracts published by Vishwakarma et al, (1992) and Tripathi *et al.* 1992 a) respectively. Both the compounds were freshly dissoloved in sterile 5% sodium bicarbonate solution for intravenous injection. Authors had also stressed the need to undertake detailed preclinical evaluation of both these water soluble artelinate compounds (α and β anomers) which could be administered by intravenous route and developed as potential antimaterial (**blood schizontocides**) for emergency treatment of cerebral malaria. The synthesis of β artelinate was published earlier by Lin et al (1987) and that of α-artelinate was reported by authors (Vishwakarma *et al.* 1989; 1992). Subsequently, the authors had also reported an additional activity of sodium β-artelinate namely its **gametocytocidal activity** against *Plasmodium cynomolqi* B. - rhesus monkey model indicating its potential to completely **block the transmission of malaria infection to vector** (*Anopheles stephensi*) by both intravenous and oral route of drug administration, though the drug had no sporontocidal effect in vector (**Tripathi *et al.* 1996).**

The present communication deals with the preclinical status of artelinic acid (sodium β-artelinate) and also reports the **fast acting blood schizontocidal efficacy of both α and β sodium artelinate** against a highly virulent **mefloquine resistant** *P. knowlesi* (W1) infection in rhesus monkey. It is hoped that the preclinical antimalarial efficacy data of α and β-artelinate against *P. knowlesi* which is an accepted cerebral malaria model (Migasena and Areekul, 1987) would stimulate further drug development efforts with both α and β - artelinate for their possible use in cerebral malaria cases.

The mortality rate of naive monkeys infected with *P. knowlesi* is 100%. In the present study each monkey was inoculated with $1x10^6$ infected RBCs and the parasitaemia was recorded daily by examining Giemsa-stained thick and thin blood smears. α and β Sodium artelinate were administered to the monkeys when they became patent. These derivatives dissolved in sterile 5% sodium bicarbonate were evaluated for

their blood schizontocidal activity by intravenous route. Freshly prepared solution of α and β sodium artelinate was given **intravenously** on three consecutive days. Blood smears from each monkey were obtained for at least 60 days after the end of the treatment and the recrudescence, if any, was recorded. Monkeys that remained negative on blood smear examination for 60 days were considered as cured. A total of ten infected monkeys were treated with β-artelinate and four monkeys were treated with α artelinate.

Both **α and β artelinate synthesized at CIMAP Lucknow** (Vishwakarma *et al.* 1989) were freshly dissolved in sterile 5% sodium bicarbonate solution and injected intravenously in the leg vein of rhesus monkeys (using 5ml drug volume). Besides β-**artelinate from WRAIR** was also used for comparison and dissolved in 5% sodium bicarbonate solution for intravenous injection in infected monkeys.

In the present investigation sodium salts of both α and β-artelinate have been evaluated for their blood schizontocidal activity against mefloquine resistant strain of *Plasmodium knowlesi* (W1) infection in rhesus monkey in order to establish their curative doses by intravenous route of drug administration. Rigorous criteria were used to establish the curative dose by checking the blood smears stained in Giemsa stain up to 60 days post-treatment and the dose showing no recrudescence up to 60 days was considered as curative dose. Both α and β-artelinate were dissolved daily in sterile 5% sodium bicarbonate solution for intravenous administration. β-artelinate doses ranging from 5.0, 10.0, 15.0 and 20.0 mg/kg. and that of α-artelinate 15.0 and 20.0 mg/kg x 3 iv injections on three consecutive days, were administered to monkey after potency was established and the results of parasitaemia clearance/ cure or recrudescence are summarized in Tables 62 and 63. Treatment with 5.0 and 10.0 mg/kg x 3 doses of β-artelinate (iv) failed to control the *P. knowlesi* infection and the monkeys died following recrudescence of parasitaemia. The dose of 15.0 mg/kg x 3 doses cured one of the two monkeys (50% cure) with α as well as β-artelinate. At highest dose i.e. 20 mg/kg x 3 doses (iv), both α and β-artelinate were curative in 2/2 monkeys each. Additional 2 infected monkeys treated with β-artelinate were also cured. No recrudescence was recorded in cured monkeys which were followed up for 60 days. At 20 mg/ kg x 3 dose level, α-artelinate cured 2/2 monkeys (100% cure rate) while β-artelinate cured 4/4 monkeys (100% cure rate).

LD_{50} dose of α- artelinate determined in mice was >1.0 g/kg. while the LD_{50} dose of β- artelinate was 670 mg/kg (ip). The study shows that α-artelinate has higher safety margin compared to β-artelinate, although both the annomers are equally effective blood schizontocides. No vascular necrosis at the site of intravenous injection was observed in monkeys treated with α- and β–artelinate formulations used in the study.

Earlier studies in which rodent malaria screening model was used, led to the identification of artemisinin derivative namely artelinic acid (and its water soluble sodium salt β-artelinate) as a potential antimalarial compound for drug development **(Lin *et al.* 1987).** Even after 28 years of its discovery, the compound sodium β-artelinate has not cleared animal pharmacology/regulatory toxicology and teratogenicity or clinical safety and tolerance evaluation in human subjects primarily because of the lack of preclinical antimalarial data to provide curative dose/ safety profile of sodium β-artelinate in simian malaria models which forms the basis of selection of new compounds for drug development. (Dutta and Tripathi 1996, 2003). Since the publication of Lin *et al.* (1987) and Brossi *et al.* (1988) major thrust in drug development efforts was focused towards development of **oil soluble injectible β-arteether** which was registered in October 2000 (TDR News No. 63) and is in clinical use. Synthesis of another oil soluble injectible compound - β-artemether had been reported by **Lin *et al.* (1987)** and **Brossi et al (1988),** and it was **approved by WHO (1996)** for clinical use. Synthesis of **third oil soluble injectible compound α and β-arteether (30 : 70)** was reported from **India in 1989 (Vishwakarma et al 1989)** and this drug was successfully **marketed in India in 1997** and is currently in clinical use in **India and African countries** (Dutta and Tripathi 2003). Another water soluble fast acting artemisinim derivative namely **artesunic acid/ sodium artesunate** for intravenous injection developed by **Lin *et al.* (1987, 1988)** and **Brossi *et al.* (1988)** is currently a **life saving drug for cerebral malaria** if **used in combination with full dose of mefloquine.**

Comparative studies on two water soluble artemisinin derivatives namely artelinic acid and artesunic acid by Lin et al (1987, 1988) had clearly established that **artelinic acid** (iv) in bicarbonate solution gave **16-fold superior blood schizontocidal** activity against *P. berghei* in mouse model as compared to artesunic acid. Stability tests in 5% Na HCO_3/D_2O further confirmed the superiority of artelinic acid because of its long term

stability in solution as compared to artesunic acid (half life about 45 days for artelinic acid vs <1 day for artesunate). Grace *et al.* (1999) had reported the efficacy of artelinic acid was partly due to its **metabolism to antimalarial metabolite dihydroartemisinin** by cytochrome P_{450} 3A4/5 in the liver.

Li et al (1998b) had carried out extensive study on toxicology, pharmacology and phasmacokinetics of β- artelinic acid in animal models. The **LD_{50} dose of artelinic acid** (IM) was reported to be **535.2 mg/kg** in rats. Toxicity evaluation of single IM dose of 100-200 mg/kg artelinic acid was found to be safe in rats, however, higher doses of 400 mg/kg produced 20% mortality, 600 mg/kg produced 60% mortality and 800 mg/kg dose produced 100% mortality of rats. Pharmacokinetic study in dog established that following 10 mg/kg dose of artelinic acid, the **Cmax was highest (17763 ± 4152 mg/ml) after intravenous** administration, which was 2-3 fold higher when same dose was given by IM or intragastric route. The plasma concentration ($AUC_{0\text{-}12h}$) was also highest (11219 ± 1356) by iv route, with **t½ β-elimination of 2.62 ± 0.58 h**. Bioavailability (%)was also highest (100%) by iv route in comparison to IM (90.2□±10.3%) and intragastric (79.7 ± 13.5%) routes. Li *et al.* (1998) also reported that artelinic acid achieved markedly higher concentrations within human red blood cells (cell-plasma ratio = 0.44) than β-arteether and artemether (0.23 and 0.28 cell: plasma ratio), suggesting that artelinic acid could be a more potent fast acting blood schizontocide in humans. **Lower toxicity of artelinic acid could be due to very low (4.3%) conversion of total artelinic acid to its antimalarial metabolite namely dihydroartemisinin (DHA)** which has much higher toxicity. In comparison to artelinic acid with only 4.3% conversion to DHA, other drivatives show very high rate of conversion to DHA, e.g. artesunate shows (up to 73%) DHA metabolite, arteether (up to 16%) and artemether (up to 12%) DHA conversion in rat, which may account for lower toxicity of artelinic acid. DHA had been reported to be most toxic causing anorectic toxicity in rat than its analogues (Brewer *et al.* 1993). On the other hand **artelinic acid was much safer** as it produced minimum anorexia at 25 mg/kg/day administered for 7 days which could be due to very low conversion rate to DHA.

The present study has established the curative blood schizontocidal activity of both α and β-artelinate against **mefloquine resistant *P. knowlesi*** in rhesus monkey model. Both the drugs are fast acting and equally curative at 20 mg/kg x 3 doses when the

drugs are administered intravenously for 3 days and no recrudescence was observed up to 60 days of observation. Curative efficacy of both α and β-sodium artelinate against a highly virulent simian malaria model justifies its potential use in cerebral malaria cases. LD_{50} data in mice show higher safety of artelinate.

In the present study intravenous inoculation of artelinic acid (both α/β) into the leg vein of the rhesus monkeys did not show any necrosis at the site of injection.

The present investigation justified further pre-clinical development of α and β-sodium artelinate individually or as (50:50) α and β sodium artelinate combination for treatment of MDR/complicated *P. falciparum* comatose case.

Another major advantage in developing **sodium artelinate** (α- and β- or α-/ β-epimers) as drug for clinical use will be its **strong gametocytocidal potential** and its ability to sterilize gametocytes and **stop malaria transmission**. Tripathi et al (1996) have reported earlier that even a single dose of β-artelinate (15 - 25 mg/kg IV or 10 - 30 mg/kg oral) produces gametocytocidal effect against human transmissible *Plasmodium cynomolgi* B infection in rhesus monkey. Gametocytocidal activity of artemisinin (Dutta *et al.* 1989) and α/β arteether (Tripathi *et al.* 1990, 1992) against simian malaria and against *P. falciparum* has also been confirmed in clinical trials (Dutta *et al.* 2001). **Sodium artelinate, thus will have dual application in malaria control strategy** as a fast acting blood schizontocide and as a **transmission blocking safe drug which could be a candidate to replace primaquine.** Price *et al.* (1996) have also proposed that artemissinin derivatives offer a promising alternative for the treatment and control of malaria transmission. Combination of artemisinin derivatives which are effective gametocytocides e.g. sodium β-artelinate, with standard antimalarials (blood schizontocides/ sporontocides) has also been proposed to **stop malaria transmission (Hog *et al.* 1998).**

It is hoped that the above up - date and **pharmacokinetic data (Li et al 1998 b) on sodium artelinate (epimers)** would help to promote further development of this drug.

1.7 PYRONARIDINE
(A fast-acting Blood Schizontocidal for Control of MDR Malarias)

1. Oral Pyronaridine for MDR Malaria

Pyronaridine for control of MDR Malarias

1. A multidrug-resistant strain of *Plasmodium yoelii nigeriensis* (MDR) showing a wide spectrum of resistance to chloroquine, amodiaquine, mepacrine, mefloquine, halofantrine, quinine, and quinidine was used in this study for ***in vivo*** **evaluation** of the blood schizonocidal activity of pyronaridine, a topoisomerase II inhibitor, in Swiss mice.
2. *Plasmodium yoelii nigeriensis* MDR produces 100% lethal infection in mice.
3. Exhibits **strong blood schizontocidal action** for *P. falciparum.*
4. A fast-acting blood schizontocide.
5. The present paper also compares the merits of a single MDR strain of *Plasmodium yoelii nigeriensis vs* a battery of different resistant lines for quick antimalarials screening.

The spread of multidrug-resistant strains of *Plasmodium falciparum* throughout Southeast Asia, Africa, and South America has been documented by several investigators, and areas of resistance to chloroquine, amodiaquine, quinine, mefloquine, sulfadoxin-pyrimethamine combination, and quinine-tetracycline combination are expanding (WHO 1984). Although several artemisinin derivatives have been recently developed for control of cerebral and severe complicated *P. falciparum* infections (Bunnag *et al.* 1991, Valecha *et al.* 1997), there is still high priority to develop new synthetic antimalarials for the control of multidrug-resistant *P. falciparum* infections which should have a different mode of action to overcome the problems of cross-resistance.

In the present study **pyronaridine**, a blood schizontocide synthesized by Chinese investigators **(Zheng *et al.* 1982)**, has been evaluated for efficacy against a multidrug-resistant strain of *Plasmodium yoelii nigeriensis*. Although the response of pyronaridine to different drug-resistant lines of *P. berghei* and *P. yoelii* has been reported by **Peters and Robinson (1992)**, however, in this investigation we have evaluated the drug

response of pyronaridine against a single MDR line of *P. yoelii nigeriensis* resistant to most of the conventional antimalarials. Mice used in the study weighed 20±1 g and were homogenous. They were kept for 1 week in the experimental wing, prior to starting the experiment. Blood-induced *P. yoelii nigeriensis* (MDR) infection in Swiss mice was used for evaluation of blood schizontocidal activity of pyronaridine. This strain of rodent malaria produced lethal infection in mice and is resistant to high doses of conventional antimalarials such as chloroquine (128 mg/kg x 4 days), amodiaquine (128 mg/kg x 4 days), mepacrine (128 mg/kg x 4 days), mefloquine (128 mg/kg x 4 days), quinine (400 mg/kg x 4 days), quinidine (400 mg/kg x 4 das), and halofantrine (128 mg/kg x 4 days) administered orally by gavage for 4 consecutive days (i.e. from day 0 to +3).

For evaluation of blood schizontocidal activity, the mice infected intraperitoneally with 1 x 10^6 parasitized RBC were treated orally with pyronaridine phosphate once a day from day 0 onward at different dose levels (0.625-5.0 mg/kg x 9 days and 1.25-30.0 mg/kg x 7 days) in order to arrive at the curative end-point dose. Tail blood smears of mice stained in Giemsa were examined to record percentage of parasitaemia and results are expressed as means ± SD. Follow-up of the treated mice was continued for 28 days **(Dutta *et al.* 2000; Tripathi *et al.* 2000).**

Blood schizontocidal activity of pyronaridine has been evaluated against a multidrug-resistant strain of *P. yoelii nigeriensis* (MDR).

Initially the response of the *P. yoelii nigeriensis* to low doses of pyronaridine (0.625 to 5.0 mg base/kg) was evaluated after 9 days oral treatment in Swiss mice and these results showed that the maximum dose of 5.0 mg/kg x 9 days was not fully curative (data not shown) and it protected 8 of 9 mice (88.8%). Curative dose determination was subsequently carried out using a series of doses ranging from 1.25 to 30.0 mg/kg on a shorter 7 day oral treatment schedule and the response of *P. yoelii nigeriensis* (MDR strain) is summarized in Table 64. The results show that doses of 1.25-5.0 mg/kg x 7 did not protect the mice completely in a 28-day follow-up. A dose of 10 mg/kg x 7 was also not fully curative as shown by the appearance of a transient parasitaemia in 1 out of 14 mice treated at this dose. The next higher dose i.e. 15 mg/kg x 7 days, was 100% curative in three replicate experiments. The **higher doses, i.e., 20.0 and 30.0 mg base/kg x 7**

days, were fully curative and survival of treated mice recorded up to 28 days follow-up was 100% at all these dose levels.

Shao (1990) had studied the blood schizontocidal activity of pyronaridine against *P. berghei* and reported that pyronaridine given orally had a far better therapeutic index (201.3) than chloroquine (14.5).

The comprehensive data on blood schizontocidal activity of pyronaridine against different parasite lines has been reported by Peters and Robinson (1992), and a highly chloroquine-resistant line of *P. yoelii* (NS) and a mefloquine-resistant (N/1100) line were reported to be fully sensitive to pyronaridine with ED90 value of 1.2 and 1.6 mg/kg, respectively. However, Peters and Robinson (1992) observed that the halofantrine-resistant line of *P. yoelii* (SH) showed high level of cross-resistance to pyronaridine (143-fold resistance).

The main **advantage of the present study** is that instead of using a large battery of resistant lines of plasmodia, **a single MDR line like *P. yoelii nigeriensis* can be a useful model for primary screen**. The major **advantage of this MDR rodent infection is that the drugs showing high activity in this model would be potentially effective in control of MDR *P. falciparum*.** This is supported by our earlier study on arteether which was initially picked up because of its curative action against this MDR screening model (Dutta *et al.* 1990) and has now become an effective drug in the treatment of multidrug-resistant *P. falciparum* in clinical cases **(Valecha *et al.* 1997).** Our study also provided evidence that pyronaridine has the potential to cure halofantrine resistance of *P. yoelii nigeriensis* which is contrary to the findings of Peters and Robinson (1992). Our study also suggests that pyronaridine could be a useful drug in halofantrine-resistant *P. falciparum* areas.

Most of the earlier *in vitro* drug sensitivity studies on *P. falciparum* clinical isolates had clearly established the efficacy of pyronaridine against chloroquine-resistant African and MDR Thailand strains (Childs *et al.* 1988; Shao 1990; Onori and Majori 1989; Ringwald *et al.* 1998). However, a few chloroquine-resistant lines showing slightly decreased sensitivity to pyronaridine have been documented by Elueze *et al.* (1996), Yang *et al.* (1997), and Pradines *et al.* (1998). Further clinical trials support the *in vitro* findings and most of the investigators are of the opinion that pyronaridine is very

effective in the areas of multi-drug resistance e.g. Thailand and chloroquine resistance of African continents (Looareesuwan *et al.* 1996; Ringwald *et al.* 1996; Ringwald *et al.* 1998).

These investigators who have used the drug clinically, strongly advocate that the drug was promising and well-tolerated and exhibited no toxicity (Fu and Xiao 1991; Winstanley, 1996). Clinical pharmacokinetic studies have shown that the $t_{1/2}$ β with oral tablet of pyronaridine (600 mg) was 65 ± 6 h and the T_{max} was 14 h. However, the low absorption rates of tablets and capsules were 19 ± 7 and 32 ± 7%, respectively, which would justify prolonged treatment for achieving a better cure rate (Fu and Xiao 1991).

Pyronaridine seems to have different mode of action than the conventional antimalarials as suggested by Chavalitschewinkoon *et al.* (1993) by virtue of its inhibition of decantenation activity of **topoisomerase II** which may be responsible for high antimalarial activity against MDR infection. Besides, pyronaridine was also reported to accumulate in the parasite food vacuoles and impair hemoglobin degeneration (Wu *et al.* 1988). The high activity of pyronaridine against chloroquine-resistant *P. falciparum* has been attributed to its **inhibitory effect of hematin polymerization** which is responsible for inhibition of *P. falciparum* growth in culture (Dorn *et al.* 1998). Recently **topoisomerase** I of *P. falciparum* has also been recognized (Bodley *et al.* 1998) and the possible effect of pyronaridine on the particular enzyme also remains to be elucidated.

2. Pyronaridine Discovery of Intramuscular Injection-Blood Schizontocidal Activity for Control of Malaria

1. **Powerful Intramuscular Blood Schizontocide against multidrug resistant malaria**.
2. Curative against MDR *Plasmodium yoelii nigeriensis.*
3. Curative by intramuscular route.
4. Pyronaridine, administered intramuscularly (im) to Swiss mice infected with the lethal multidrug-resistant *Plasmodium yoelii nigeriensis*, was found to exert high blood schizontocidal activity. The efficacy of doses of pyronaridine ranging from 0.625 to 30 mg (base/kg) was evaluated using a 4 day treatment schedule (drug was administered at 0, 24, 48 and 72 hrs). It was found that doses of 2.5 mg/kg and higher protected animals completely from the lethal effects of the parasite.

The same degree of protection was found when the treatment duration was reduced to 3 days. This study shows that pyronaridine is a potentially useful antimalarial drug that could be exploited for the **control of multidrug-resistant malaria infection.**

Blood schizontocidal activity of pyronaridine was evaluated against multidrug-resistant *P. yoelii nigeriensis* infection in Swiss mice. This strain produces 100 percent lethal infection in control mice. Pyronaridine was administered by the intramuscular route for 4 consecutive days at doses ranging from 0.625-30.0 mg/kg, and the parasitaemia was followed up to 28 days post-infection. Doses of 2.5-30.0 mg/kg of pyronaridine were found to be curative and no recrudescence was observed (Table 65). Incidentally, one of 6 mice at 15 mg/kg dose and another mouse at 5 mg/kg showed very transient parasitaemia which was subsequently cleared on its own. The lower doses of 0.625 and 1.25 mg/kg were not curative.

Pyronaridine was further evaluated in a shorter 3 day schedule in which the two doses were given on day 0 (at 0 and 8 h) followed by two subsequent doses on the 1st and 2nd days. Four doses ranging from 2.5 mg/kg to 30.0 mg/kg were administered at 0, 8, 24 and 48 h. The drug was curative at all doses tested, and the **lowest dose (2.5 mg/kg) was again curative** and no recrudescence of parasitaemia was observed up to 28 days **(Dutta *et al.* 2000; Tripathi *et al.* 2000)** (Table 66).

(The compound Pyronaridine was kindly provided to the Author through the courtesy of Late Mr. A.V. Modi, Unichem Laboratories, Mumbai, India).

Pyronaridine-Artesunate ACT Combination

Ramharter *et al.* (2008) have reported that development of a fixed-dose combination of **Pyronaridine-Artesunate** for pediatric patients treatment suffering from uncomplicated *P. falciparum* in Gabon is **considered a high priority area** in the field of drug research. The authors report that **Medicines for Malaria Venture (Geneva) and Shin Poong Pharmaceuticals (Seoul, Republic of Korea)** are jointly developing a blood schizontocidal **ACT pyronaridine-artesunate combination**, for treating uncomplicated *P. falciparum* and *P. vivax* malaria using 1 daily dose x 3 days. Although, pyronaridine alone is known to control chloroquine resistant *P. falciparum*, but there is strong recommendation not to use pyronaridine monotherapy because of the possibility of emergence of resistance against *P. falciparum.* Current studies phase I and II have shown that pyronaridine-artesunate ACT combination provide a good safety profile and it would be a suitable ACT for **control of both *P. falciparum* as well as a blood schizontocide for treating *P. vivax*.**

The tablets formulated contained three dose levels:

A. Pyronaridine salt 100 mg = 56.93 mg + 16 mg Artesunate (6:1)
B. Pyron 72 mg + 24 mg AS (3:1)
C. Pyron 96 mg + 32 mg AS (3:1)
D. Granule co-formulation = 60 mg pyron tetraphosphate + 20 mg artesunate (Paedriatic)

Treatment was given daily x 3 days

15 patients of uncomplicated *P. falciparum* were treated with each dose level (June-December, 2006). By day 28, 53 patients who completed treatment, showed 100% cure rate. The **PCR corrected efficacy was 89%-100% in all the four groups**. The authors concluded that pyronaridine-artesunate therapy was **well tolerated at all dose levels.** The granules co-formulation for young children showed good efficacy and tolerance with the pediatric 2-14 years patients. This study constituted **Phase II evaluation** of pyronaridine-artesunate (ACT) among young children suffering from uncomplicated *P. falciparum*.

Poravuth *et al.* (2011) conducted the **phase III trial** of Pyronaridine-Artesunate combination for the treatment of acute *P. vivax* versus chloroquine in randomized, double

blind, non-inferiority trial across five clinical centres across **Cambodia, Thailand, India**, **and Indonesia**. Patients aged 3-60 years suffering from *P. vivax* (mono-infection) were randomised (1:1) to receive the antimalarial comprising of pyronaridine-artesunate (target dose 7.2:2.4 mg/kg to 13.8:4.6 mg/kg) or chloroquine (standard dose) once daily for 3 days. 14 days cure rate were 99.55 with pyronaridine-artesunate (217/218) and 100% (209/209) with chloroquine. The authors observed that pyronaridine-artesunate cure rates were non-inferior to chloroquine on day 21-42. Parasite clearance was faster with pyronaridine-artesunate (23.0 h) versus chloroquine (32.0 h, $p<0.001$). The authors finally recommend that pyronaridine-artesunate antimalarial therapy is a **promising antimalarial in areas where *P. vivax* and *P. falciparum* infections are prevalent.**

1.8 DISCOVERY OF SYNTHETIC 1,2,4-TRIOXANES (FENOZANS) AS BLOOD SCHIZONTOCIDES

1. **Fenozans 7**
2. **Fenozans 11**
3. **Fenozans 45**

1. Fast-acting oral synthetic endoperoxide blood schizontocides.
2. Curative synthetic drugs for control of multidrug resistant malarias.
3. Curative against MDR *P. yoelii nigeriensis*.
4. Potential synthetic endoperoxides for the control of multidrug resistant *P. falciparum* malaria identified.
5. Possible substitute for artemisinin.

Peters *et al.* (2002) had reported that their earlier published data on synthetic 1,2,4-Trioxanes, in which DMSO (10%) was used for formulating Fenozan 50F (=B07/fenozan7), showed wider discrepancies between different experiments because the compound B07 had been found to be unstable in a DMSO-based aqueous formulation. Evaluation of drug formulated in DMSO if stored for several days, indicated that earlier antimalarial activity data had under-estimated the activity of this compound. They further report that Fenozan-50F (B07) (as racemic. form) is five to seven times more active against *P. berghei* N when given orally in 'DMSO-solution' than originally reported (Jefford *et al.* 1995). They have pointed out that loss of activity may result from chemical interaction between compound B07 and DMSO in which cyclopentanone moiety would be replaced by DMSO, thus producing less active derivative or alternately B07 possibly crystallized out of DMSO solution on freezing drug formulation. They have **cautioned against the use of DMSO formulation for drug testing of Fenozans and other endoperoxide antimalarials** because this could clearly lead to erratic and misleading antimalarial screening results. In view of the above reported controversial role of DMSO in *in vivo* antimalarial screening, refined neutral groundnut (peanut) **Arachis** oil has been successfully used as vehicle for formulating the compounds in this study **(Tripathi, Jefford and Dutta, 2006).**

Blood schizontocidal activity of ten selected cis-fused cyclopenteno- 1,2,4-trioxanes [namely Fenozan compound nos. 6,7,11,27,32,39,44,45,48 and 51 have been reinvestigated to establish their curative doses against multidrug resistant *Plasmodium yoelii nigeriensis* strain, which produces lethal infection in Swiss mice. Freshly prepared formulations of these compounds prepared either in neutral groundnut (peanut) oil or in DMSO-Tween-water were compared for their antimalarial activity. **Only two** compounds, namely **Fenozan derivatives 11 and 45** formulated in neutral groundnut oil for **oral** administration, showed highest activity amongst Fenozan compounds evaluated with **100% cure rate against MDR *P.yoelii nigeriensis* infected mice**, while the DMSO-Tween- water formulations were inactive. In case of fenozan7, the oil and DMSO-Tween formulation produced 92.3 and 76.0% cures respectively. Fenozan derivatives no. 6, 27, 32, 39, 44 and 51 were not protective either in groundnut oil or DMSO-Tween oral formulations. The present study has applied more **rigorous criteria for selection of active compounds**, and identified 3,3-spirocyclopentane derivative namely **Fenozan 11**, and 3,3-spirohydropyran derivative namely **Fenozan 45**, as potential blood schizontocides which do not show delayed recrudescence but can completely eliminate multidrug resistant malaria infection in mice (Table 67). **Both these compounds can be considered as candidates for preclinical development.** Present study advocates the preferred use of oil vehicle for oral evaluation of potential antimalarial trioxanes/fenozans instead of the DMSO formulation, which gives inferior curative efficacy.

Fenozan 11 (Fig. 1) formulation in neutral groundnut oil was very effective orally against MDR *P. yoelii nigeriensis* in mice at 30mg/kg/dayx5 doses as shown by complete clearance of parasitaemia (day 4 - 28), with high cure rate (10/10 mice). However its formulation in DMSO-Tween-water administered orally was inactive (Table 67). Revalidation of Fenozan 11 oral formulation in neutral oil also showed that the compound was fully curative, protecting 8/8 mice at both 20 and 30 mg/kg x 5 doses, while lower dose (15mg/kgx5) was partially curative (3/8 mice). The results of 3 dose administration schedule at 15 mg/kg/day showed partial protection of 5/8 of mice. The cure rate of Fenozan 11 in oil formulation administered orally seems to be better than Fenozan-7 (Table 67).

Fenozan 45 (Fig. 1) showed very good blood schizontocidal activity at 30 mg/kg/dayx5 days when administered orally in oil formulation with complete clearance of parasitaemia beyond 28 days and 100% protection of mice (10/10 mice), whereas its DMSO-Tween formulation was not protective. In revalidation tests also oral formulation in oil was 100% curative and protected 8/8 mice (Table 67).

Fenozan – 7 (Fig. 1) at 30-mg/kg/dayx5 doses showed initially 90% curative action against MDR *P. yoelii nigeriensis* when administered orally in neutral groundnut oil formulation (with survival of 9/10 mice). The second formulation in DMSO-Tween was less active as shown by survival of 7/10 mice, (Table 67). In revalidation test, oral formulation in oil at 30 mg/kg/day x5 doses treatment also cured 15/16 mice (cure rate 93.7%) while the oral formulation in DMSO-Tween was less active (p value =) with the cure rate of 81.2% (13/16 mice protected. The results of intramuscular administration of Fenozan-50F showed that IM formulation in oil at 30mg/kg/day was inactive, while the IM-formulation at 30 mg/kg in DMSO-Tween was curative with survival of 7/8 mice(cure rate 87.5%) after 5-day treatment (Table 67).

(*Fenozan compounds were kindly provided to the Author through the courtesy of Professor Emeritus C.W. Jefford,Dept. of Organic Chemistry, University of Geneva, CH-12, Geneva, Switzerland)*

Antimalarial Studies on Synthetic substituted 1,2,4-trioxanes by Singh C and Puri S K : US Patent No. 6,316,493)

Extensive preclinical studies on the **synthesis of new substituted 1,2,4-trioxanes as antimalarial agents have been patented from CDRI (US Patent 6,316,493) by Singh C. and Puri S.K. (2001)**. Large number of synthetic trioxanes had been successfully screened against MDR *Plasmodium yoelii nigeriensis* rodent parasite in Swiss mice model. The trioxanes were dissolved in sterile groundnut oil sodium bicarbonate solution and administered for 4 days (day 0-3) via IM or oral route in Swiss mice. Follow-up studies were conducted up to 28 days post-treatment. According to the authors out of large number of compounds screened, compound nos: 25a, 27a, 27b, 27c, 27f, 28a, 31a1, 35a1, 41a, 41b were found to be curative by **im route**, and compounds (27b, 41a, 41b) were curative by **oral route** also against MDR *P. yoelii nigeriensis*.

The authors continued the antimalarial evaluation of 27a and 27b and their hemisuccinate derivatives 41a and 41b for blood schizontocidal activity against *P. cynomolgi* B infections in rhesus monkeys.

Evaluation against *P. cynomolgi* B

Compound	Dose mg/kg	Regimen	Cure rate of monkeys
27a (IM)	10.0x4 days	Divided	2/2
(Oral)	20.0x5 days	Divided	2/2
(Oral)	20.0x5 days	Divided	3/3
	15.0x5 days	Divided	3/3
27b (IM)	15.0x4 days	Divided	2/2
	10.0x4 days	Divided	2/2
(Oral)	20.0x4 days		
	15.0x4 days		
41a (IM)	10.0x4 days	Divided	4/4
(IM)	10.0x4 days	Single	2/2
(Oral)	20.0x5 days	Divided	3/3
(Oral)	20.0x4 days	Divided	2/2
41b (IV)	10.0x4 days	Divided	2/2
(IM)	10.0x4 days	Divided	2/2
(Oral)	20.0x5 days	Divided	2/2
(Oral)	10.0x4 days	Divided	2/2

Compound 27a at 15 and 20 mg/kgx4 days (divided) doses was also curative by IM route against 2/2 monkeys infected with *P. knowlesi.*

Like-wise oral dose of 20 mg/kg was also **curative against *P. knowlesi.***

Compound 41a at 20 mg/kg (divided dose) was curative in 2/2 monkeys.

Compounds **27a and 41a** were also tested for **gametocytocidal tests against *P. cynomolgi* B** and *P. yoelii nigerneisis* and the gametocytocidal action was confirmed in rhesus monkey model and *P. yoelii nigeriensis* model.

Candidate drugs under development (Singh C and Puri SK, unpublished)

1. **CDRI 97/78**
2. **CDRI 99/411**

Compound CDRI 97/78, a fully synthetic 1,2,4-trioxane derivative has been identified for development as a **alternative to artemisinin derivatives** for use against drug resistant *P. falciparum* and cerebral malaria case. Compound has successfully completed preclinical efficacy and regulatory tests and Phase I clinical trials at IPCA Pharmaceuticals Ltd.,Mumbai.

Another antimalarial trioxane compound **CDRI 99/411** with increased half life to allow for extended exposure dosage has also completed preclinical efficacy and regulatory studies.

Clinical development of both the above compounds is being pursued under **licensing agreements with IPCA Pharmaceuticals Ltd., Mumbai**.

Preclinical experimental studies on antimalarials

Follow up studies with 99-411 (Handed over to IPCA 29/8/2007)

Synthetic endoperoxide compound 99-411 had been identified for drug development earlier.

Curative dose: 24 mg/kg x 4 days regimen against *P. yoelii* – MDR Swiss mice model.

Curative dose : 20 mg/kg dose x 5 days regimen against *P. cynomolgi* rhesus monkey model.

Recently shorter regimens with 99-411 have been established : *P. yoelii* MDR

Rodent curative dose :**48 mg/kg in two doses**.

Curative dose :**96 mg/kg in single dose** against rodent model.

Monkey : Initial studies in two monkeys showed curative response with 20 mg/kg x 3 days against *P. cynomolgi.*

Revalidation in progress.

Combination with piperaquine and lumefantrine - had been extensively used as partner drugs with other artemisinin derivatives e.g. dihydroartemisinin and artemether by earlier investigators including MMV.

Additive effects 97-78 / 99-411 with piperaquine and lumefantrine under investigation.

In a recent study on pharmacokinetics and pharmacodynamics of 1,2,4-trioxanes CDRI 97/78 compound have been studied by **Gupta and Srivastava (2014).** They have reported that 75% of the **CDRI 97/78 is metabolized to CDRI 97/63.** They have further proposed that CDRI 97/78 and 97/63 are **new trioxane class** of antimalarial compounds. There is a **very little possibility that the parasite could develop resistance against this new class of synthetic trioxanes.** According to them, the metabolism of CDRI 97/78 was completely inhibited by CYP3A4 specific inhibitors. Since 70% of the CYP_{450} is in the form of CYP3A4, this explains the reason behind the very rapid metabolism of CDRI-97/78 and its low bioavailability. This also explains, the reason for drug metabolism by microsomal fraction, as most of CYP's are located in microsomes only. It is therefore suggested that the inhibitor of CYP3A4 can be supplemented as an add-on to increase the bioavailability of CDRI 97/78.

Gupta, P and Srivastava, P (2014) Hepatic metabolic studies of 1,2,4-trioxanes derivative: A new antimalarial; *J. Pharamcol. Clin. Toxicol*. **2**(1) 1024

1.9 AZITHROMYCIN AND DRUG COMBINATION FOR CONTROL OF MULTIDRUG-RESISTANT MALARIA

1. DISCOVERY OF AZITHROMYCIN (BLOOD SCHIZONTOCIDE FOR MDR MALARIA)

1. Initial antimalarial study involving several antibiotics evaluation against MDR *P. yoelii nigeriensis* in *in vivo* mouse model showed that tetracycline, erythromycin, ciprofloxacine, non-floxacine, etc. failed to protect *P. yoelii nigeriensis* MDR infected mice.
2. The macrolide azithromycin at 200 and 100 mg/kg administered orally for **seven consecutive days** was found to protect mice infected with **MDR *P. yoelii nigeriensis* infection** and discovery of exceptional cure rate of the azithromycin had established the antimalarial efficacy in the management of MDR malaria (**Tripathi, Dhawan and Dutta, 2005**).
3. *P. yoelii nigeriensis* produces a fulminating infection in Swiss mice producing 100% mortality of infected mice. Azithromycin has been reported to be **exceptional safe antibiotic which can be used in combination with blood schizontocides such as chloroquine as a safer drug combination effective for control of MDR malarias.**
4. Azithromycin provides a safe antimalarial therapy for infants and for pregnant women.
5. The present study shows that only azithromycin has the potential to control an MDR *P. yoelii nigeriensis* infection in Swiss mice, provided the treatment with a dose of 50-100 mg/kg/day by oral route is continued for a period of 7 days.

Previous studies on the antimalarial efficacy of various antibiotics had been reviewed by Puri and Dutta (1982) and amongst the various groups of antibiotics, the macrolides had shown high potential for the control of malaria. The antimalarial activity has been specifically reported for rifampicin (Strath *et al.* , 1993), erythromycin (Gingras and Jensen 1993), spiramcyin (Hill 1975), midecamycin (Puri and Dutta 1989), clindamycin (Lewis 1968), minocycline (Dutta and Singh 1979), fluoroquinolones (Singh and Puri 1996), and azithromycin (Anderson *et al.* ,1995; Gingras and Jensen 1993).

More recently **Puri and Singh (2000)** have shown **blood schizontocidal activity of azithromycin against *Plasmodium yoelii neigeriensis* N-67 strain** which shows low level of resistance to chloroquine only. Azithromycin dose of 70 mg/kg per day × 4 days, as well as 40 mg/kg per day × 7days was reported to be curative in the above study (Puri and Singh, 2000). Andersen *et al* (1995) had reported the curative action of azithromycin against *Plasmodium berghei* and only 71% cure rate was obtained at a dose of 128 mg/kg per day dose administered for 3 days. **Anderson *et al.* (1995)** had also reported its curative action against ***Plasmodium falciparum* infection in *Aotus* monkey at 100 mg/kg** dose administered for **seven consecutive days**, whereas its lower dose (30mg/kg × 7 days) was able to clear the parasitaemia initially, but there was recrudescence in one of the two monkeys. Besides, the curative dose of azithromycin against *Plasmodium cynomolgi B* in rhesus monkey had been reported to be 25 mg/kg per day × 7 day schedule **(Puri and Singh 2000)**.

The *in vitro* antimalarial activites of ciprofloxacin, norfloxacin and ofloxacin and numerous other antibiotics against *Plasmodium falciparum* had been reported by Tripathi *et. al* (1993) and Mahmoudi *et al.* (2003). *In vitro* antimalarial activity of a variety of other antibiotics such as cycloheximide, erythromycin, tetracycline, chloramphenicol, clindamycin, actinomycin D, rifampin and isoniazid against *Plasmodium falciparum* had also been reported earlier by Geary and Jensen (1983).

A battery of more than one dozen drug resistant lines of rodent malaria, had been developed by Peters (1987) and Peters *et al.* (1993 a, b, c) for antimalarial screening programme which would need enormous inputs, time and expertise to obtain ED_{50}/ED_{90} values of the test compounds in *in-vivo* systems. **In preference to a battery of antimalarial screens, the authors prefer to use a single MDR strain of *Plasmodium yoelii nigeriensis*** with a very wide spectrum of resistance to antimalarials/antibiotics, **as a blood schizontocidal screen** in random bred Swiss mice. In the present study, the MDR strain of *Plasmodium yoelii nigeriensis* which produces 100% mortality in mice, was used to evaluate the **blood schizonotocidal activity of azithromycin alone, in combination with α/β arteether** and several other antibioticas.

Blood schizontocidal activity of other antibiotics against MDR parasite

Tetracycline (500 mg x 4), oxytetracycline (500 mg x 4), doxycycline (25 mg x 4), erythromycin (250 mg x 4), ciprofloxacin (50 mg x 5) and norfloxacin (50 mg x 5), were evaluated for blood schizontocidal activity against *Plasmodium yoelii nigeriensis* (MDR) strain and the treatment was given orally for 4-5 consecutive days. The results presented in Table 68 show that the strain is fully resistant to high doses of these antibiotics and the mortality after treatment was 100% in all the groups. Even combinations of tetracycline and ciprofloxacin with chloroquine (32 mg/kg x 5) were not protective. However norfloxacin and chloroquine combination protected 3 out of 10 mice.

The MDR strain of *P. yoelii nigeriensis* used in this study shows high level of resistance to different antimalarials such as chloroquine, amodiaquine, mepacrine, mefloquine, quinine, quinidine and halofantrin.

Blood schizontocidal activity of azithromycin against MDR *P. yoelii nigeriensis* has been investigated in Swiss mice. Azithromycin was administered orally at 12.5, 25 and 50 mg/kg doses for seven consecutive days, starting from day 0 today + 6. The lowest dose of 12.5 and 25 mg/kg was not curative and the number of animals surviving were 3 and 4 respectively in each group. At 50 mg/kg dose, the transient parasitaemia (0.02±0.04) was recorded on day 6 but the parasitaemia was finally cleared and all the eight mice survived beyond 28 days (Table 69). In the second experiment the dose of 50, 100 and 200 mg/kg was evaluated and in this experiment also although 50-mg/kg dose showed transient parasitaemia on day 5 and 6 but the parasitaemia was cleared and all the eight mice survived. Similarly at 100 and 200 mg/kg doses parasitaemia showed a transient patency on day 5 but parasitaemia was finally cleared up to 28 days of observation. The study showed that 50-mg/kg/day dose was curative by oral route, against the multi-drug infection in 7-day treatment schedule in 2 experiments. Both of the control batches, which were not, treated showed 100% mortality and the mice developed very high parasitaemia before death.

In another experiment **azithromycin was administered with α/β arteether by the oral route of administration.** Doses of 30, 15 and 3.75 mg/kg of azithromycin were given with 25, 16.6 and 5.5 mg/kg doses of α/β arteether respectively for 4 days. A dose

of 30 mg/kg azithromycin with 25 mg/kg α/β arteether provided 100% curative effect against MDR strain. There was no trace of parasitaemia during 28 days of observation, whereas the lower dose i.e. 15 + 16.6 mg/kg produced only 60% cure. Results show that with the appropriate dose combination, these **two drugs can produce 100% protection and cure of MDR strain** (Table 70).

Besides the antimalarial action, azithromycin possesses a wide spectrum of antimicrobial activity against *Mycoplasma pneumoniae, Chlamydia pneumoniae, Chlamydia trachomatis, Mycobacterium avium, M. intracellulare complex, Helicobacter pylori, Campylobacter sp. , Bordetella pertusis (*Zuckermann *et al.* 2003; Tarlow *et al.* (1997), *Entamoeba histolytica* and *Giardia lamblia (*Lode *et al.* 1996), and *Toxoplasma gondii* (Degerli *et al.* 2003).

Prophylactic efficacy of azithromycin against *Plasmodium falciparum* infection was reported to be 83% by de Vries *et al.* (1999) and 71.6% by Taylor *et al* (1999, 2003). Andersen *et al.* (1998) had reported that the daily administration of 250 mg azithromycin for 10 weeks, gave 82.7% prophylactic protection against *P. falciparum* while Andersen et al (1995) had shown only 40% protection (protecting 4 out of 10 volunteers) in first batch treated with 250 mg daily dose, while the second batch of 10 volunteers treated with above dose was fully protected against *Plasmodium falciparum* infection.

An earlier report had shown that a short 3 day azithromycin therapy was inadequate for achieving complete cure of *Plasmodium falciparum* cases even when azithromycin was combined with artemether. Combination of 300mg artemether together with 500mg azithromycin followed by 250 mg azithromycin at 24 and 48 hrs was evaluated for treatment of MDR *Plasmodium falciparum* infection, and the **recrudescence** rate of drug resistant falciparum infection **was 53.3% (Na Bangchang *et al.* 1996).**

Clinical studies on the blood schizontocidal efficacy of azithromycin in combination with artesunate and dihydroartemisinin against multi-drug resistant *P. falciparum* have also been conducted (Krudsood *et al.* 2000, 2002). In one of the trials, artesunate 200 mg together with azithromyci 50 mg once daily for 3 days gave a partial cure rate of 56% among uncomplicated *P. falciparum* cases in Bangkok (Krudsood *et al.* 2000). In another clinical trial dihydroartemisinin (80 mg dose daily x 3 days) was given

in combination with azithromycin (500 mg dose daily x 3 days) which resulted in 69.7% cure rate among MDR *P. falciparum* cases in Thailand (Krudsood *et al.* 2002). Partial radical cures reported in the above studies were mainly due to inadequate dosages of azithromycin and short-term treatment.

In earlier studies azithromycin in combination with artemisinin derivatives did not prove to be very effective and 52%, 56% and 69% cure rates were achieved with azithromycin + artemether, artesunate and dihydroartemisinin (DQHS) respectively. In the present study **we achieved 100% cure with the combination of azithromycin with α/β arteether**. α/β arteether is a very potent antimalarial and its **LD_{50} (i.e. <1250 mg) is more than artemether, artesunate and DQHS**. We are convinced that doses used in earlier combination studies in humans were inadequate and that the number of doses was also not sufficient. Four days treatment with azithromycin and α/β arteether will certainly improve the cure rate of *P. falciparum*-infected humans.

In vitro antimalarial studies with azithromycin against *Plasmodium falciparum* carried out by Yeo and Rieckman (1995) also showed that the antibiotic was slow acting and maximum activity was obtained at 96 hrs, with MIC ranging from 0.04-0.08µg/ml. In the present study also action of azithromycin on clearance of asexual blood stages of *Plasmodium yoelii nigeriensis* was slow at the curative dose i.e. 50mg/kg, as well as at 100 and 200 mg/kg doses and slide positive parasitaemia was observed upto day 5-6 of treatment. Because of slow blood schizontocidal action of azithromycin against MDR rodent strain, the treatment was extended to 7 day course in order to achieve complete cure. The curative action of tetracycline, oxytetracycline, doxycycline, ciprofloxacine, norfloxacine and erythromycin against MDR *Plasmodium yoelii nigeriensis* could not be achieved in the present study even when high doses of these antibiotics were used for treatment of mice. The **MDR strain developed in the study exhibits high level of resistance to the above antibiotics excepting azithromycin to which the strain is still sensitive**. Combinations of several antibiotics and chloroquine (32mg/kg) were tested but norfloxacine and chloroquine combination were only partially protective (3/10 mice.)

The *P. yoelii nigeriensis* MDR strain selected in this study would be a **useful antimalarial screening model to** identify new compounds/antibiotics which could be developed to tackle the problem of multi-drug-resistant malaria. The MDR strain has

acquired the wide spectrum of drug resistance similar to the MDR *Plasmodium falciparum* isolates from the field.

We emphasize that out of several antibiotics screened against *Plasmodium yoelii nigeriensis* (MDR) **only azithromycin was found to eliminate the multi-drug resistant parasites**, although the rate of clearance of parasitaemia was slow. Besides azithromycin, **minocycline is another antibiotic**, which is potentially very active against malaria and has been reported by **Dutta and Singh (1979)** to cure fulminating *P. knowlesi* infection in rhesus monkey.

The present study also shows that the single MDR line of *P. yoelii nigeriensis* which produces 100% mortality in mice, could be a useful model for antimalarial screening and it has obvious advantage over the conventional method of screening compounds against a battery of resistant lines of rodent malaria infections as has been proposed by earlier investigators (Peters, 1987, Peters. *et al.* 1993a, b, c). Instead of determining ED_{50}/ED_{90} values of the potential antimalarials, the determining of 100% curative dose in mice infected with wide spectrum of MDR *Plasmodium yoelii nigeriensis* seems to be a more rigorous and realistic criterion for selection of highly active compounds/antibiotics for further drug development.

2. CLINICAL TRIALS WITH AZITHROMYCIN/CHLOROQUINE COMBINATION AGAINST *PLASMODIUM FALCIPARUM* MALARIA IN INDIA

Azithromycin (an azalide antibiotic) had been discovered by the authors (Tripathi, Dhawan and Dutta,2005) to show curative antimalarial activity against multidrug resistant *P. yoelii nigeriensis* by oral route of drug administration compared to other antibiotics such as Tetracycline, Oxytetracycline, Doxycycline, Erythromycin, Ciprofolxacin, Norfloxacin. The Azithromycin had also showed curative action against highly MDR *P. yoelii nigeriensis* strain in Swiss mice model. Further **multicentric clinical studies were planned in India by Dunne *et al.* (2005) in collaboration with Dr. N Valecha (Malaria Research Centre, New Delhi) to assess the efficacy of Azithromycin in combination with chloroquine** in the multicentric trials against uncomplicated *P. falciparum* cases. In the interim analysis, *P. falciparum* cases from Vadodara and Guwahati were treated with Azithromycin (1 g x 3 days) (14 *P. falciparum*

cases) and chloroquine (1500 mg over 3 days) (15 cases). All these cases were analyzed for efficacy of two drugs. After interim analysis, 63 selected uncomplicated *P. falciparum* cases were treated with Azithromycin plus chloroquine combination (these cases belonged to **states of Gujarat, Delhi, Madhya Pradesh, and Assam where there was stable malaria transmission throughout the year)**. The results of this multicentric clinical trials are presented in Table 71.

The *P. falciparum* diagnosed fever cases administered **azithromycin/ chloroquine/combination of azithromycin + chloroquine** were followed-up for 28 days and the cure achieved with Azithromycin alone was 36% (5/14 cases), chloroquine alone 27% (4/15 cases) while the treatment with **combination of Azithromycin + chloroquine showed 97% cure rate (61/63 cases)**. This trial results showed that resolution of *P. falciparum* parasitaemia was inadequate with individual treatment with Azithromycin and chloroquine, but combination of Azithromycin + chloroquine was significantly better, giving 97% cure rate in *P. falciparum* cases. Only 2 cases out of 63 reported failure after 28 days follow-up. According to the authors, the combination therapy of Azithromycin and chloroquine may be a promising for the treatment of malaria in areas of chloroquine resistance.

Chico and Chandramohan (2011) had reported that combination therapy using azithromycin and chloroquine had demonstrated **adequate parasitological response >95%** in clinical trials on non-pregnant adults in sub-Saharan Africa. They propose that this combination could replace sulfadoxin-pyrimethamine as the first line therapy against *P. falciparum* in Africa, where sulfadoxin-pyrimethamie resistance is a serious problem. This combination can be effectively used to prevent and/or treat *P. falciparum* or *P. vivax*. The authors conclude that azithromycin-chloroquine can be considered as a potential alternative to sulfadoxin-pyrimethamine, since efficacy studies had shown curative effect against *P. falciparum* among non-pregnant adults in Sub-Saharan Africa, Colombia and India, even in areas which were carrying chloroquine resistant *P. falciparum*.

3. CLINICAL TRIALS WITH MEFLOQUINE/AZITHROMYCIN/ ARTESUNATE COMBINATION

Krudsood *et al.* (2000) had conducted clinical trials with combination of Azithromycin and artesunate in Thailand against uncomplicated *P. falciparum*. Group I of 68 patients were administered arteunate 200 mg once daily x 3 day. Group II patient were administered artesunate 200 mg together with 10 mg/kg, mefloquine for the first 2 days and artesunate 200 mg together with 5 mg/kg mefloquine on the third day, Group III was administered artesunate 200 mg together with Azithromycin 50 mg once daily x 4 days. The 28 day cure rate was reported to be 44.3, 98.2 and 56.4% respectively. The authors pointed out that Azithromycin dose (50 mg x 4 days) used in combination with artesunate was probably inadequate and suggested further trials in view of clinical safety of Azithromycin.

4. *IN VITRO* STUDIES ON AZITHROMYCIN

Ohrt *et al.* (2002) had carried out extensive *in vitro* antimalarial study with Azithromycin in combination with chloroquine and several other antimalarials. The combination of chloroquine-azithromycin show a range of activities from additive to synergistic interactions for eight chloroquine resistant *P. falciparum* isolates. Combinations of azithromycin with quinine, tafenoquine and primaquine show additive to synergistic response with azithromycin. The authors concluded that azithromycin combination with chloroquine should be evaluated for malaria prophylaxis. Further *in vitro* interaction of azithromycin or erythromycin with chloroquine/quinine etc., against multidrug-resistant *P. falciparum* was investigated by Nakornchai and Konthiang (2006) concluded that best drug combinations against multidrug-resistant *P. falciparum* would be quinine-azithromycin and quinine-erythromycin combination. Nakornchai and Konthiang (2006) quote the earlier *in vitro* study which had indicated that azithromycin is 10-fold more potent than erythromycin against chloroquine resistant P. falciparum FCR 3TC isolates (Gingras and Jensen, 1992). Further *in vivo* studies against chloroquine-resistant *P. berghei* and *P. yoelii nigeriensis* in mice, also had reported that azithromycin was 60-fold and 31-fold more potent that erythromycin, respectively as reported by Gingras and Jensen (1993) and Puri and Singh (2000). The data generated by Nakornchai

and Konthiang (2006) also showed that chloroquine in combination with azithromycin or erythromycin for 96 h incubation acted synergistically against multidrug-resistant *P. falciparum* K1 strain.

5. AZITHROMYCIN FOR PROPHYLAXIS OF *P. VIVAX*

Heppner *et al.* (2005) conducted the **efficacy studies of azithromycin for prophylaxis against *P. vivax* malaria** in 276 adults in **Western Thailand** in a randomized, double-blind, placebo-controlled trial. Following antimalarial suppressive treatment, all volunteers were randomized in a 2:1 ratio to either treatment regimen with azithromycin or placebo, respectively. The treatment with azithromycin was given for an average 74 days. The azithromycin group comprising of 179 adults had five cases which develop parasitaemia (1 *P. vivax* and 4 *P. falciparum*). The placebo group included 97 adults and the 28 day endpoint parasitaemia was recorded (21 *P. vivax*, 5 *P. falciparum* and 2 mixed infections). The **protective efficacy of azithromycin was 98% for *P. vivax*** (95% confidence interval [CI] = 88-100%). There were too few cases to assess the efficacy of azithromycin against *P. falciparum* (PE = 71%, 95% C = -14-94%). This clinical trial with azithromycin conducted in western Thailand clearly **established azithromycin efficacy for the prevention of *P. vivax* malaria**. In addition, the authors also point out that azithromycin was **safe, well tolerated and showed high efficacy against *P. vivax*.**

2. DISCOVERY OF NEW GAMETOCYTOCIDES TO BLOCK MALARIA TRANSMISSION (A STEP TOWARDS MALARIA ERADICATION)

2.1 ARTEMISININ (QINGHAOSU) – A NEW GAMETOCYTOCIDE

a) A **new gametocytocidal drug for malaria discovered (1989)**

b) The drug **stops malaria transmission**

c) 5 mg/kg dose **kills gametocytes** of *Plasmodium cynomolgi* B in monkey

d) This is **first report to show artemisinins can kill sexual stages of Plasmodia**

e) The drug kills gametocytes but has no sporontocidal action

The status of the development of new gametocytocidal/sporontocidal drugs which can interrupt transmission of both *Plasmodium falciparum* and *Plasmodium vivax* malaria has not changed since the publication of the WHO report on Chemotherapy of Malaria (1955). **Primaquine is the only gametocytocidal drugs in clinical use to stop transmission of all the species of human malaria**. Besides, pyrimethamine and proguanil exert powerful sporotoncidal action. According to a WHO report on Tropical Disease Research (1982), studies on the gametocytocidal efficacy of sesquiterpene lactones including qinghaosu (artemisinin) and its derivatives namely artesunate, artemether and arteether had been initiated but no results had been presented in that report. **A Chinese group (1982)** published review on chemistry and **clinical applications of artemisinin and its derivatives (artemether and artesunate)** suggested that **these compounds did not kill the gametocytes of *P. falciparum***. Besides **no gametocyte infectivity studies in mosquitoes had been conducted.**

Mosquito infectivity experiments using simian malaria model **(*Plasmodium cynomolgi* B infection in rhesus monkey)** provides **a valuable test system for the demonstration of gametocytocidal/sporontocidal efficacy of primaquine (Omar *et al.* 1973).**

The details of doses of **artemisinin used in 5 monkeys to study the gametocytocidal activity** of this drug together with the percent infectivity data of mosquitoes and the mean oocyst count (±SD) in each batch of mosquitoes, fed before and after artemisinin administration to infected monkeys are given in the **Table 72** Pretreatment (control) feedings were invariably carried out at -1 h and in some cases 24 or 48 h before drug administration and all the batches of mosquitoes fed on different

monkeys showed high infection rate ranging between 57.14 and 95.00% with mean oocyst count ranging from 5.5±5.5 to 154.05±96.69. Initial infectivity experiments carried out after administration of **3 doses of 10 and 20 mg/kg of artemisinin at 24 hour intervals showed that within 24 h of the first dose the mosquito infectivity rate and oocyst count was completely abolished**. Post-treatment feedings at 48 and 72 h i.e. 24 h after the 2nd and 3rd dose, respectively, also showed no infectivity. When a **single dose of 10 mg/kg of artemisinin** was given the mosquitoes infectivity was reduced within 6 h from 86.36% (pretreatment control) to 73.07% and oocyst count was likewise reduced from 154.05±96.69 to 14.73±13.95. Feedings carried out at 18 and 24 h post-treatment showed no infectivity in the mosquitoes as shown by gut examination and oocyst count was nil. Experiments carried out with **5 mg/kgx2 doses of artemisinin**, also showed no oocyst development. Finally, **a single 5 mg/kg dose was tested in 1 monkey** (No. 4905) and this dose was **found to abolish infectivity within 24 h as well as at 48 h**.

The blood slide examination showed that **artemisinin is a very quick acting drug**, which started affecting the morphology of both the asexual parasites and gametocytes, and their numbers started declining within 4 h of treatment, by 18 h there was progressive clearance of asexual parasites and some gametocytes were still persisting till 24 h though they were non-infective. There was recrudescence of parasitaemia after 5 and 10 mg/kg doses since the curative blood schizontocidal action was obtained at 20 mg/kg x 3 day dose schedule.

The results (Table 73) show that percent mosquito infectivity and mean oocyst counts of the mosquitoes fed on **vehicle (oil)-injected monkeys** were not appreciably affected or reduced by oil administration.

For assessing the **sporontocidal activity** of the drug, 1-day to 4-day-old infected batches of mosquitoes were given a blood meal on healthy (normal) monkey administered two doses of artemisinin (20 mg/kg) prior to the blood meal. The results of the experiment (Table 74) show that **developing oocysts on the gut were not affected** indicating the **lack of sporontocidal action** of the drug on development stages of the oocysts.

Omar *et al.* (1973) have reported that ***P. cynomolgi* B infection in *A. maculatus*** provides an **ideal screening model for gametocytocidal/sporontocidal activities of**

antimalarials. Primaquine, the known gametocytocidal drug administered at 1.95 mg/kg oral dose to a *P. cynomolgi* infected rhesus monkey, was reported to abolish the mosquito infectivity and produced highly significant reduction of mean oocyst count in the gut when the batch of mosquitoes was fed 24 h after primaquine administration.

In the present study, the pretreatment feedings done on 8 gametocyte-carrying monkeys, which were subsequently given artemisinin, showed on average good infectivity and mean oocyst counts in different batches of mosquitoes. **Artemisinin administration at doses ranging from 5 to 20 mg/kg completely abolished mosquito infection as well as oocyst development**. Even **a single intramuscular dose of 5 mg/kg artemisinin produced complete sterilizing action on gametocytes within 18-24 h.**

The primary action of the drug seems to be gametocytocidal as well as blood schizontocidal. The sporogonic developmental stages in the mosquitoes do not seem to be affected by the drug. The gametocytocidal action reported here is due to specific action of artemisinin. Since the parasite used in this study (*P. cynomolgi* B) is biologically related to *P. vivax* in its relapse patterns and its chemotherapeutic response to standard drugs, it is hoped that **artemisinin which has shown gametocytocidal activity** in our simian model would be **useful for interrupting transmission of *P. vivax* and *P. falciparum*.**

Toxicity studies on **qinghaosu** published in linterature show that **the drug is relatively safe, the LD_{50}** in mouse being **5,105 mg/kg orally** and **2,800 mg/kg intramuscularly**. In monkeys the dose of 24 mg/kg in oil suspension for 14 days was safe and well-tolerated.

Our studies have shown that even **one dose of 5 mg/kg artemisinin** is able to **abolish mosquito infectivity** though its **curative action as blood schizonticide** against *P. cynomolgi* B is achieved at **20 mg/kg x 3-day intramuscular** treatment schedule.

Subsequent to the **discovery of the gametocytocidal action of artemisinin** against *Plasmodium cynomolgi* B (a human transmissible simian malaria infection) by the authors laboratory (Dutta, G.P., Tripathi, R. and Vishwakarma, R.A. (1989) Chemotherapy, Basel, 35, 200-207) which first reported the transmission blocking activity of artemisinins because of the sterilizing effect on the gametocytes in the monkey, **further experimental evidence was provided in series of publications** by the author's group on gametocytocidal action of **other artemisinin derivatives** including

gametocytocidal effect of single dose of **α/β-arteether** (both injectible and orally administered drug) by **Tripathi, Dutta and Vishwakarma 1990,1992,1996).**

STUDY OF THE GAMETOCYTOCIDAL/SPORONTOCIDAL ACTION OF QINGHAOSU (ARTEMISININ) BY ELECTRON MICROSCOPY

A search for a **new gametocytocidal drug to stop malarial transmission has continued** since the **first such drug, primaquine, was discovered (Sinton and Bird, 1928).** Efforts are continuing to **replace primaquine**, the most active gametocytocidal drug, because of its **adverse side effects** (**Covell *et al.*, 1955**). Studies on "the gametocytocidal efficacy of sesquiterpene lactones, including qinghaosu (artemisinin), have been initiated by the World Health Organization, but results are unavailable to date".

Preliminary studies by Chinese scientists suggested that artemisinin and its derivatives (**artemether and artesunate) failed to eliminate or kill the gametocytes** of *Plasmodium falciparum* (**Anonymous, 1982**). However, these investigators did not conduct gametocyte infectivity studies in mosquitoes. Peters *et al.* (1986) reported the absence of gametocytocidal activity of artemisnin against *Plasmodium yoelii nigeriensis*. They exposed the parasite to *in vivo* action of the drug for only 1 hr. Gametocytocidal efficacy of primaquine against *Plasmodium cynomolgi* was reported by Omar *et al.* (1973), whereas proguanil and pyrimethamine were found to be sporontocidal (Omar *et al.*, 1974). The **gametocytocidal efficacy of artemisinin at 5 mg/kg single intramuscular** dose was reported using the *P. cynomolgi-Anopheles stephensi* model (**Dutta *et al.* 1989**). The present communication provides **scanning electron microscopical evidence** for the gametocytocidal action of artemisinin. No sporontocidal effect was found.

Gametocytocidal test

As summarized in Table 75, the infectivity data of control *A. stephensi* fed on gametocyte-carrying rhesus monkeys show a high infection rate of the mosquitoes (86.36 and 95.00%). The mean oocyst count of different batches was 154.05±96.69 and 25.52±15.48, respectively.

SEM study of infected gut showed numerous oocysts that develop to a large size by day 8 (Figs.1,2).

To establish the gametocytocidal activity of artemisinin, the drug was administered at 5-10 mg/kg doses to gametocyte-carrying monkeys, and mosquito feeding was done 18-24 hr after drug administration. Dissections of these mosquitoes 8-10 days later showed **complete absence of oocyst development in the guts** (Table 75). A dose of 10 mg/kg of artemisinin could **sterilize gametocyte infection in 18-24 hr, and 78 mosquito dissections** in this batch showed **no infctivity**. Likewise a **5 mg/kg dose abolished infectivity in 246 mosquitoes** that were dissected individually.

Mosquito infectivity was abolished partially within 6 hr of artemisinin treatment. The oocyst number per gut was considerably reduced (Fig. 3). The action of drug in 6 hr was incomplete.

Treatment with 5 mg/kg artemisinin was found to abolish oocyst development on the gut 18 hr after treatment (Fig. 4). The high dose (10 mg/kg) also abolished oocyst development completely.

Sporontocidal test

One-day-old infected mosquitoes, given a blood meal on a drug-treated healthy monkey, developed normal oocysts at an 82.60% infectivity rate (mean oocyst cound 42.50±30.66). Similarly, 2-day-old infected mosquitoes that were allowed blood meals had 96% infectivity and normal oocyst development (36.24±31.12) (Fig. 5). The infectivity of 3-day-old mosquitoes after drug treatment was 96.00% and the mean oocyst count was 25.6±16.76 (Fig. 6). Similarly, after drug treatment, 4-day-old mosquitoes showed 95.00% infectivity with a mean oocyst count of 41.95±30.50. Overall, results show that **sporogony in 1-4-day-old infected mosquitoes is not interrupted or affected by artemisinin.**

Action of vehicle (ground nut oil) on infectivity

Three milliliters of oil given to a gametocyte-carrying rhesus monkey did not affect the mosquito infectivity or the oocyst counts on the mosquito's gut (Table 75).

The choice of a reliable gametocytocidal drug, which should be active against all species of human malaria, is limited to **primaquine**. Because of its toxicity and side effects, including methemoglobin toxicity, hemolytic anemia in G-6-PD-deficient cases,

epigastric distress, and hepatotoxicity, it **is not universally acceptable**. **Efforts are continuing to develop a safe non-8-aminoquinoline gametocytocidal drug (Wernsdorfer and Trigg, 1987). Qinghaosu (artemisinin) is known to be a highly active gameto-cytocidal drug** against *P. cynomolgi* B. A single intramuscular dose of 5 mg/kg is capable of abolishing gametocyte infectivity of the mosquitoes (Dutta *et al.* , 1989). The present **study provides SEM evidence** to demonstrate the gametocytocidal action of atemisinin though it has no sporontocidal effect.

2.2 ARTEETHER α/β – A NEW GAMETOCYTOCIDE

2.2.1 Areether α/β (30:70) Intramuscular Formulation

A New Safe Gametocytocide – Transmission Blocking Drug for *P. falciparum*

10. **Fast-acting blood schizontocide** for *P. falciparum*, *P. vivax* **(Dutta *et al.* 1987/Patent 1990 CSIR-CDRI/CIMAP).**
11. Active against chloroquine/Mefloquine/Quinine resistant/**multidrug resistant complicated malarias (*P. falciparum/P. vivax*).**
12. Active against **uncomplicated/severe complicated malarias**.
13. Powerful injectible α/β-arteether containing 150 mg drug, **formulated in double refined, neutralized ground-nut oil for the control of comatose/cerebral malaria cases.**
14. Safe intramuscular injection (3 x doses),having successfully completed Phase I, II, III, **post-marketing (IV) clinical trials**.
15. α/β-arteether exerts **strong gametocytocidal (transmission blocking action)** against *P. cynomolgi* B and *P. falciparum.*
16. α/β-arteether is effective against **simian malaria *P. knowlesi, P. cynomolgi* B, *P. fragile*)** and **zoonotic *P. knowlesi* (monkey) malaria infections/epidemics** in human subjects.
17. α/β-arteether **pharmacokinetics** showed that each injection dose is **effective beyond 30 hrs**, and **antimalarial action of 3 dose** (IM injections) **extends beyond 120 hrs.**

18. **Both α and β annomers (30:70)** in α/β-arteether exert **powerful synergistic antimalarial action** against MDR malaria/cures highly multidrug resistant *Plasmodium yoelii nigeriensis* MDR strain (in standard 4 dose IM treatment schedule in Swiss mice) developed at CDRI.

α/β-Arteether Major Activities

10. α/β-Arteether is a semi-synthetic compound derived from Artemisinin (Qinghaosu) in 1987. This **compound has been developed jointly by CSIR-**Central Drug Research Institute, and CIMAP, Lucknow. After extensive preclinical, clinical and toxicological safety studies, the compound has been well accepted as the **most effective emergency drug against virulent MDR *P. falciparum*** and chloroquine/mefloquine/quinine resistant malarias.
11. It rapidly kills asexual stage of MDR *P. falciparum* and considered most efficacious in the treatment of severe complicated *P. falciparum* malaria.
12. Indian experience has demonstrated that arteether provides **93-100% cure rate in uncomplicated *P. falciparum* malaria** and **91.5-100% cure rate in complicated *P. falciparum* malaria.**
13. The drug α/β-arteether is lipophilic, helps greater accumulation in brain tissue and is a **drug of choice for the treatment of dreaded cerebral malaria** caused by *P. falciparum/P. vivax.*
14. No drug related side effects. Drug is safe and ensures good patient compliance.
15. Discovery of **gametocytocidal action of artemisinin (Qinghaosu)** was first discovered by Dutta *et al.* (1989) indicating the transmission blocking activity of artemisinins. The α/β-arteether also **exerts gametocytocidal action against simian parasites** (Tripathi *et al.* 1990, 1996) **and *P. falciparum*** (Dutta *et al.* **2001 US Patent**) 6326023.
16. **National Drug Policy on Malaria – India** Directorate of National Vector Borne Disease Control Ministry of Health and Family Welfare Programme (**Government of India,** 2010)

    Arteether injection for severe malaria approved by NAMP.

17. **Malaria WHO Report: RBM** – The Malaria Problem in South-East Asia Region **Success Stories in Malaria Control from the SEA Region**

    α/β-Arteether : α/β-arteether (30:70 ratio) is racemic mixture of ethyl derivative of artemisinin developed at the Central Drug Research Institute, Lucknow, India. Mulcti-centric studies in over 500 patients have confirmed the efficacy of drug in uncomplicated and complicated falciparum malaria with recrudescence rate of about 8%. The drug is produced by Themis Chemicals, Mumbai, India. It is available throughout the country and has been introduced in the treatment of severe malaria by the NAMP.

18. **CDS/RBM Report by Dr. Andrea Bosman**: Roll Back Malaria dated 18 March, 2002 on α/β-arteether : α/β-arteether was registered as **E-MAL®** in India for the treatment of acute uncomplicated **multi-drug resistant falciparum malaria** and **for severe malaria in January 1997**. The drug released for marketing in **April 1997**. The external packaging and the package insert clearly indicate that the drug is for **hospital use only**.

α/β-arteether : Capability of Production (1997-2001) : Production of α/β-arteether is by a plant which is **WHO approved for GMP** and conforms to international standards. Themis reports that it has a **100,000 ampoule/month** capacity for production and, since **registration 1997**, 1,752,233 adult treatment doses have been sold in India (129 891 in 1997, 262 117 in 1998, 356 160 in 1999 and 1,004,065 in 2000 and 2001).

WHO Recommendation

Although WHO guidelines on the specific use of α/β-arteether do not exist, the Company's recommended 3-day dosage regimen does not **conform with WHO recommendations that state that monotherapy with regimens of artemisinin or its derivatives of less than 7 days should not be used because of the unacceptable high levels of recrudescence. No comparative clinical trial of artemotil and α/β-arteether available. α/β-Arteether is probably cheaper to produce as it does not require the chemical step to produce the pure β form from the chiral mixture.**

Authors Comment: Roll Back Malaria/WHO/TDR/Any International Agency may kindly conduct three clinical trials to compare curative action of 3 dose α/β-arteether (IM)

and Artemotil (3 doses). Three doses (150 mg injection or oral formulation can sterilize *P. falciparum* gametocytes and block malaria transmission).

Criteria Used For Selection of α/β-Arteether (30:70) Intramuscular

- **No *in vitro* screening test was used for selecting α/β-arteether**.
- Rodent Malaria Parasite – *Plasmodium yoelii nigeriensis* MDR selected at CDRI was used for **Drug Discovery of Antimalarial α/β-Arteether and several other blood schizontocides in the Primary In vivo Screening Protocol using rodent model (Random bred Swiss mice) at CDRI.**
- *P. yoelii nigeriensis* MDR is 100% fatal in Swiss mice 18-22 g).
- This strain was procured from the London School of Tropical Medicine & Hygiene (showing innate resistance to only chloroquine). This strain was subsequently exposed to a large variety of standard drugs and antibiotics or combinations and has been designated as MDR *P. yoelii nigeriensis* CDRI strain, which was developed by the author and cryopreserved from time to time and also passaged in Swiss mice. This strain has been used at CDRI for drug discovery of new antimalarials (fast-acting blood schizontocide) including α/β-Arteether.
    7. *P. yoelii nigeriensis* has also been used as primary strain for selection of α/β-Arteether (Dutta *et al.* 1989b).
    8. *P. knowlesi* WI simian malaria (Bajpai *et al.* 1989a).
    9. *P. cynomolgi* B simian malaria (Dutta *et al.* 1988, Tripathi *et al.* 1991)
    10. *P. fragile* (Tripathi *et al.* 1996)
    11. *P. coatneyi* (Dutta *et al.* 2003)
    12. *P. falciparum* (Dutta *et al.* 1990) and U.S. patent 2001

α/β ARTEETHER (INTRAMUSCULAR) – A NEW GAMETOCYTOCIDE

a) A new sesquiterpene lactone **α/β-arteether is a fast-acting** and **safe gametocytocide** discovered (1989, 1990).
b) Transmission blocking activity against *P. cynomolgi* B malaria established (1990).
c) Three IM doses (150 mg x 3 injections) sterilize the early stages/maturing gametocytes (stage I, II, III) of *P. falciparum* from clinical cases.
d) Potential drug for control of multi-drug resistant malaria (*P. falciparum* and *P. vivax)*.
e) The drug has no sporontocidal action.

Efforts to develop a non-8-aminoquinoline which would be able to interrupt malaria transmission have been in progress ever since the toxicity of primaquine was recognized (Covell *et al. ,* 1955). The World Health Organization had accorded a **high priority** towards the development of a new, **safe gametocytocidal drug** to substitute primaquine (**WHO, 1984, 1987**).

We have recently reported high gametocytocidal action of artemisinin against the simian malaria parasite *Plasmodium cynomolgi* B. This paper **reports gametocytocidal efficacy of an α/β-arteether** against *P. cynomolgi* B.

Gametocytocidal activity

Pre-treatment (control feeding) data on 4 monkeys (nos. 5128, 5384, 5648 and 5971) presented in Table 76 show that *An. stephensi* fed on gametocyte-carrying monkeys produced high infectivity (69.2-100%) with a mean oocyst count of 44.1±21.3 to 126.6±103.4 per gut. Mosquito feeding done within 4-5 hr of **drug administration (5 mg/kg α/β-arteether single dose im)** was able to reduce the infectivity and oocyst count partially. Feeding done at 18-20 hr showed either no infectivity or significantly reduced infectivity (35.7-45%) and the oocyst count per gut was also nil or greatly reduced (2.6±1.9 to 4.4±3.6). **Feeding carried out at 24-26 hr showed complete loss of infectivity.**

Pre-treatment (control) infectivity in the 2nd batch of monkeys (nos. 4541, 5650, and 5758) was 83.33-100%. Administration of 2.5 mg/kg α/β-arteether produced complete sterilization of the gametocytes within 24 hr as shown by complete loss of gut infectivity.

Parasitemia (asexual and gametocytemia) records before and after arteether treatment are given in Table 76. A single 2.5 or 5 mg/kg dose of arteether was effective in clearing asexual parasites within 24 hr of treatment, while the gametocyte number was significantly reduced at 24-26 hr post-treatment. However, these gametocytes were non-infective for *An. stephensi*. Data on oil vehicle control showed normal infectivity 48 and 72 hr after 2 im doses of neutralized arachis oil.

In order to establish that there was no sporozoite development in mosquito batches found negative for oocysts on day 8 after single 5 mg/kg dose administration, homogenates of 30 mosquitoes each from 20 hr and 26 hr batches fed on monkey no. 5971 (Tables 76) were inoculated into 2 naïve monkeys. Neither monkey developed patent infection during the observation period (60 days). Another naive monkey inoculated with homogenates of 19 mosquitoes fed 4 hr after drug administration developed patent infection on day 9 (Table 77), showing that sporozoites were viable. This group had 81.2% infectivity and a mean oocyst count of 98.9±81.9. The salivary gland dissections on day 14 also showed normal sporozoite development at 4 hr, while those taken at 20 and 26 hr did not show any sporozoites.

Sporontocidal action

The study shows **no evidence** of a sponrotocidal effect of α/β-arteether against *P. cynomolgi* B. Further sporozoite transmission studies were carried out to determine the viability of sporozoites in 3 batches of infected mosquitoes, drawn from Exp. 4 and 5 (Table 78), which were fed on α/β-arteether-treated monkeys (40 and 50 mg/kg doses). Homogenates of these 3 batches of mosquitoes gave patent infections in naïve monkeys between days 8 and 12 (Table 79). These transmission studies show no sporontocidal effect, since the sporozoits were fully infective.

Earlier reports from China on *P. falciparum* cases suggested that **artemisinin had no action on circulating gametocytes (China Co-operative Research Group, 1982).** However, these authors did not use mosquito infectivity methods to support their observations. Peters and others (1986) reported a lack of gametocytocidal action of

artemisinin against *P. yoelii nigeriensis.* Consistent gametocytocidal effect was found in rhesus monkeys after 18-24 hr of drug action.

The blood schizontocidal activity of α/β-arteether has been reported and its curative action has been established against several malaria parasites, including *P. cynomolgi* B (Dutta *et al.* , 1989), *P. berghei* (sensitive strain) (Dutta *et al.* , 1987), *P. knowlesi* (Bajpai *et al.* , 1989), and multidrug resistant *P. yoelii nigeriensis* (Dutta *et al.* , 1989). The drug has no causal prophylactic or radical curative activity against *P. cynomolgi* B (Bajpai *et al.* , 1989). The present communication adds to the antimalarial spectrum of α/β-arteether. Like artemisinin (Dutta *et al.* , 1989), α/β-arteether has no sporontocidal action.

2.2.2 α/β-ARTEETHER – A NEW ORALLY ADMINISTERED GAMETOCYTOCIDE TO BLOCK *P. FALCIPARUM* TRANSMISSION

a) A **fast-acting blood schizont** (discovered in 1987); and it is **very good gametocytocide** (published in 1988 and 1996).
b) New transmission blocking drug (**effective by oral administration**).
c) Effective against **MDR malaria**.
d) Safe drug to replace primaquine.
e) α/β-Arteether is metabolized in body to form **dihydroartemisinin** which also possesses high antimalarial activity.
f) α/β-Arteether administration also eliminates early stages of gametocytes of *P. falciparum* in human patients, by virtue of its gametocytocidal activity.
g) Arteether (IM) was marketed in 1997.

Chloroquine-resistant malaria is spreading at an alarming rate and this spread has occurred presumably through infected individuals moving from village to village, seeding local anophelines along the way and over long distances. It is, therefore, important to **treat gametocyte carriers in order to block the transmission of malaria through the use of a safe gametocytocide**. Primaquine had been the only available drug that was in clinical use for the last sixty years in order to stop the transmission of chloroquine-resistant MDR *Plasmodium falciparum*. The drug **primaquine is contraindicated** during pregnancy as well as in infants, children and glucose-6-phosphate-deficient subjects (Bajpai *et al.* , 1989). Earlier, we had reported that an injectable intramuscular (im) preparation of α/β-Arteether was a fast-acting blood schizontocide that was highly effective against *P. knowlesi* infection in the rhesus monkey, which is an experimental model for cerebral malaria (Bajpai *et al.* , 1989). Its gametocytocidal action against *P. cynomolgi* when administered by the im route, had also been reported (Tripathi *et al.* , 1990). Since it would be difficult to provide this injectable arteether therapy in rural areas without a paramedical staff, it was decided to evaluate the **gametocytocidal activity** of this preparation, which could be administered **by the oral route**.

Gametocytocidal activity. Doses of arteether ranging from 1.25 to 15 mg/kg were tested for gametocytocidal action against *P. cynomolgi* B. Pretreatment (control)

mosquito infection based on oocyst detection was 65-100% and the mean±SEM oocyst count was 16.52±2.35-55.75±5.37 in different experiments. The infection rates of the mosquitoes after drug treatment are given in Table 80. Results indicate that single doses of 1.25 and 2.5 mg/kg given orally to gametocyte-carrying monkeys marginally decreases oocyst development in the mosquitoes, while the next highest dose, i.e., single dose of 5 mg/kg, was able to block oocyst development in the mosquitoes temporarily (20-18 hr). **The end-point effective dose, which could completely block both oocyst development and sporozoite formation in mosquitoes was established at 10 mg/kg given in two divided doses or at a single dose of 10 mg/kg.**

Mosquitoes fed on drug-treated monkeys were maintained up to 14 days and salivary gland dissections showed no sporozoites at doses of 10 and 15 mg/kg. Homogenates of these mosquitoes, when inoculated into naïve monkeys, did not produce any infection, as evidenced by the negative blood films examined daily for 60 days (Table 81).

Sporontocidal activity: This study showed no evidence for a sporontocidal effect of areether.

In this study, we have shown the gametocytocidal activity of α/β-arteether after oral administration. We had previously reported the gametocytocidal action of this drug at a single dose of 2.5 mg/kg given by the im route (Tripathi *et al.* , 1990). This study showed that oral doses of 1.25 and 2.5 mg/kg failed to block mosquito infections, while a dose of 5 mg/kg eliminated infections in mosquitoes fed on monkeys between 20 and 48 hr after drug administration. However, two divided doses of 5 mg/kg or a single dose of 10 mg/kg by the oral route produced a complete gametocytocidal effect within 24 hr after administration. This dose was sufficient to sterilize the gametocytes in the host and they failed to develop into oocysts/sporozoites in the vector. This is the **first report in which the gametocytocidal action of α/β-arteether by oral administration has been shown**. **This drug will be helpful in the control of malaria transmission, especially in areas where** multidrug-resistant malarias are prevalent and primaquine cannot be given for mass therapy because of its toxicity (conversion of hemoglobin to methemoglobin) and its restricted use in infants, children, and pregnant women (Carson, 1984). It was anticipated that data provided on the gametocytocidal action of artemisinin, α/β-areether

as well as **β-arteether against *P. cynomolgi* B** would be applicable to sterilize *P. falciparum* gametocytes since the activity of artemisinin reported in 1989 with *P. cynomolgi* transmission blocking model was confirmed in *P. falciparum* clinical cases (Chen *et al.* , 1994). Areether is safe and nontoxic up to a single dose of 1 g/kg in mice, and it is known to **produce no metHb toxicity in normal or pregnant rhesus monkeys**. **Brewer and others (1994)** have reported that arteether (5-10 mg/kg) administered for 28 days **did not produce any neurotoxicity in dogs**. International Patent (Dutta, G.P. *et al.* 2000) further established the gametocytocidal action of α/β-arteether which can block *P. falciparum* transmission.

Transmission blocking activity of α/β-arteether formulation in clinical malaria case (*P. falciparum*)

P. falciparum clinical cases (No.=8) which were gametocyte carrier were administered arteether formulation parenterally (intramuscular) at 150 mg dose (in neutralized ground nut oil) x 3 days for treatment of malaria, and within 48 h both asexual parasitaemia and gametocytes were completely cleared from the blood stream. Out of 8 cases, 7 cases were cleared of gametocytes within 24 h of treatment while in the 8^{th} case (Case No. 3) gametocytes were cleared by 48 h. The study shows strong gametocytocidal action of arteether formulation. All cases remained parasite free up to 28 days observation and drug cure rate was 100 percent. For control and total clearance of asexual parasites arteether 150 mg/kg x 3 days treatment is necessary but to achieve gametocyte clearance even one dose (150 mg/kg/60 kg adult) may be given once a week or maximum twice a week to knock out *P. falciparum* gametocytes (Table 49).

2.2.3 SEM EVIDENCE: STUDIES ON THE GAMETOCYTOCIDAL ACTION OF α/β-ARTEETHER IN *P. CYNOMOLGI* B INFECTION

a) SEM evidence is provided to establish the gametocytocidal action of α/β-arteether.
b) **α/β-arteether has potential to block the transmission of *P. cynomolgi* B**.
c) Very low dose of drug sterilizes the parasite development in mosquito mid-gut.

INTRODUCTION

No safe drug is available to stop the malaria transmission. Primaquine is the only drug which is able to interrupt the malaria transmission but this drug is not universally accepted because of its **potential toxicity for pregnant women and infants**, and it is not recommended for administration to **red-cell G-6-PD deficient subjects** (Covell *et al.* , 1955). Efforts are continuing for the last six decades to develop a safe gametocytocidal drug. Authors have recently reported a new sesquiterpene lactone- α/β-arteether as a new gametocytocidal agent (Tripathi etg al., 1990). The present communication presents **SEM evidence regarding the gametocytocidal action of α/β-arteether** (This drug has been **marketed since 1997**).

MATERIAL AND METHODS

Plasmodium cynomolgi B strain was used for rhesus monkey inoculation. One million parasitized RBCs were inoculated intravenously and the course of parasitaemia/gametocytaemia in rhesus monkey was monitored in Giemsa-stained blood films. Fresh lots of 3-4 day-old *Anopheles stephensi* mosquitoes were fed on gametocyte-carrying rhesus monkeys anesthetized with 30 mg/kg thiopanton sodium (May & Baker) intravenously. The fully engorged female mosquitoes were maintained under standard insectary conditions (27±1^{o}C) and dissected after 8-10 days for recording the number of oocysts/gut.

One hour after control mosquito feeding, the drug α/β-arteether was administered to infected monkeys at a doses of 2.5 and 5 mg/kg (single intramuscular dose) dissolved in 1 ml sterile neutralized ground nut oil (oil arachis). Fresh batches of mosquitoes were used for post-treatment feeding on arteether-treated monkeys at different periods 4, 8 and

24 hr after drug administration to study the effect of the drug on mosquito infectivity and oocyst development.

To study the effect of arteether on sporogonic stages, batches of 1-2 day-old infected mosquitoes were given a blood meal on a drug-treated normal monkey that had received single intramuscular dose of 20 and 50 mg/kg drug. Three hours after drug administration the infected mosquitoes were given a blood meal on this monkey. All the mosquitoes were dissected on day 8 after infection and the number of oocysts/gut were recorded.

For scanning electron microscopy, mosquito guts were fixed in 2% paraformaldehyde in 0.1M sodium cacodylate buffer, pH 7.2) at 4°C. Following fixation for 3 hr, the samples were washed in 0.1M cacodylate buffer pH 7.2 twice for 3 hr, then dehydrated in graded alcohols and amyl acetate after which they were dried by critical point method using CO_2 as critical point drying agent. The specimens then were coated with gold and palladium alloy in a Polaron 2000 sputter coater and examined in Philips 515 scanning electron microscope.

RESULTS

Gametocytocidal test

The control infectivity data of *A. stephensi* fed on gametocyte-carrying rhesus monkeys showed a high infection rate of the mosquitoes (77.77% and 89.00%). The mean oocyst count of different batches were 70.37±8.12 and 23.60±2.89 (Table 82). SEM study of infected guts showed numerous oocysts that develop to a large size by day 8 (Figs. 1 and 2). To establish the gametocytocidal activity of arteether, the drug was administered at 2.5 and 5.0 mg/kg dose to gametocyte carrying monkeys and mosquito feeding was done at 4-8 and 24 hr after drug administration. Dissections of these mosquitoes 8 days post-feeding showed the presence of reduced number of oocysts on the guts 4-8 hrs after drug treatment (Fig. 3), but after 24 hrs **complete absence of oocyst development on the guts** had been observed (Fig. 4, Table 83).

Sporontocidal test

Control (pre-treatment) feedings on gametocyte carrying rhesus monkeys showed good infection (72.22 to 100.00%) with mean oocyst count ranging from 29.77-46.1.

Two days old infected mosquitoes were allowed to feed on healthy monkeys treated with 20 or 50 mg/kg doses of arteether, and dissection of these mosquitoes on day 8 postfeeding showed normal oocyst development with infectivity rate 72.72-76.92% and mean oocyst count of 49.37-93.1 per gut. The overall results show that sporogony in 2-day old infected mosquitoes is not interrupted or affected by arteether (Figs. 5 and 6, Table 83).

DISCUSSION

Efforts are continuing for the last six decades to develop a safe non 8-aminoquinoline gametocytocidal drug that can replace primaquine which produces adverse toxic effects including methemoglobinemia, hepatotoxicity and haemolytic anaemia in G-6-PD deficient subjects (Wernsdorfer and Trigg, 1987). **Qinghaosu (artemisinin) a non-8-aminoquinoline had been reported by Dutta *et al.* (1989)** to be highly active **gametocytocidal drug** against *P. cynomolgi* B. Since the above report, the authors have shown gametocytocidal action of a new derivative of artemisinin i.e. α/β-arteether (Tripathi *et al.* , 1990) which is much more potent blood schizontocide than artemisinin (Dutta *et al.* , 1989). The scanning electron microscopic studies have confirmed the gametocytocidal activity of α/β-arteether and a single dose of 2.5 mg/kg has been found to abolish mosquito infectivity completely in 24 hrs after drug administration.

2.3 ARTELINIC ACID – A NEW GAMETOCYTOCIDE

Gametocytocidal Action Of Artelinic Acid-A Fast-Acting Blood Schizontocide

1. **Li *et al.*** (1998a,b) Trans. Roy. Soc. Trop. Med. Hyg. **92**,332-340
   (i) Li *et al.* (1998a,b) had conducted the toxicological and pharmacokinetic study on Artelinic acid. Its sodium salt Artelinate is **fast-acting, water soluble** blood schizontocide and its LD_{50} in rat is 535 mg/kg.
   (ii) Oral bioavailability 79.7% in dogs.
   (iii) Artelinate conversion to dihydroartemisinin by IV, IM, Oral routes in dogs was **very low (0.1-0.5% of total dose) indicating stability of intravenous (IV) administered drug *in vivo***.
   (iv) **Half-life of labeled drug (21-49 h).**
   (v) Artelinic acid is considerably **much more stable than Artesunic acid in plasma.**
2. **Li *et al.*** (1998b) J. Pharm. Pharmacol. 50 173-82-in another study on artelinic acid further reported that:
   (i) After IV injection the **highest plasma level** was obtained with Artelinic acid.
   (ii) Artesunic acid IV showed fastest conversion to DQHS whereas **artelinic acid showed least conversion to DQHS indicating prolonged action *in vivo* and slowest rate of elimination.**

   Conclusion: Artelinic acid IV will be superior to artesunate in acute malarias.
3. **The above Authors Li *et al.* (1998a,b) reported from Walter Reed (USA) that:**

   Artelinic acid (Sodium β-artelinate) seems to be a good candidate; shows highest plasma concentration, high binding to RBC, highest oral bioavailability, longest eliminiation half-life, lowest metabolic rate, lowest toxicity at equivalent dose level as compared to other artemisinins.

Toxicity Studies on Artelinic Acid

Noker, P.E. (2004): Preclinical pharmacodynamic and pharmacokinetic studies of investigational new drugs. U.S. Pentagon Reports (01-March 2004) stated that **no effect**

dose of artelinic acid for the production of neurotoxicity in rats was 20 mg/kg/day qd x 14 days. No effect dose in dogs was 80 and 200 and <400 mg/kg.

Noker, P.E. (1999): U.S. Pentagon Reported that the **radioactivity of C14 artelinic acid in dog was detectable in whole blood and plasma for greater than 192 hrs after oral/iv administered of labeled drug.**

Gametocytocidal Activity of β-Artelinate

The author conducted extensive study on the gametocytocidal action of both α and β-artelinate by IV route (Tripathi, Puri and Dutta, 1996b) and reported the strong gametocytocidal action of β-artelinate against *P. cynomolgi* B infection in rhesus monkey model. The drugs were safe by IV route of administration. The results presented in Table 84 show **complete gametocytocidal action** of sodium β-artelinate against human transmissible *P. cynomolgi* B malaria infection (Tripathi *et al.* 1996b). Sodium β-artelinate is fast-acting gametocytocide at dose ranging from 10 mg/kg-30 mg/kg administered to rhesus monkey by **orogastric tube**, which results in 100% sterilization of gametocytes as indicated by absence of any oocyst development on the mosquito *Anopheles stephensi* gut. Sodium β-artelinate administered by **intravenous route** at 15 mg/kg to 25 mg/kg single dose also sterilizes the gametocyte of *P. cynomolgi* B, resulting in complete loss of gametocyte viability as indicated by absence of oocyst on the mosquito gut. β-Artelinate is water soluble and can be easily administered by IV or oral route. It is an **important new drug to sterilize the sexual stages and stop malaria transmission to mosquito.** This drug has no sporontocidal action (Tripathi, R.,Puri, S.K. and Dutta, G.P. 1996b, *Exptl. Parasitol*. **82** 251-254) (Table 85).

2.4 ELUBAQUINE (BULAQUINE) (CDRI-80/53) – A NEW GAMETO-CYTOCIDAL DRUG

For the last 65 years, **primaquine (an 8-aminoquinoline) is the only drug in clinical use for radical cure of relapsing *Plasmodium vivax*** as well as **for sterilizing gametocytes of *P. falciparum* (WHO, 1955)** which are **responsible for blocking transmission** of *P. falciparum*. National Malaria Eradication Programme of India also recommends the primaquine as the only drug to stop the transmission of *P. falciparum*. Inherent toxicity of primaquine which causes haemolysis and methaemoglobin toxicity in G-6-PD red cell deficient cases, is a universal problem, and efforts of all International Agencies to achieve Malaria Eradication have been unsuccessful. Relentless Global efforts are continuing to develop primaquine substitute which can sterilize gametocytes of *P. falciparum,* thereby interrupt malaria transmission from gametocyte carriers. The Central Drug Research Institute has been successful to develop a New Drug named **CDRI 80/53 (Elubaquine = Bulaquine)** as a primaquine substitute which was first marketed in year 2000 (under the name Bulaquine). **First report of the discovery of this compound CDRI 80/53** was published by Dutta, Puri, Bhaduri *et al.* (1989b) and update review on this compound was published by Dutta and Puri (2003).

a) Bulaquine (Elubaquine) is **8 times safer than primaquine** as shown by **LD_{50} value.**

b) The drug shows **high gametocytocidal activity** against *P. falciparum*.

c) The methaemoglobin toxicity is **4-times lower** as compared to primaquine.

d) The drug has been approved by the Drugs Controller General of India for **control of relapsing malaria.**

e) Among the 8-aminoquinolines evaluated so far, Ebulaquine/Bulaquine is much safer than primaquine.

f) A dose of 75 mg (single dose) blocks malaria transmission in *P. falciparum* by killing the gametocytes.

g) In spite of its methaemoglobin safety, the drug is not recommended in pregnancy, and infants as yet, pending clinical trials in these patients.

h) The compound CDRI 80/53 (Bulquine) had **cleared 3 months Regulatory Toxicity for Antirelapse activity** in healthy Rhesus Monkey Model.

Primaquine, despite induction of haemolytic anaemia in G-6-PD deficient subjects and production of methemoglobinemia, cyanosis, abdominal discomforts, and pruritus in normal patients (Clyde, 1981), **remains the standard for radical cure of *Plasmodium vivax*** as well as having **gametocytocidal activity against *P. falciparum***. Elubaquine is an 8-aminoquinoline anti-malarial, which has shown anti-relapse activity against established sporozoite induced infections with *P. cynomolgi* B in rhesus monkeys (Dutta *et al.* , 1989). The major problems associated with the use of 8-aminoquinolines mainly relate to their toxicity. Compared to primaquine, elubaquine (compound CDRI 80/53, bulaquine) is less toxic since **3-4 times less methemoglobinemia is formed** after multiple treatments in beagle dogs (Puri *et al.* , 1989b). Comparison of methemoglobin levels in human volunteers during Phase I clinical trials also showed that while there was no alteration in the methemoglobin values after elubaquine (25 mg/kg x 7 days) administration, a fourfold increase in methemoglobin levels occurred after primaquine (15 mg/kg x 7 days) treatment (Dutta *et al.* 1994).

Gametocytocidal activity

The assessment of the gametocytocidal activity of the compound has been based on the comparison of mean number of oocysts per infected mosquito and the proportion of mosquitoes with oocysts in mosquito batches sequentially fed on gametocyte carrying rhesus monkeys, before and after, administration of specified doses.

The results of the efficacy tests at **1.25 mg/kg in one** monkey, **at 1.87 mg/kg** in 2/2 monkeys, and at **2.50 mg/kg** in one monkey were identical. The mosquito batches fed at +5 h post-treatment showed **marked decrease in the number of oocysts** though these oocysts were able to complete the sporogonic cycle as indicated by the presence of sporotozoites in salivary glands on days 15-16. The **mosquito batches fed on these monkeys at +24 h and +48 h did not develop any oocysts,** nor were any sporozoites demonstrable in their salivary glands.

Oocyst development was completely blocked in the mosquito batches fed at +5 h as well as at 24 and 48 h post-treatment in one monkey treated at 2.50 mg/kg one monkey treated at 3.75 mg/kg and 2 monkeys treated at 5.0 mg/kg dose. Moreover the salivary gland dissections from these batches carried out between days 14-20 post-infective blood meal also did not show any sporozoite. The asexual parasitemia and gametocytaemia

levels for different monkeys are shown in Table 86. Although the gametocytes persisted in the circulation at +24 and +48 h post-treatment, they were not infective for *A. stephensi* mosquitoes as demonstrated by the absence of oocysts in the midgut preparations.

The present study provides evidence that **elubaquine exhibits marked and rapid gametocytocidal activity,** adding to the anti-malarial spectrum of this drug as an **anti-relapse** (Dutta *et al.* ,1989) and **causal prophylactic** agent (Puri and Dutta, 1990). The results provide an argument for the assessment of gametocytocidal activity of elubaquine against *P. falciparum* malaria in humans. Based on comparisons with human dose for radical cure, the expected **dose for gametocytocidal activity would be in the close to 75 mg single dose.** A limited clinical study employing this dose (Gogtay *et al.* 2004) has shown that the **clearance of *P. falciparum* gametocytes was better than with primaquine**. An extended clinical trial for gametocytocidal efficacy could provide the evidence for transmission blocking activity.

GAMETOCYTOCIDE

Current Status Of New Approaches To Develop Gametocytocidal Agents To Stop Transmission Of *Plamosdium Falciparum* And *P. Vivax* Malaria

Primaquine (an 8-aminoquinoline) discovered nearly 60 years ago, is the only gametocytocidal antimalarial drug in clinical use to interrupt transmission of malaria in spite of its inherent toxicity in terms of production of methemoglobinaemia, cyanosis and haemolysis particularly in G-6-PD red-cell deficient subjects, and it is contraindicated for pregnant women, children and infants. According to **WHO (2010) report primaquine is an essential drug as a gametocytocide for interrupting malaria (*Plasmodium falciparum*) transmission and as a radical curative drug for treating relapsing stages (hypnozoites)** in the liver of patients infected **with *P. vivax* and *P. malariae* infections**. Global efforts are continuing to develop safe primaquine substitute to be used as gametocytocide and as safe anti-relapse/radical curative drug.

Global efforts have been continuing to replace primaquine with a safe alternative gametocytocide to stop transmission of multi-drug resistant *P. falciparum* as well as to block transmission of primaquine-resistant gametocyte strains of *P. falciparum* which are emerging in India and other tropical countries where **viable gametocytes survive in spite of use of WHO prescribed primaquine dose (45 mg x 1) for adults.** Reports have also appeared where gametocytes have been found to **carry drug resistant genetic markers** to powerful antimalarial drugs such as chloroquine and sulfadoxine/pyrimethamine etc., which **confer survival advantage to the *P. falciparum* gametocyte producing strains.** There are numerous authentic published reports showing that the drug resistant *P. falciparum* strains are fully resistant to chloroquine, sulfadoxine/pyrimethamine, mefloquine, etc. These **resistant strains produce** significantly **higher level of gametocytaemia capable of producing 15-38 fold higher gametocyte levels in human patients** which promote higher transmission risk and efficiency in spread and transmission of virulent *P. falciparum* strains (**Hallett *et al.* , 2004**). **Pregnant women** carrying still 2-5 fold higher risk of asexual parasitaemia, are likely to be **carriers of higher gametocytaemia levels causing more efficient transmission mechanism and source of spread of malignant *P. falciparum*** in slum areas which are not protected by mosquito nets or insecticide impregnated nets. There are a large number of **symptomless**

***P. falciparum* gametocyte carriers** which pose a potential threat of MDR *P. falciparum* malaria in U.P.

First report on the gametocytocidal action of artemisinin (Qinghaosu) was published from CDRI in 1989 (Dutta, Bajpai and Vishwakarma, 1989) and that of arteether α/β was reported by Tripathi, Dutta and Vishwakarma (1990) using intramuscular route of drug administration. Further studies in eight human *P. falciparum* gametocyte carriers have shown clearance of gametocytes from the blood smears within 48 hr of arteether (150 mg/kg daily) treatment, suggesting gametocytocidal potential of α/β arteether (Table 49). Our studies with simian model (*P. cynomolgi* B) have also shown that **α/β arteether given by oral route** at 10 mg/kg also exerts the gametocytocidal action and the drug **abolishes mosquito infectivity** by sterilizing the gametocytes (Tripathi, R., Dutta, G.P. and Vishwakarma, R.A. 1996. Amer. J. Trop. Med. & Hyg. **54,** 652-654; Dutta and Tripathi, R. 1996. Japanese J. Trop. Med. & Hyg. **24**, Suppl. 1, 65-69; Dutta and Tripathi, 1996. XIV Intern. Congr. Trop. Med. Malaria. Abs. B-83-5, p. 149). Chinese investigators have confirmed our findings on gametocytocidal action of artemisinin and its derivatives (Chen Pei-quan *et al.* , 1994. Chinese Medical Journal **107**, (9), 709-711) and three abstracts (Chen, P. Q. *et al.* a,b; Li Go *et al.* , 1996. XIV Intern. Congr. Trop. Med. & Malaria Abstr. Nos. 0-22-2, 0-22-3 and 0-22-4,p. 211) presented at the XIV International Congress of Tropical Medicine and Malaria (Nov. 17-22, 1996) at Nagasaki.

Sodium β-artelinate has been identified as a **potent gametocytocide** against *P. cynomolgi* B in rhesus monkey model. A dose of 15 mg/kg by iv route and 10 mg/kg by oral route is able to produce 100% gametocytocidal effect, stopping transmission of *P. cynomolgi* B to *Anopheles stephensi* (Tripathi, Puri and Dutta, 1996. Exper. Parasitol. **82**, 251-254). This is a water soluble fast acting artemisinin-derivative which can be given by iv route.

Emergence of multiple drug resistant strains of *Plasmodium falciparum* and transmission of highly drug resistant *P. falciparum* are threatening the global efforts to control the spread of malaria in most of the tropical countries. Spread of strains of *P. vivax* resistant to chloroquine combined with failure of standard primaquine regimen (e.g. 15 mg/kg x 7-14 days) to produce radical cure of *P. vivax* have contributed to increased

prevalence of certain *P. vivax* malaria strains. Emergence of primaquine resistant strains of the gametocytes of *P. falciparum* are also being reported in clinical studies from India (**Gogatay *et al.* 1999**) with the result that the primaquine standard dose (45 mg/kg x 1) used since 1945, now fails to stop transmission of malignant *P. falciparum* in many tropical countries. Although asexual blood stages of *P. vivax* have acquired chloroquine resistance, there is great possibility that *P. vivax* gametocytes which were earlier sterilized by chloroquine therapy, would also show chloroquine resistance resulting in its failure to stop transmission of *P. vivax*.

The problem of drug resistance of asexual stages and gametocytes of *P. falciparum* as well as lack of curative effect of radical curative primaquine regimens against *P.vivax*, are compounded because of emergence of insecticide DDT/Malathione resistance among the Anopheline vector species.

Studies carried out at **this Institute** have given a **new hope to deploy artemisinin and its derivatives as safe gametocytocides to stop malaria transmission** to laboratory bred *Anopheles stephensi* by virtue of their sterilizing action on gametocytes. **First report** on gametocytocidal action of artemisinin (from plant *Artemisia annua* Lin.) in simian malaria was published in 1989 (Dutta *et al.* , 1989, 1996) which was reported to be a **safe gametocytocide** due to its sterilizing action on gametocytes of *Plasmodium cynomolgi* B which is human transmissible simian malarial parasite.

Asymptomatic gametocyte carriers from areas **with multidrug resistant *P. falciparum*** (resistant to chloroquine, quinine and sulfadoxine/pyrimethamin etc.) have been reported from Calcutta (West Bengal) adjoining Bangladesh Border. These gametocytes carriers which remain undiagnosed also cause a potential threat of transmission of drug resistant *P. falciparum*. There are also reports in literature that viable gametocytes continue to circulate after quinine therapy for more than 20-25 days. Even in hospitalized cases treated with sulfadoxine/pyrimethamine, bone-marrow aspirations have shown high gametocytaemia level of immature stages in *P. falciparum* cases.

Transmission potential of drug resistant markers (pfcrt76T and pfmdr1) carrying gametocytes of *P. falciparum* was reported by **Hallett *et al.* (2004)** following treatment of the Gambian children for three days with chloroquine alone or chloroquine +

artesunate combination. Following the drug treatment with the antimalarials, the gametocyte transmission potential was estimated on the basis of number of oocysts on the gut of mosquito following drug treatments.

Hallett *et al.* (2004) studied the effect of oral chloroquine dose (25 mg/kg over 3 days) and compared the data with the oral artesunate (4 mg/kg/day x 3 days) – chloroquine combination.

Table 87

Resistant genotype	Treatment	No. of mosquitoes analyzed	Mean oocyst count	p
pfcrt-76T allel produces 38 x higher oocysts	CQ CQ + AS	592 456	38.29 (12.18-120.3) 0.987 (0.349-2.794)	<0.001 <0.981
Pfmdr1-86Y allel produces 14 x fold higher oocyst	CQ CQ + AS	619 494	14.58 (1.831-116.2) 0.266 (0.076-0.928)	<0.011 <0.038

Hallett *et al.* (2004) reported the results of trials in Farafenni, the Gambia, based on the mosquito infectivity in children carrying gametocytes with resistant markers (pfcrt-76T and pf-86Y). The authors major conclusion are summarized below:

1. Malarial parasites carrying genes conferring resistance to antimalarials such as chloroquine (CQ), and sulfadoxine-pyrimethamine, were reported to provide selective advantage responsible for higher rates of transmissibility to mosquitoes from drug treated patients.
2. The gametocytes from the *P. falciparum* patients with CQ-resistance-associated alleles pfcrt-76T prior to treatment were reported to produce infected *Anopheles*

gambiae mosquitoes with **38 times higher *P. falciparum* oocysts** burden than the gametocytes from patients infected with sensitive parasites (P<0.001). They further reported that the gametocytes from the parasites carrying resistance-associated allele pfmdr1-86Y produced **14-fold higher oocyst burden** than gametocytes infected with sensitive parasites (P=0.011).

3. The antimalarial CQ-artesunate combination treatment, was reported to overcome the transmission advantage enjoyed by the drug-resistant parasites.
4. The authors finally observed that after CQ monotherapy, the **resistant parasites enjoyed 14 to 38-fold higher intensity of transmission to mosquitoes.** Further they observed that the CQ-artesunate only temporarily reduced the gametocyte infectivity but the gametocyte number again showed increase by 3rd and 4th week after treatment.

Hallett *et al.* (2004) provided experimental evidence that CQ/AS combination failed to provide long-term benefit in reducing the spread of drug-resistant *P. falciparum*. Earlier transmission studies in the Gambia had reported that **sulfadoxine-pyrimethamine + artesunate combination** gave more sustained reduction in the gametocyte carriage (Targett *et al.* , 2001). In another earlier study, von Seidlein *et al.* (1998) had discovered that **sulfadoxine-pyrimethamine + co-artemether** combination in the same study area in Gambia, also gave more sustained reduction in the gametocyte carriage.

Chutmongkonkul *et al.* (1992) examined the gametocytocidal action of chloroquine on *in vitro* produced gametocytes of *P. falciparum*. In this study, five-day old gametocytes (stages I and II) of *P. falciparum* obtained from *in vitro* cultures exposed for 48 hour to antimalarial drugs, were fed to mosquitoes to determine the mosquito infectivity. The authors obtained *P. falciparum* gametocytes (stages I and II; 5-6 day old) from *in vitro* cultures, and exposed them to three antimalarials: (1) $5x10^{-8}$M chloroquine (CQ), $5\text{-}7x10^{-9}$M halofantrine, and 10^{-7}M pyrimethamine, for 48 hrs *in vitro*, and the exposed gametocytes were fed to the mosquitoes to assess the inhibitory effect of these drugs on gametocyte development. The authors observed that chloroquine inhibited 51% gametocyte development, halofantrine produced 78% inhibition and pyrimethamine treatment produced 48% inhibition of *P. falciparum* gametocytes compared to the control

cultures. The authors reported that all the three antimalarials did not completely kill the *P. falciparum* gametocytes after 48 hour exposure, and drug treated gametocytes were infective to mosquitoes (*Anopheles stephensi*). Suppression of maturation of earlier/young developing gametocytes of *P. falciparum* was reported to confirm the earlier observations first described by Jeffery *et al.* (1956), Smalley (1977) and Smalley and Sinden (1977).

Earlier investigations by Ramkaran and Peters (1969) and Rosario *et al.* (1988) had reported that chloroquine enhanced the infectivity of chloroquine-resistant strains of rodent malaria infection. Besides Wilkinson *et al.* (1976) had also observed that chloroquine enhanced the infectivity of CQ-resistant *P. falciparum* to *A. balabacersis.*

Chutmongkonkul *et al.* (1992) also demonstrated that pyrimethamine exerted sporontocidal activity – if fed to the mosquitoes with infective blood meal but not after 4 days of infective blood meal. Pyrimethamine was also reported to exert gametocytocidal action on 50% gametocytes. The gametocytes which survived drug action were capable of infection to mosquitoes with higher infection rate than the control. Infection rate:

Control 5.81% vs Pyrimethamine treated= 51.80%

Control 3.70% vs Pyrimethamine treated = 59.37%

After pyrimethamine – all asexual parasites were inhibited by pyrimethamine (10^{-7}M).

Pyrimethamine acts on immature gametocytes (stages I and II) and on young stages of sporogonic cycle of *P. falciparum* in mosquito mid-gut as a result of indirect inhibition of DNA synthesis.

Sporontocidal action of pyrimethamine was reported earlier also by Foy and Kondi (1952); Shute and Maryon (1954), Gerberg (1971); and Teklehaimanot *et al.* (1985).

According to Graves (2005) both chloroquine and sulfadoxin-pyrimethamine (CQ and SD-PY) have been reported to have very limited life time regarding their clinical usefulness for control of *Plasmodium falciparum* because this drug combination has become ineffective in Asia as shown by clinical trials. Besides their poor clinical efficacy in sub-Saharan African countries (White *et al.* , 1999), it has also been claimed that adequate treatment of *P. falciparum* malaria infection would not control the disease transmission through mosquito bites on treated patients. The presence of mature

gametocytes which are unaffected by above combination of drugs, continue to develop into mature gametocytes which are responsible for post-treatment transmission of parasites carrying drug resistant alleles (Sutherland *et al.* , 2002). **With the introduction of ACT (3 days artesunate in combination of standard antimalarials)**, the number of **gametocytes were drastically reduced** resulting in **decreased incidence of malaria transmission (Nosten *et al.* , 2002).** Besides the CQ and SD-Py combination, a six-dose artemether-lumifantrine (co-artemether) therapy in the Gambian children was recorded to reduce gametocyte prevalence, duration of gametocyte carriage as well as infectiousness of the mosquitoes in comparison to treatment with CQ and SD-Py combination. The later gave infectivity of 37% on day 7 after treatment whereas **co-aremether treatment showed no infectivity in the mosquitoes (Southerland *et al.* , 2005).** It may be mentioned that subsequent studies showed that mosquito infectivity was restored beyond 14 days.

Several investigators have reported that adition of artesunate to SD-Py combination was effective in reducing the proportion of gametocyte carriers when compared to SD-Py alone in the Gambian children population.

14 out of 20 children treated with SD-Py alone had shown gametocytaemia on follow-up compared to 4 out of 20 children treated with artesunate and SD-Py combination. Both the treatments failed to eliminate the gametocytaemia when observations were continued for 28 days (Doherty *et al.* , 1999).

Drug resistance in gametocytes against artemisinin and artesunate: *P. chabaudi chabaudi* was exposed to prolonged treatment with artemisinin and artesunate in mice by Afonso *et al.* (2006) and the authors reported to have achieved genetically stable resistance which was reported to be **15-fold against QHS** and **6-fold against artesunate**. Further the resistance up to 300 mg of artemisinin and 60 mg/kg/day of artesunate was stable after cloning and transmission to the mosquitoes. The transmission of gametocytes resistant to high level of artemisinin and artesunate which are proposed to be an integral component of ACT combinations, could eventually lead to the spread of resistant malaria. Consequently, the use of artemisinins as effective component of treatment of multi-drug resistant falciparum malaria, would become doubtful if not in effective. Additionally the demonstration of cross-resistance among the clones to the artemisinin and artesunate,

constitute an additional threat to the successful control of the artemisinins resistant gametocyte carriers.

Doherty *et al.* (1999) reported reduction in *P. falciparum* gametocyte carrier rates in artesunate and S/D combination compared to SD/Py therapy alone. In their study, 70% of SD/PY treated patients were gametocytaemic at some stage of study compared to 20% cases of children treated with combination of artesunate/sulfadoxine. They demonstrated that **artesunate dose was more efficient in treating gametocyte carriers**.

Gogtay *et al.* (1999) reported extraordinary resistance of *P. falciparum* gametocytes following treatment of 45 mg primaquine dose. They further emphasized that 32 out of 49 CQ RI resistant cases (65%) failed to clear gametocytaemia at the standard gametocytocidal dose compared to CQ sensitive parasite infected patients (27%). As **transmission blocking strategy, they have proposed higher doses of primaquine** to be **introduced to eradicate gametocytaemia**.

According to Sowanmi and Fateye (2003) clinical study in Nigerian children showed that SD/Py monotherapy was associated with significant gametocyte carriage and increased gametocytaemia may encourage transmission of SD/Py resistant infection. They also suggested that the continuing use of CQ in CQ-resistant areas and use of SD/Py monotherapy could lead to enhanced transmission of CQ and SD/Py resistant infections.

Gogtay *et al.* (2006) also compared the efficacy of **primaquine 45 mg** with **75 mg dose of bulaquine** for the treatment of gametocyte carriers of *P. falciparum* in Mumbai. They claimed that **bulaquine is a safe alternative to primaquine as a gametocytocidal drug**. On day 15 and beyond, all patients were gametocyte free. Their data further showed that on day 8, 16/31 PQ cases and 7/59 bulaquine cases showed gametocyte viability. Moreover at day 15 and beyond, gametocytes from both the groups were cleared from circulation. The contribution of **Gogtay *et al.* (2006)** clearly established that **bulaquine (single 75 mg dose) was able to clear gametocytes from *P. falciparum* patients faster compared to 45 mg standard primaquine dose.**

Van Seidlein *et al.* (2001) had also observed that use of chloroquine in choloroquine resistant *P. falciparum* areas, or use of pyrimethamine-sulfadoxin in Py/SD resistant areas, showed **enhanced gametocytaemia** from 2.2 to `8% post-treatment in

CQ resistant areas and from 5.5% gametocytaemia to 56.3% following Py/SD treatment in Gambian children where Py/SD is used as first line treatment (Table 88). In these areas, **addition of artesunate to SD/Py was able to reduce post-treatment gametocytaemia**.

Enhanced gametocytaemia associated with emergence of drug resistant Malaria

Problem of enhanced gametocytaemia in drug resistant strains of *Plasmodium falciparum* following antimalarial treatment has been reported by several investigators.

According to results reported by Sowunmi and Fateye (2003), the gametocytaemia in children infected with *P. falciparum* (chloroquine sensitive strain) showed higher level of gametocytaemia compared to the children infected with chloroquine resistant strain. The authors further noted that following chloroquine (30 mg/kg over 3 days) treatment, the chloroquine resistant *P. falciparum* infected 81 children showed significantly higher gametocytaemia (42.0%) on day 7, compared to chloroquine sensitive infected children (n = 141) who showed lower gametocyte counts (12.8%). The authors also pointed out that **chloroquine showed gametocytocidal action against the immature stages** but the **drug was not gametocytocidal against the mature *P. falciparum* gametocytes** as observed earlier by Sinden (1982, 1983).

Suputtamongkol *et al.* (2003) also reported that drug treatment (mefloquine (MFQ) 25 mg base/kg in two divided doses + artesunate (AS) 4 mg/kg x 3 days) combination did not kill mature gametocytes of *P. falciparum* and the combination neither prevented further gametocyte development by their antimalarial action against the precursors of the asexual parasites as well as against the asexual parasites. Another drug **combination (comprising of meflqouine (MFQ) 25 mg/kg + primaquine (PR) 30 mg base single dose) was found to eradicate mature gametocytes of *P. falciparum***. The investigators also reported that primaquine alone was not able to eradicate gametocytes (both on presentation as well as in prevention of subsequent gametocytaemia) when compared to artesunate treatment. Observations on follow-up studies on patients during 42 days post-treatment were further observed to **develop gametocytaemia in 5 patients treated with MFQ + AS combination** and **in 10 patients treated with MFQ + PR combination. Earlier studies on the efficacy of primaquine (Jeffery *et al.* , 1956**;

Rieckman *et al.* , 1969) had reported the gametocytocidal action primaquine against mature gametocytes of *P. falciparum*.

In another study in Senegal, a high level of chloroquine resistance (45%) among *P. falciparum* cases was recorded by Robert *et al.* (1996). A dose of 25 mg/kg of chloroquine (administered in 3 days) showed chloroquine sensitivity in 62% cases, 16% showed RI, 15% showed RII level of resistance. Following chloroquine treatment, the gametocyte rate was reported to be double on day 4-7 post-treatment and incidence of gametocytaemia was three times higher on day 14 in the *P. falciparum* chloroquine resistant cases, as compared to sensitive group.

Fleck *et al.* (1996) had reported the inhibitory action of Atovaquone (compound 566C80) on the very early (intraerythrocytic) stages of *P. falciparum* (NF-54 strain) (i.e. before the stage 2 gametocyte development). They suggested that the site of action of hydroxynaphthoquinones was inhibition of the cytochrome-bc1 complex of the mitochondrial electron transport chain. Atovaquone was reported to inhibit maturation of gametocytes through the blockade of gametocyte recruitment from the merozoite produced by the preceding schizogony.

Ringwald *et al.* (1999) had observed that **neither pyronaridine (32 mg/kg) nor chloroquine (25 mg/kg dose) showed potent gametocytocidal activity against *P. falciparum* gametocytes.** According to their findings, 47 patients (64%) of the 73 *P.falciparum* cases administered pyronaridine, and 96 (21%) of the 77 patients treated with chloroquine which showed no detectable gametocytes on day 0 (before treatment), had subsequently developed gametocytaemia on follow-up.

The authors point out that chloroquine had shown activity against immature gametocyte stages which were sequestered in deeper organs during the maturation process. **Patients treated with pyronaridine were observed more likely to develop post-treatment gametocytaemia than those treated with chloroquine.** The authors finally concluded that **pyronaridine had either exhibited no gametocytocidal activity or lesser activity than chloroquine treatment against immature gametocytes.**

In an earlier study, Bhasin and Trager (1987) had reported the both pamaquine and primaquine blocked the gametocyte infectivity to mosquito, but both were ineffective against the developing oocysts in the mosquitoes. **Their study clearly established the absence of sporontocytocidal activity of pamaquine and primaquine.**

3. NEW ANTI-RELAPSE (HYPNOZOITICIDAL/RADICAL CURATIVE AGENTS FOR TREATMENT OF *PLASMODIUM VIVAX/P. MALARIAE*

3.1 CDRI 80/53 (ELUBAQUINE = BULAQUINE) A NEW ANTI-RELAPSE DRUG

Justification for selecting compound CDRI 80/53 as new radical curative drug

Substitution of primaquine with a safe antimalarial drug for the radical cure of relapsing *P. vivax* malaria and for sterilizing gametocytes of *P. falciparum* has assumed highest priority for National Malaria Eradication Programme because of the inherent toxicity of primaquine.

Although during the last four decades several new 8-aminoquinoline analogues had been synthesized at the Central Drug Research Institute with potential radical curative activity against relapsing simian malaria, the new compound CDRI 80/53 (an analogue of primaquine) was selected by the CDRI-Malaria Group for preclinical development because of its unique biological activities.

1. Significant lower toxicity (**LD_{50} value**) in mice. The compound **80/53 is 8 times less toxic as compared to primaquine.**
2. Definitive **radical curative (anti-relapse),** and **causal prophylactic activities** of compound 80/53 have been established and the compound is superior to primaquine in its antimalarial potentials and safety profile.
3. Compound 80/53 has a weak blood schizontocidal activity like primaquine.
4. Methaemoglobin toxicity of compound 80/53 in beagle dogs is 4-times lower and the compound is, therefore, considered 4-times safer in comparison to primaquine.
5. In view of higher therapeutic index, compound 80/53 was selected for preclinical studies and recommended for general pharmacological screening, long term sub-acute toxicity, teratogenicity and carcinogenicity studies in animal models.
6. The dossier entitled “Data on compound 80/53 – anti-relapse antimalarial” was submitted to Drugs Controller of India and permission to conduct phase I clinical trials was received by the Institute. Bulk production of the drug for clinical trials was taken up in 1988 by late Dr. S.K. Chatterjee.

7. Amongst the radical curative antimalarials (tissue schizontocides) synthesized in India and elsewhere, compound CDRI 80/53 was the first to enter the phase I clinical trials stage.
8. After completing the clinical trials with CDRI 80/53 compound, the Institute received the marketing permission from Drugs Controller General of India in 2000. This is the only compound which can be considered as safe substitute for primaquine.

New Antimalarial Drug Development in India: Radical Curative Agents CSIR-CDRI 80/53 (Bulaquine/Elubaquine) and WR 238605 (Tafenoquine)

Global efforts had been continued for the last 75 years, to find a safe radical curative (anti-relapse) drug to replace primaquine for the treatment of *Plasmodium vivax* and *P. ovale.* Throughout the world, more than 500 potential tissue schizontocides had been synthesized and screened, and two new primaquine derivatives had been **identified at CDRI in primate malaria radical curative tests** which could be considered as the **candidate drugs to replace primaquine**.

INTRODUCTION

The **anti-relapse drug available today** for treatment of relapses of *Plasmodium vivax* and *P. ovale* is **primaquine** which also exerts **strong gametocytocidal action** against *P. falciparum* gametocytes. Primaquine and other 8-aminoquinolines are known to produce a variety of **adverse side-effects** in human subjects such as methaemoglobinaemia with cyanosis, haemolytic anemia in G-6-PD deficient individuals, hepatotoxicity, gastrointestinal distress, epigastric discomfort, anorexia, vomiting, headache and derangement of leucocytes levels (**Clyde, 1981**). Because of its toxicity, primaquine is **contraindicated in pregnancy** and not recommended for use in **infants and children** as well as in individuals whose red cells possess **genetic G-6-PD deficiency**.

Although several other **analogues of primaquine** such as **pamaquine**, **pentaquine, isopentaquine** and **quinocide** had been clinically evaluated, these were found to be either less effective or more toxic than primaquine and were, therefore, superseded by **primaquine** which continues to be the **only radical curative agent in**

clinical use at present (**Covell *et al.* 1955, Davidson *et al.* 1981**). Global efforts had been continuing for the last six decades to discover a safer anti-relapse drug for replacing primaquine, which would not produce significant levels of methaemoglobinaemia and hemolytic anemia, and which could be administered to infants, children and pregnant women without any adverse effect. The **CHEMAL Steering Committee of TDR** of the World Health Organization had also given top priority to study the pharmacokinetics, metabolism and toxicity aspects of primaquine, with a view to identify some less toxic but more active metabolites of primaquine but no viable lead had emerged (Wernsdorfer & Trigg, 1987).

Even before the discovery of tissue phase of malaria by Short and Garnham (1948), the relapses of *P. vivax* had been well-known. **Garnham (1977)** had reported that **relapses were caused by activation of dormant tissue stages**. Krotoski *et al.* (1980) had identified these dormant stages (as **hypnozoites**) in the liver (hepatocytes) *P. cynomlogi* infected rhesus monkeys, which had been infected through sporozoites. Though primaquine was known since long to cure the relapses of *P. vivax* (Edgcomb *et al.* 1950), the demonstration of its efficacy against relapse stages of ***P. cynomolgi* B in rhesus model** was reported by **Schmidt *et al.* (1966).**

Although rodent and avian antimalarial screens for the tissue schizontocidal activity had been extensively used earlier (Davey 1946, Singh *et al.* 1952, Hill 1975, Peters 1980), the predictive value of these models to select compounds that would be active against hypnozoite stages of *P. vivax* in man, had been reported to be very poor (Clark 1946, Schmidt 1983a).

The establishment of sporozoite induced ***Plasmodium cynomolgi* infection** in rhesus monkey through the bite of infected *Anopheles* vector by Schmidt *et al.* (1966) was the starting point for screening of new tissue schizontocides against this simian parasite which **resembles *P.vivax*** in its biological characteristics. ***P. cynomolgi* B strain** is of special importance since it is known to be **human transmissible** and is considered ideal parasite for screening potential radical curative (anti-relapse) agents (**Dutta & Puri 1988, Dutta, Puri, Tripathi and Gupta, 1998**) New Antimalarial Drug Development and Prophylaxis against Malaria. Monograph Published by Central Drug Research Institute, Lucknow (Nov. 1998, pp 1-110).

1. CDRI 80/53 (ELUBAQUINE=BULAQUINE)

In our search for new 8-aminoquinoline analogues with potential radical curative activity against relapsing simian malarial parasite (*P. cynomolgi* B),the compound CDRI 80/53 (Elubaquine – an analogue of primaquine) was selected for preclinical development because of its **anti-relapse (radical curative) activity against *P. cynomolgi* B**, and its higher level of **safety in LD_{50} assay, very low level of methaemoglobin levels produced in beagle dogs, high therapeutic index**, safety in general pharmacological screen and **successful clearance/safety in 90 days sub-acute regulatory toxicity tests in rats and monkeys.** The drug showed complete safety in phase-I clinical trials in volunteers. Anti-relapse efficacy of CDRI 80/53 (25 mg/kg x 5 days) dose schedule is comparable to primaquine (15 mg/day x 5 days) dose. Relapse rate during non-transmission period indicated that placebo (chloroquine) group showed higher relapse rate (10.6%), whereas relapse rate with CDRI 80/53 was (4.9%) and primaquine (3.0%). The major advantage of CDRI 80/53 over primaquine was that it did not produce significant methaemoglobin levels in treated cases. **CDRI 80/53 has been found to be a safe primaquine substitute for radical cure of relapsing *P. vivax* malaria.** Its safety in G-6-PD deficient cases, infants, children and pregnancy has not been established so far.

3.2 TAFENOQUINE (WR 238605) (CSIR-CDRI COLLABORATION WITH WRAIR, WASHINGTON)

Possibilities in the Development Of Malaria Drugs (FrS-12-4)

New alternatives to primaquine

MILHOUS WK, THEOHARIDES AD,SCHUSTER BC, PURI SK, DUTTA GP, HEISEY GB, KYLE DE, ODUOLA AM, DHAR MM, HEIFFER MH, REID WA & DAVIDSON DE JR (1988) (This is the **First Publication on the discovery of WR 238605 by WRAIR & CDRI)**

WR238605 (8-[(4-amino-1-methylbutyl)amino]-2,6-dimethoxy-4-methyl-5-(3-tri-fluoromethylphenoxy) quinoline succinate) has already completed clinical development at the Walter Reed Army Institute of Research as a replacement drug for primquine (PQ), a drug which has a poor therapeutic index (especially in G-6-PD deficient patients).

Tissue schizontocidal cures of established sporozoite-induced *P. cynomolgi* B infections in rhesus monkeys had been achieved at CDRI, Lucknow, at doses of 0.1, 0.316 and 1.0 mg/kg/day given for seven consecutive days by oral administration in combination with a completely suppressive blood schizontocidal regimen of chloroquine. **Calculated CD50's of 0.172 mg/kg/day have demonstrated that WR238605 is 7.4 times as active as PQ as a tissue schizonticide.** WR238605 is also **effective as a causal prophylactic agent** against the pre-erythrocytic tissue stages of sporozoite-induced *P. cynomolgi* malaria in the rhesus model. Animals were challenged with sporozoites on day zero and drug was administered orally on day -1, 0, and +1. Patency was successfully prevented at daily oral doses of 0.1, 0.316, 1.0 and 1.78 mg/kg. The resulting calculated causal prophylactic ED50 of 0.124 mg/kg/day is 10.5 times more effective than PQ against pre-erythrocytic stages of *P. cynomolgi*. Against *P. falciparum in vitro*, WR238605 was found at WRAIR to be 5-fold more active than PQ against chloroquine susceptible parasite clones and 15-fold more active against chloroquine resistant parasite clones.

1. The improved efficacy of WR238605, combined with its **reduced toxicity, good oral bioavailability and longer half-life** make this drug an excellent potential candidate to replace PQ.
2. Besides Tafenoquine, large number of Radical Curative/Causal Prophylactic-Active Compounds have been screened at Primate Malaria Research Facility at CDRI (Professor S.K. Puri, Director, CDRI and G.P. Dutta – unpublished data) under CDRI-WRAIR (Walter Reed) International Collaboration with U.S.

WR238605 (TAFENOQUINE)

Definitive radical curative (anti-relapse) activity of WR 238605 had been established in simian malaria *P. cynomolgi* B screening model, under WRAIR-CDRI collaborative malaria programme. Besides, it has gametocytocidal action also.

Further drug development studies with WR 238605 were carried out at WRAIR Washington. The compound shows high therapeutic index and has successfully cleared 28 days subacute/chronic toxicity, **declared safe in phase-I clinical trials and its high radical curative activity against *P. vivax* (at 300 mg/day x 7 days) has been reported**

by US. Investigators in limited Phase-II clinical trials conducted in Bangkok. The major drawback of WR 238605 is that it raises methaemogloin level significantly comparable to that of primaquine, and its **radical curative dose is very high** and its clinical trials are based on very small number of *P. vax* cases. Further research on clinical trials and its safety in G-6-PD deficient cases has been continuing till to-date. Besides the compound Tafenoquine (WR 238605) has shown potential **prophylactic efficacy against falciparum malaria. It is hoped that compound WR 238605 which has a longer half-life (14 days), will be useful primaquine substitute for treatment of relapsing malaria.**

SEARCH FOR NEW RADICAL CURATIVE (ANTI-RELAPSE) ANTI-MALARIALS

Major efforts had been made in the past by the Walter Reed Army Institute (WRAIR), Washington to organize a large-scale synthesis and screening of new tissue schizontocides on behalf of U.S. Army antimalarial programme and the results had been summarized/published by Davidson *et al.* (1981), Schmidt (1983a,b) and Sweeney (1984). Davidson *et al.* (1981) listed 24 new compounds (Table 92) which showed radical curative efficacy against relapsing parasite *P. cynomolgi* B in rhesus monkey model. The highest activity was observed in 4-methyl-5-phenoxy-8-aminoquinolines (WR 232956, 232584, 233195, 233078 and 225448) which were 4-5 times more active than primaquine.

None of the 24 compounds listed in Table 89, which were found to possess anti-relapse activity, have been pursued further for preclinical development presumably either due to higher toxicity or lower therapeutic index as compared to primaquine.

The biological/antimalarial activity of these very compounds had also been reported by Schmidt (1983a,b) and Sweeney (1984). Out of 200 8-aminoquinoline compounds screened, 98 compounds were found to be active as radical curative agents against *P. cynomolgi* B-rhesus model. Compound WR 225448 was identified as most promising radical curative agent whose activity was 5-times more than that of primaquine. However, a related 4-methyl-5-phenoxy substituted primaquine analogue identified as WR 238605 **(Tafenoquine) was later found to be much more safer by WRAIR** because of its **comparatively lower methaemoglobin toxicity in dogs** and

identified as a candidate drug molecule for development as primaquine substitute (**Milhous *et al.* 1988**).

Chemical synthesis at Central Drug Research Institute attempted **suitable substitution at the primary amino group in primaquine to obtain a still more safer derivative.** This led to the **selection of compound CDRI 80/53 (Bulaquine, Elubaquine)** for further development as radical curative agent.

CDRI 80/53 (Elubaquine=Bulaquine) a New Oral Radical Curative (Anti-relapse) Drug Development

It must be emphasized that although we had identified in 1987 several 8-aminoquinolines as potential anti-relapse agents under our primate anti-malarial screening programme (Bhat *et al.* 1984), the compound CDRI 80/53 was specifically picked-up for further drug development because of its proven anti-relapse activity in simian model, with 8-fold safety on LD_{50} basis compared to primaquine and its very low levels of met-Hb toxicity in comparison to the standard drug primaquine.

Antimalarial Activity

1. Radical Curative Action Against *P. cynomolgi* B

Radical curative (anti-relapse) activity of 80/53 (Elubaquine) was tested against *P. cynomolgi* sporozoite induced infection in rhesus monkey using 7-day radical curative test, and the results were compared with primaquine (Table 90 & 91).

(a) 7- day radical curative test

Compound 80/53 exhibited radical curative (anti-relapse) activity at 1.25 mg base/kg x 7 days in rhesus monkeys (9/9) and cure-rate was 100%. Like-wise higher doses (2.0 to 4.0 mg/kg x 7 days) were also 100% curative in 13/13 monkeys (Table 93, 94) (Dutta, Puri, Bhaduri and Seth 1989, Dutta *et al. ,* 1994). Primaquine produced 100% radical curative activity at 1.0 mg base/kg x 7 days in 12/12 rhesus monkeys.

(b) 3-day radical curative test

Compound 80/53 showed 90% radical curative activity at 2.92 mg/kg x 3 day drug schedule (Dutta, Puri, Bhaduri and Seth, 1989).

2. **Causal Prophylactic Activity**

   CDRI 80/53 curative causal prophylactic dose against sporozoite induced *P. cynomolgi* B was 3.16 mg/kg x 3 days in rhesus monkey model (Table 93 & 94).

3. **Blood Schizontocidal Activity Against *P. berghei* (Sensitive Strain)**

   Compound 80/53 shows weak blood schizontocidal acting against sensitive strain of P. *berghei* and its ED_{90} value is 7.8±0.12 mg/kg and ED_{50} being 3.67±0.64 mg/kg x 4 doses (Kazim *et al.* 1989). The compound showed weak activity against *P. cynomolgi* B.

Methaemoglobin Toxicity

Methaemoglobin (met-Hb) toxicity in beagles is the most important parameter for assessment of the safety/tolerance of new 8-aminoquinolines before any one of them can be selected as a potential candidate compound for further preclinical drug development. Experimental studies with primaquine have shown that beagle dogs (colony bred) are the only animal model suitable for met-Hb toxicity assays since in this model met-Hb levels are raised significantly within 3-7 days of primaquine administration.

The results of met-Hb and related toxicity following 7 days oral administration of compound CDRI 80/53 at 3x times its radical curative dose (3.75 mg/kg/day) showed that the compound is 3-4 times safer than primaquine (Puri *et al.* 1989; Dutta *et al.* 1994) (Table 92).

Test compound CDRI 80/53 was screened for any observable side-effects or toxicity in animal models using a battery of biochemical/pharmacological tests to detect any adverse drug reaction etc. (Tables 93, 94) (Kar *et al.* 1988). The compound was found to be safe and no drug related effect was observed. **On the basis of LD_{50} data CDRI 80/53 has 8-fold safety over primaquine.** Single dose lethal toxicity tests in baby rhesus (4 months age) with CDRI 80/53 also had established high level of safety of this drug (administered intraperitoneally) as compared to primaquine.

Sub-acute Long-term Toxicity

The sub-acute toxicity studies with CDRI 80/53 were conducted in two animal species namely rats and monkeys. Three groups of 20 rats each were administered, **doses of 6.25, 12.50 and 25.0 mg/kg/day orally for 90 days**, respectively. These doses did not show drug related toxicity in rats. Another 20 rats were administered the placebo and all the animals were kept in the same condition of feeding and handling during the period of experiment.

In primate species also, three groups of 4 monkeys each received **drug by intubation at 3.12, 6.25 and 12.5 mg/kg/day and 4th group was given placebo for a period of 90 days**. These doses were **calculated as 2.5, 5.0 and 10.0 times the radical curative dose of the compound**. A daily record of the behavior and general health of all animals was maintained. Body weights and haematological parameters were done initially and then at monthly intervals till the end of the treatment. Biochemical estimation, gross and microscopic examinations of the tissues were carried out after the sacrifice of animals at the end of the 90 day administration. Sections of the organs were prepared, and in addition urinary bladder and gall-bladder of monkeys were processed and studied for histopathology.

The compound CDRI 80/53 was found **safe in long-term sub-acute toxicity** and therefore, recommended for phase I clinical trials in human subjects (volunteers) (Sethi *et al.* 1993, Dutta *et al.* 1994).

Teratogenicity Study

So far as its teratogenicity is concerned, at the dose level of 6.25 and 12.50 mg/kg body weight/rat/day in rats and 3.25 and 6.50 mg/kg body weight/rabbit/day in rabbits, this compound did not show any significant effect on the rate of implantation and resorption. The litter size and weight, etc. were also found to be normal. The compound was administered orally during the period of major organogenesis (Sinha & Sethi, 1994).

Clinical Trials

Clinical Trials (Phase-I)

a) **Single Dose Tolerance Study**:

Phase I single dose tolerance study was undertaken by **Asthana *et al.*** at Central Drug Research Institute, Lucknow. Thirty six human subjects, who executed a written informed consent to undergo the clinical trials as per approved plan and were not found deficient in G-6-PD, completed the study. The dose of the compound 80/53 was gradually escalacted from 5 to 75 mg. In this study, 7 volunteers received placebo, while 4 each were given 5, 10, 20, 30, 40, 50, 60 mg and only one volunteer received 75 mg of compound 80/53 as a single oral dose.

b) **Multiple Dose Tolerance Study**

Multiple dose tolerance study was undertaken at CDRI Clinical Pharmacology Unit, Seth G.S. Medical College, Bombay. Twenty healthy male human volunteers who were not found deficient in G-6-PD, were administered compound 80/53 as 25 mg OD per oral dose for one week. A critical appraisal of the data indicates that no untoward reaction, experiences or drug-related abnormalities could be detected in clinical and laboratory parameters (see Dutta *et al.* 1994).

c) Multiple Dose Tolerance Study with Primaquine

With a view to have a comparative data, 6 healthy human volunteers, who executed a written informed consent, were administered primaquine (15 mg once a day) orally for one week. Comparative data on initial (0 day pre-drug) and final (+ 7 day post-drug) values of haemoglobin, methaemoglobin, prothrombin time, partial thromboplastin time, and fibrinogen in healthy human subjects treated with primaquine (15 mg ODx7 days) and CDRI compound 80/53 (25 mg/ODx5 days) have shown that **one week primaquine treatment leads to rise in methaemoglobin levels from 3.97% to 16.32%,** which is highly significant in comparison to the **2.29% and 3.02% levels of methaemoglobin before and after 7 days treatment with CDRI compound 80/53**, respectively. Thus it is evident that primaquine treatment produces rise in methaemoglobin, while compound 80/53 does not cause appreciable rise in plasma methaemoglobin levels, offering a significant advantage over primaquine (Table 95) (Dutta *et al.* 1994).

Phase II/III Clinical Trials with CDRI 80/53

Phase II/III clinical trials with CDRI 80/53 had been under-taken at **Malaria Research Centre, Delhi.** The results of clinical trials on the anti-relapse efficacy of primaquine (15 mg daily x 5 days was compared with CDRI 80/53 (25 mg daily x 5 days) using standard 5 day regimen for treatment of *P. vivax* cases. Short term follow-up during non-transmission period (Mid November to March) showed relapse rate of 4.9% for CDRI 80/53 and 3.0% for primaquine in comparison to placebo (chloroquine) which gave 10.6% relapse rate. This analysis showed that anti-relapse efficacy of primaquine and CDRI 80/53 was comparable. Long term follow-up studies upto one year showed a relapse rate of 26.8% after primaquine therapy, as compared to 29.6% after treatment with CDRI 80/53 (Table 95) (**Valecha *et al.* 2001**). The relapse rate of untreated (placebo) control *P. vivax* was around 40.1% in Delhi area. In the long term relapse data, the cases showing true relapse and re-infections from endemic area cannot be distinguished. Anklesaria *et al.* (1994) had shown on the basis of *in vitro* study that **CDRI 80/53 would be relatively safer for G-6-PD deficient individuals compared to primaquine**. It may be mentioned that clinical trials in G-6-PD deficient individuals, infants, children and pregnant women had not been conducted so far to ensure the safety of the drug CDRI 80/53 in these cases.

The **drug CDRI 80/53 (Elubaquine=Bulaquine) has been marketed since 2000 in combination with chloroquine as Aablaquin-a safe radical curative (anti-relapse) kit** for treatment of *P. vivax* cases.

Revised Chemical Nomenclature of CDRI Compound 80/53 as per WHO Drug Information, Vol. 13, No. 4, 1999 Proposed List INN : List 82
(CDRI Compound 80/53/Bulaquine/Elubaquine) Anti-relapse Activity of dihydro-3-[1[[4-[(6-methoxy-8-quinolyl)amino]pentyl]amino]ethylidene]-2(3H)-furanone by Krudsood *et al.* (2006) Korean J. Parasit. 44, 221-228

(Figure)

A recent comprehensive clinical study on the **safety, tolerability, toxicity and anti-relapse efficacy of elubaquine (bulaquine = CDRI 80/53)** in *Plasmodium vivax* treatment had been conducted by **Professor Krudsood and Colleagues (2006) in Thailand**. According to this leading report published in (Korean Journal of Parasitology (2006) 44,221-228), the current *P. vivax* treatment comprises of a 3-day course of chloroquine (total dose 1500 mg base), is followed by primaquine 15 mg a day x 14 days. This treatment although resolves acute symptoms and clearance of parasitaemia, nevertheless a substantial number of cases show relapse due to persisting hypnozoites in the liver as reported by Bunnage *et al.* (1994), Looareesuwan *et al.* (1997) and by Wilairatana *et al.* (1999). I wish to report that the chemical structure and nomenclature of CDRI 80/53 (Elubaquine=Bulaquine) reported by Professor Krudsood *et al.* (2006) (based on CDRI 1st publication on anti-relapse compound 80/53 reported by Dutta, Puri, Bhaduri and Seth (1989) in American Journal of Tropical Medicine & Hygiene 41, 635-637, as well as by Dutta *et al.* (1994) in Tropical Diseases: Molecular Biology and Control Strategy, **CSIR Golden Jubilee Symposium Proceedings (Eds. Kumar, S., Sen, A.K., Dutta, G.P. and Sharma, R.N.)** Publication and Information Directorate, Council of Scientific & Industrial Research, New Delhi, India) as N-(3-acetyl-4-5-dihydro-2-furanyl)-N-(6-methoxy-8- quinolinyl)1,4-pentanediamine) **Code Name Compound 80/53 CDRI, has been revised by World Health Organization** and **named as bulaquine (=elubaquine) according to WHO Drug Information Vol. 13, No. 4, p. 268 (proposed INN List 82) and CDRI 80/53 compound is named as dihydro-3-[1-[[4-[[6-methoxy-8-quinolyl] amino]ethylidene]-2(3H)-furanone**. Krudsood *et al.* (2006) evaluated the anti-relapse activity of elubaquine (compound CDRI 80/53) against 141 *P. vivax* malaria cases. Both elubaquine and primaquine were evaluated in two batches of patients following treatment with standard chloroquine, 30 mg/kg given over 3 days. Group A n = 71 patients were administered 30 mg primaquine once daily for 7 days and Group B n = 70 patients were administered 25 mg elubaquine (compound 80/53) (bulaquine) once daily for 7 days. According to their findings, **all patients cleared *P. vivax* blood stage parasites within 7 days after chloroquine treatment. One of the 71 patients treated with primaquine relapsed on day 26 while none of the 70 patients treated with elubaquine (bulaquine) showed any relapse**. As shown in Table 96

patients in group A completed 28 day follow-up (95.7%0 while 64 patients in group B (94.3%) completed 28 day follow-up. **Only one patient in primaquine group relapsed whereas no relapse was recorded in elubaquine (bulaquine) treated group.** Fever clearance time in group A was 18.65 hour (range 4-70 hour) and in group B 22.74 hour (range 4-90 hour). Parasite clearance (mean time) was 43.68 hour in group A and 44 hour in group B. **Serious adverse effects** (cyanosis, abdominal cramps, hypertension, arrhythmias, CNS symptoms etc.) **were not observed in both group A or B**. Both primaquine and elubaquine were **well-tolerated in *P. vivax* cases**. The **authors also observed that 4 patients with G6PD deficiency who were treated with primaquine had clinically significant fall in hematocrit, whereas 3 patients with G6PD deficiency treated with elubaquine did not show significant fall in hematocrit**. The authors finally conclude that elubaquine (bulaquine) (Group B, n=3) treated patients with G-6-PD deficiency did not show clinically significant hemolysis.

WR 238605 (TAFENOQUINE) POTENTIAL ANTI-RELAPSE AGENT

WR 238605 succinate an 8-aminoquinoline was originally synthesized by the Walter Reed Army Institute of Research (WRAIR), Washington, D.C. as a replacement drug for primaquine.

Preclinical Antimalarial Studies

The compound WR 238605 had undergone preclinical primate anti-malarial screening in rhesus monkey – *P. cynomolgi* B model at Central Drug Research Institute under WRAIR-CDRI collaborative programme, and the results were published by Milhous *et al.* (1988), Heisy *et al.* (1988), Puri *et al.* (1994) and Puri and Dutta (1997).

1. **Radical Curative (Anti-relapse) Activity**

Using sporozoite induced *P. cynomolgi* B infection in rhesus monkey, the CD_{50} of WR 238605 given orally was found to be 0.172 mg/kg/day x 7 days. **On CD_{50} basis compound WR 238605 was 7.4 times more active than primaquine in radical curative tests (Milhous *et al.* 1988)**. The **100% radical curative (anti-relapse) dose** of WR 238605 was found to be **0.316 mg/kg/day x 7 days** while the corresponding

primaquine curative dose was 1.00 mg (base)/kg x 7 days (Puri *et al.* 1994). WR 238605 was reported to be 3xtimes more active than primaquine (Table 93, 97).

2. Causal Prophylactic Activity

The CD_{50} of WR 238605 was 0.124 mg/kg/day (x3 doses) in causal prophylactic test against *P. cynomolgi* B and its **primaquine index was calculated to be 10.5 (Milhous *et al.* 1988)**. The 100% curative **causal prophylactic dose** of WR 238605 against *P. cynomolgi* B was **0.316 mg/kg x 3 days** (**Puri *et al.* 1994**) (Table 98). *In vitro* activity of this compound against exoerythrocytic stage of *P. cynomolgi, P. cynomolgi* B and *P. knowlesi* had been reported by Fisk *et al.* (1989).

3. Blood Schizontocidal Activity

WR 238605 exhibited **curative blood schizontocidal action** against both *P. cynomolgi* B and *P. fragile* at 3.16 mg/kg x 7 days. Primaquine used for comparison was curative in 1 out of 4 monkeys at 10 mg/kg x7 days (Table 99) (**Puri *et al.* 1994, Puri & Dutta, 2003**). Earlier *in vitro* studies had shown that WR 238605 was **more active than primaquine as blood schizontocide** as well as **tissue schizontocide** against the pre-erythrocytic stages of plasmodia (**Milhous *et al.* 1991, 1992, Vennerstrom *et al.* 1999**). Four to 100 times higher activity of WR 238605 as compared to primaquine had been reported against drug resistant lines of rodent malaria (Peters *et al.* 1993). Cooper *et al.* (1994), however, reported slow clearance of *P. vivax* infection in *Aotus* following WR 238605 treatment. Obaldia III *et al.* (1997) had described **synergistic effect of** combination of **WR 238605 and chloroquine** against chloroquine resistant *P. vivax* in *Aotus.*

4. Gametocytocidal Activity

WR 238605 exhibits gametocytocidal activity at single 2 mg/kg dose against *P. cynomolgi* B and the activity is comparable to primaquine which is also gametocytocidal at 2 mg/kg x single dose (Table 100) (Puri *et al.* 1994). The compound seems to have **potential to stop transmission of malaria**. Coleman (1990), and Coleman *et al.* (1992) had also reported gametocytocidal/sporontocidal actions of this drug against *P. berghei* Anka strain. Clinical trials for gametocytocidal activity have not been undertaken so far.

WHO (1991-92) had reported that WR 238605 was 7-10 times more potent as a gametocytocide than primaquine.

In a further report on radical curative and causal prophylactic activity of WR 238605 based on primate *P. cynomolgi* relapsing malaria model at the Central Drug Research Institute, **Puri *et al.* (1996a)** had demonstrated enhanced radical curative properties (7-fold more active than primaquine) and causal prophylactic properties (10-fold more active than primaquine), again confirming improved efficacy, reduced toxicity, excellent oral bioavailability and better half-life than primaquine which has a narrow therapeutic index. The authors have also reported a potential **synergistic blood schizontocidal effect when WR 238605 is co-administered with halofantrine**. **Puri *et al.* (1996b) and Puri and Dutta (1997)** had **reported improved efficacy of WR 238605 in combination with halofantrine** for the control of both blood and relapsing stage of malaria. Halofantrine at 10 mg/kg x 7 days has curative blood schizontocidal action against *P. cynomolgi* B while the curative anti-relapse dose of WR 238605 is 0.316 mg/kg x 7 days in this model. The curative **blood schizontocidal dose of halofantrine could be reduced** from 10 mg/kg to 3.16 mg/kg when it is combined with 0.316 mg/kg dose of WR 238605. This combination would be useful alternative regimen for treatment of chloroquine resistant *P. vivax* cases.

Pharmacology

Methaemogloin Toxicity

Anders *et al.* (1988) had reported increased methaemoglobin toxicity of WR 238605 compared to primaquine in dogs (MetHb 16.05 vs 6.3%).

Acute Toxicity

LD_{50} of WR 238605 by oral route was reported to be 0.78 m moles/kg in rats and 0.64 m moles/kg in guinea pigs and this compound was claimed to be less toxic than primaquine whose LD_{50} is 0.46 m moles/kg in rats and 0.12 m moles/kg in guinea pigs (Brueckner & Fleckenstein 1991).

Sub-chronic and Chronic Toxicity

Compound WR 238605 was judged to be less toxic than primaquine in sub-chronic and chronic toxicity studies in dogs. Primaquine at 3 and 9 mg/kg/day administered orally for 28 days resulted in muscle necrosis, coma and death in dogs (Lee *et al.* 1981), while WR 238605 administered **up to 16 mg/kg/day for 28 days did not produce similar toxicity changes (Brueckner & Fleckenstein 1991).**

Half-Life Studies

Half-life of WR 238605 in dogs was estimated to be **very long (19.70 h)** in comparison to **primaquine whose half-life is only 2 hr** (Brueckner & Fleckenstein 1991). Its half-life in rodents had been reported to **be 60 hr, and in monkeys 52 hr**. Brueckner *et al.* (1998a) had reported that elimination **half-life of WR 238605 was about 14 days in humans.**

Clinical Trials

Clinical Trials (Phase-I)

WR 238605 was administered to 48 healthy subjects at 400-600 mg (base) dose by oral route. Like primaquine, WR 238605 can cause gastrointestinal distress and at **300-600 mg dose**, 12 out of 48 individuals showed headaches (2 cases), lightheaded dizziness (3 cases), heartburn and gas (4 cases), vomiting (1 case) and diarrhoea (1 case) etc. **WR 238605 had not been administered to human with G-6-PD deficiency** (Brueckner *et al.* 1998a). **The half-life of WR 238605 in humans is fairly long (14 days) compared to 4-6 hr for primaquine but it is less toxic.** The authors conclude that the drug is well-tolerated but with possible gastrointestinal side-effects (Brueckner *et al.* 1998a).

Clinical Trials (Phase-II/III) for Radical Curative Efficacy

The results of first clinical trial with WR 238605 for radical curative (anti-relapse) activity carried out among *P. vivax* infected cases in Bangkok and the **levels of methaemoglobin produced** by the treatment have been published by **Walsh *et al.* (1999).** The results of this limited trials on **23 *P. vivax* cases** (administered 3 different regimens), who completed a short-term follow-up (2-6 months) are summarized in Table

95. The results of short-term and extended (one year) **anti-relapse trials with CDRI 80/53** and standard drug **primaquine carried out in India** (**Valecha *et al.* 2001**) are also summarized in Table 95. for comparison with WR 238605 efficacy/met Hb toxicity data.

Overall relapse rate within 2-6 months of follow-up with three regimens of WR238605 was 2/23 cases (8.7%).

Regimen 1: 300 mg x 7 days – relapse Nil/7 (met-Hb 13.5%)

2: 500 mg x 3 days (repeated)-relapse 1/9 (met-Hb 14.7%)

3: 500 mg x 1 dose – relapse 1/7 (met-Hb 6.4%)

Placebo (chloroquine 1500 mg)-relapse 4/7 (57%).

Corresponding relapse rate with one year follow-up with **CDRI 80/53** (25 mg x 5 days) was 29.6% among 219 total cases treated, and with **primquine** (15 mg x 5 days) was 26.8% among 220 total cases treated. Indian *P. vivax* cases (224) treated with placebo (**chloroquine**) showed **40.1% relapse rate** over one year of follow-up. During short **non-transmission period relapse rate was low 4.9%, 3.0% and 10.6% respectively.**

A comparison of anti-relapse trials among *P. vivax* cases shows that different regimens of WR 238605 produce exceptionally **high levels of methaemoglobin** (6.4, 14.7 and 13.5%) which are **similar to primaquine** (16.32%) indicating that **high dose WR 238605 anti-relapse therapy** (500 mg to 3000 mg course dose) would **raise methaemoglobin to significantly high level**. **CDRI 80/53 is comparatively** safe as it is used at very low dose (125 mg course dose) and does not produce significant changes in normal level of methaemoglobin. **Safety of WR 238605 in G-6-PD deficient cases has not been established** and treatment of infants, children and pregnant woman is contraindicated both with WR 238605 and CDRI 80/53.

The precise comparison of two drugs can be made only if identical doses of WR 238605 and CDRI 80/53 are used for future clinical trials. WR 238605 had undergoing phase-II/III clinical efficacy trials at WRAIR with larger number of patients with follow-up preferably up to one year. Major advantage of WR238605 is that it has **longer half-life (14 days) than primaquine,** which would definitely improve drug compliance in field studies. WR 238605 has the potential to develop as a **candidate prophylactic drug**

for *P. falciparum* infections also (Brueckner *et al.* 1998b). CDRI 80/53 has proven causal prophylactic efficacy against simian malaria model.

Nasveld *et al.* (2002) have reported high efficacy of both WR 238605 (Tafenoquine) and primaquine as drugs for **post-exposure (terminal) prophylaxis of *vivax* malaria** among defence personnel returning from Papua New Guinea where Chesson type *P. vivax* infections are endemic. Efficacy of 400 mg base Tafenoquine x 3 days was compared with 22.5 mg base primaquine x 14 days. Within one year of follow-up, 7 volunteers on tafenoquine (out of 378), and 6 volunteers on primaquine (out of 214) had developed vivax malaria. The study concludes that **both drugs (3 day Tafenoquine or 14 day primaquine) are equally effective in preventing vivax malaria.**

Conclusion: Two new anti-relapse drugs had been identified under primate anti-malarial screening at CDRI.

AABLAQUIN

The anti-relapse tablets (combination of Chloroquine (3 day treatment) and Bulaquine (5 day treatment) has been patented (CDRI 501/MUM/2000; Grant 31.05.2000 IN) and the dose schedule is shown in the Table 101). Chloroquine total dose is 1500 mg (over 3 days) and Bulaquine (Elubaquine) is 25 mg daily x 5 day.

Aablaquin oral anti-relapse regimen for P. vivax comprising of Chloroquine (x 3 days) and Bulaquine (x 5 days) (CDRI Patent 501/MUM/2000 Grant 31.05.2000 IN. Mumbai).

1. **CDRI 80/53 (Elubaquine-Bulaquine)**
2. **WR238605 (Tafenoquine)**

1. Preclinical and clinical studies show that both CDRI 80/53 and WR 238605 which are primaquine analogues, can be safe substitutes for primaquine. The relapse rate with different drugs is varying because of different dose regimens

   Non-transmission period:

   Primaquine 15mg daily x 5 days-relapse rate 3.05

   CDRI 80/53 25mg daily x 5 days-relapse rate 4.9%

   Placebo chloroquine 1500mg – relapse rate 10.6%

WR 238605 (500mgx1 day, 500mgx3 days, (Repeated), 300mgx7 days-relapse rate 8.7%

2. CDRI 80/53 has **cleared long term (3 months) sub-acute toxicity** in two animal models and the compound was declared safe, whereas **WR 238605 was reported to be safe in 28 day short term toxicity study.**
3. Both drugs (CDRI 80/53 and WR 238605) have cleared human safety studies in volunteers, and **both can be used as primaquine substitutes for anti-relapse activity** against relapsing *P. vivax/P.ovale* infections. However, further clinical trials are urgently needed to establish the safety of these drugs in G-6-PD deficient individuals/children/infants/pregnant women.
4. Judging from the methaemoglobin levels produced in *P. vivax* cases with two new primaquine derivatives, the drug **CDRI 80/53 (Elubaquine),** seems to be much safer (**metHb 3.025**) because the prescribed anti-relapse treatment dose is very low (25 mg daily x 5 days) while **WR 238605 (Tafenoquine**) was used at very high dose (1500 mg in 3 days, repeated or 2100 mg in 7 days) in clinical trials which raised metHb level significantly **(13.5-14.7%).**
5. Since primate anti-relapse studies show that **WR 238605 is 4 times more potent than CDRI 80/53**, therefore, further clinical trials with WR 238605 would be justified to reduce the treatment dose.
6. The half-life of CDRI 80/53 is not known, but it is expected to be very short (a few hours) like primaquine. Paliwal and Gupta (1998) have reported terminal half-life of **95.3±43.5 min (i.v.) and 10-28 min (oral)** in rabbits. However, WR 238605 being long acting (half-life 14 days), treatment with this compound would improve patient compliance.
7. The available limited data on *P. vivax* clinical trials suggest that **both CDRI 80/53 and WR 238605** are candidate drugs which can eventually replace primaquine as effective **anti-relapse agents for treatment of relapsing malaria**.
8. Besides the drug **WR 238605 has a potential gametocytocidal activity** and also exhibits **causal prophylactic activity**.
9. **CDRI has already marketed Aablaquin** - a combination kit of blood schizontocide chloroquine (1500 mg) and the anti-relapse component CDRI 80/53

(Elubaquine=Bulaquine) 25 mg/day x5 days as a **standard radical curative treatment for *P. vivax*** (Table 96).

10. **CDRI 80/53 (Elubaquine=Bulaquine)** – a drug for **recurring (relapsing) malaria** has received **Jai Vigyan Recognition from the Govt. of India, Ministry of Science and Technology (on 19 Nov 2002).**
11. BULAQUINE. CDRI Patent

    Pratap, R. *et al.* (U.S. 7183291 Feb. 27, 2007) E.P. 1055427 Oct. 13, 2004.

12. Aablaquin – Patent CDRI. 501/MUM/2000 Grant 31.0-5.2000 IN. Mumbai.

4. NEW CAUSAL PROPHYLACTIC DRUGS

Causal Prophylaxis Activity of Two New Compounds (Bulaquine/Elubaquine and Tafenoquine)

4.1 Bulaquine (Elubaquin) (CDRI Compound 80/53) (dihydro-3-[1-[[4-[(6-methoxy-8-quinolyl) amino]pentyl]amino]ethylidene]-2(3H)-furanone)

As reported above in this document, this 8-aminoquinoline derivative synthesized at CDRI 80/53, has already undergone evaluation for radical curative activity against sporozoite induced *P. cynomolgi* B in Rhesus monkey as well as for gametocytocidal activity against *P. cynomolgi* B. The compound has undergone detailed drug development, and safety **Phase I studies** in human subject and also successfully completed **Phase II/III evaluation** for radical curative activity against *P. vivax* at **Malaria Research Centre (ICMR), New Delhi**.

Causal prophylactic activity of Bulaquine CDRI 80/53 against *P. cynomolgi* B in Rhesus monkey.

1. 3.16 mg/kg x 3 doses in curative
2. Single dose of 80/53 at 10 mg/kg on day 0 was also curative in Rhesus monkeys.

Only experimental studies have been undertaken and the drug is safe.

Reference

Dutta G P, Puri SK, Pandey V C, Seth M, Bhaduri A P, Chatterjee SK, Asthana O P and Gupta K C (1994) New potential antimalarials: antirelapse compound CDRI 80/53; Proceedings of Tropical Diseases Molecular Biology and Control strategies (editors: Sushil Kumar, A.K. Seth, G.P. Dutta and R.N. Sharma) Publications & Information Directorate, New Delhi pp 286-300.

4.2 Causal Prophylactic Activity of Tafenoquine (Compound WR238605)

The causal prophylactic activity of WR238605 was evaluated in 22 rhesus monkeys at doses in the range 0.03-1 mg (base)/kg (Table 93, 98) using 3 day treatment schedule. All the four monkeys treated at 0.03 mg/kg dose became patent on day 8. Likewise, 6 out of 7 monkeys treated at 0.1 mg/kg also developed patency between days 8 and 17, while one monkey was protected. Nine out of 9 monkeys at 0.316 mg/kg and

2/2 monkeys at 1 mg/kg were protected. Hence, a dose of **0.316 mg (base/kg) was recorded as the prophylactically curative dose. Primaquine**, used as reference drug in this model, showed 100% prophylactic activity in 4/4 monkeys at **1.78 mg (base/kg).** Another four monkeys kept as vehicle control developed patent infection on days 8,8,8 and 9.

Tafenoquine Prophylaxis

Tafenoquine is an analogue of primaquine with a longer (14 days) half-life than primaquine besides having activity against liver-stage malaria parasites. It is also a drug of choice for prophylaxis. A randomized, double blind study on malaria prophylaxis with tafenoquine was published by Faucher *et al.* (2000) in which out of 2144 cases living in Lambarene Gabon, having *P. falciparum*, 426 selected cases were treated with four dose levels (250 mg, 125 mg, 62.5 mg or 31.25 mg) of tafenoquine daily for three days. A total of 417 cases had received initial curative treatment with halofantrine, and 410 completed the assigned tafenoquine prophylaxis therapy. By 77 day of follow up, a total of 34 positive blood smears were recorded. Out of 79 tafenoquine (31.25 mg dose) cases, 16 became slide positive, while 62.5 mg dose showed three positive out of 86 cases, while **one out of 79 tafenoquine (125 mg) cases became positive. None of the 84 cases treated with tafenoquine (250 mg dose) developed malaria**. The study showed the tafenoquine (250 mg dose) could be used for malaria prophylaxis.

Edstein *et al.* (2001) evaluated tafenoquine for prophylaxis of both *P. falciparum* and *P. vivax* among Thai soldiers and they reported that **short course (3 day) tafenoquine prophylaxis was considered better than 14 day primaquine course. Shanks *et al.* (2001)** reported that the prophylactic dose of **200 mg or 400 mg tafenoquine taken weekly for about 13 weeks** was **highly efficacious in providing protection against *P. falciparum*** malaria in studies conducted in Kenya. **Walsh *et al.* (2004)** had reported that **monthly tafenoquine was safe and well-tolerated**. Initial tafenoquine daily dose 400 mg (base) for 3 days followed by single monthly 400 mg dose for 5 months was reported to be effective. Tafenoquine gave an overall **97% protection against *P. falciparum/P. vivax*** infections. The author reported **six months prophylactic protection** in Thai soldiers following **monthly 400 mg dose regimen**.

Crockett and Kain (2007) recommended that tafenoquine cannot be used in pregnant women and individuals who are deficient in the enzyme gluco9se-6-phosphate dehydrogenase. They reported that **tafenoquine was highly effective** in both **radical cure of relapsing malaria** and **causal prophylaxis of *P. vivax* and *P. falciparum***, with protective efficacy of > or = 90%.

References

Puri S K, Dutta G P, Milhous W S, Schuster B G and Davidson (Jr) D E (1994) W.R.: 238605 : A potential broad spectrum antimalarial. In "Tropical Diseases Molecular Biology and control Strategies" Editor, S. Kumar, A.K. Sen, G.P. Dutta and R.N. Sharma. CSIR Publication, pp 276-285.

4.3 Causal Prophylactic Activity of Non-8-aminoquinolines

Most of the 8-aminoquinolines are toxic to human subjects whose RBC shows G-6-PD deficiency, which is responsible for methaemoglobin toxicity and haemolysis of RBC, efforts have been continuing to screen non-8-aminoquinolines for causal prophylactic activity against sprozoite induced *P. cynomolgi* B infection using Rhesus monkey as a standard model for evaluation of causal prophylaxis activity. Our long-term collaboration with Walter Reed Army Institute (WRAIR) provided more than a dozen non-8-aminoquinoline compounds for causal prophylaxis activity. Puri and Dutta (2000) have published the results of screening Non-8-aminoquinline compounds provided by Walter Reed have been evaluated for causal prophylaxis activity against sporozoites induced *P. cynomolgi* B and results have been published in J. Parasitic Dis. 24,67-72 (2000). Further studies are proposed on these Non-8-aminoquinolines in collaboration with WRAIR, Washington.

References

Puri S K and Dutta G P (2000) Causal prophylactic activity of non-8-aminoquinoline compounds against sporozoite induced *Plasmodium cynomolgi* infection in rhesus monkey. *J. Parasitic Dis*. **24**: 67-72

5. LIFE SAVING RECTAL ANTIMALARIAL SUPPOSITORIES

5.1 STATUS OF RECTAL SUPPOSITORIES OF ARTEMISININ DERIVATIVES

ARTEMISININ SUPPOSITORY

Several reports have been published which support the observation that rectal suppository preparations of artemisinin can exert antimalarial effect in reducing the *P. falciparum* parasitaemia in critically sick and severe cases including cerebral complications (Li *et al.* 1994, Trans. R. Soc. Trop. Med. Hyg. 88 (Suppl.1), 5-6; Arnold *et al.* 1990, Trans. R. Soc. Trop. Med. Hyg., 84, 499-502; Hien *et al.* 1991, Trans. R. Soc. Trop. Med. Hyg. 85, 202-211; Hien *et al.* 1992, Trans. R. Soc. Trop. Med. Hyg. 86, 582-583; Li *et al.* 1985, J. Trad. Chinese Med. 5, 159-161;Cao *et al.* 1997, Trans. R. Soc. Trop. Med. Hyg. 91, 335-342). The suppository treatment was found satisfactory for parasite clearance and for fever clearance in patients. However, a **very high dose of artemisinin (2800 mg in 48-56 h) needs to be administered to produce complete parasite clearance.** Besides the high dose of artemisinin administered, may approach the toxicity level and there are frequent problems of recrudescence with this drug in children.

5.2 ARTESUNATE SUPPOSITORY

Artesunate suppositories have also been used for control of severe *P. falciparum* cases. Although they produce fast parasite clearance and fever clearance, the recrudescence rate is high. Artesunate suppositories were given at a **total dose of 1600 mg (in 8 doses) over 3 day course and in order to prevent recrudescence a long-acting antimalarial mefloquine (1250 mg) was administered** to reduce recrudescence rate (92%) (Looareesuwan *et al.* 1995, Ann. Trop. Med. Parasitol. 89, 469-475). Looareesuwan also administered 1600 mg artesunate suppository which was followed by mefloquine (1250 mg) which produced 96% cure rate and Bhatt *et al.* 1996 (Jpn. J. Trop. Med. Hyg. 24 (Suppl. 1), 59-63) also used 1600 mg artesunate suppositories followed by 1000 mg mefloquine. In spite of double treatment, **13.6% mortality was recorded (cure rate 86.4%).** Gomez (Gomez Landires, 1996, Jpn. J. Trop. Med. Hyg. 24 (Suppl. 1) 16-24) employed 1400 mg artesunate supossitory which was followed by 750 mg mefloquine. Thwe *et al.* (1996, Jpn.J. Trop. Med. Hyg. 24 (Suppl. 1), 25-32) administered 1200 mg artesunate suppositories (in 3 days) followed by 1250 mg,

mefloquine for treatment of severe malaria and achieved a cure rate of 92.3%. Halpaap *et al.* 1998, (Am. J. Trop. Med. Hyg. 58, 365-368) administered two 50 mg artesunate suppositories at 4 h interval in children **followed by sulfadoxin/pyrimethamine**, and Sabchareon *et al.* (1998) administered 200 mg artesunate suppository (x 3 days) followed **by mefloquine (2 doses).**

Most of the trials recommend the **administration of a second antimalarial like mefloquine or sulfadoxin/pyrimethamine with artesunate suppository**. Wilairatana *et al.* 1997, (Ann. J. Trop. Med. Parasitol. 91, 891-896) also reported that artesunate suppository was useful in reducing and clearing parasitaemia in severe *P. falciparum* cases. Kyaw *et al.* (1996, Jpn. J. Trop. Med. Hyg. 24 (Suppl. 1), 55-58) compared the efficacy of artesunate suppositories (200 mg x 3 days) and artemether suppositories (200 mg x 3 days) and the cure rates among adult uncomplicated falciparum cases were 50 and 43.9% respectively.

5.3 ARTEETHER FORMULATION

Major Advantages

1. α/β-Arteether is a **very fast-acting blood schizont**.
2. It can control highly MDR malaria infections.
3. The **suppository can revived comatose cerebral malaria cases**.
4. Suppository can serve as emergency treatment for cerebral malaria.

Rectal formulation of α/β-arteether

α/β-Arteether mixture dissolved in neutralized groundnut oil was administered to *P. yoelii nigeriensis* (MDR II) infected mice at 10 mg/kg to 20 mg/kg dose from day 0 to day 2 (for 3 consecutive days) by intra-rectal route. Drug was administered with 0.05-0.25 ml oil (vehicle) in single dose or in 2 divided dose daily. Parasitaemia was recorded daily from tail blood smears stained with Giemsa stain and results are given as % parasitaemia (means±SD). All the doses of arteether namely 10.0, 15.0 and 20.0 mg/kg suppressed the parasitaemia even after treatment (up to 5 days) as shown by blood smear examination. The study shows that till day 5, the control mice had developed very high level of parasitaemia (57.6±24.7) and had starting dying whereas the groups of mice administered 15-20 mg/kg α/β-arteether formulation by rectal route treatment were fully

protected as shown by negative parasitaemia (Table 102). The study shows fairly rapid drug absorption of this formulation by rectal route which was effective in initially controlling multi-drug resistant (II) *P. yoelii nigeriensis* infection in mice. α/β-Arteether rectal route controls the severity of infection and prevents mortality in severe complicated comatose malaria cases and will be specially useful in infants, children and those having frequent vomiting who can not be given oral formulation initially. Rectal formulation easily controls highly multi-drug resistant malaria infections which do not respond to drugs like chloroquine, amodiaquine, mepacrine, mefloquine, halofantrine, quinine, quinidine, pyrimethamine + sulfadoxin etc. in MDR areas. Rectal route α/β-arteether formulation remains 1st line of emergency treatment of comatose *P. falciparum* cases in areas of MDR.

5.4 DIHYDROARTEMISININ RECTAL SUPPOSITORIES FOR CONTROL OF MDR AND COMATOSE SEVERE *PLASMODIUM FALCIPARUM*

Major Advantages

1. DHA suppositories can be administered to **uncomplicated/severe/cerebral malaria** cases as emergency therapy as a fast-acting blood schizontocidal therapy.
2. DHA suppositories can be administered 2-3 times daily to **revive comatose malaria** cases and even administered for 5-7 days for substantial recovery.
3. Effective for control of MDR malarias in children, to reduce mortality.
4. Additional use as an **effective gametocytocide** to stop malaria transmission.
5. DHA suppositories can be used as **emergency drug** in rural/primary health centres or even in rural areas by unskilled health workers to gain time till the patient is shifted to proper malaria clinic.
6. **DHA patent granted internationally in 16 countries** (Table 56).

Formulation of dihydroartemisinin (DHA) of formula (1)

Dihydroartemisinin (DHA) 5 mg was dissolved in sterile refined neutralized (10 ml) groundnut oil/sesame oil and similar oil with better solubility by mild heating at 80-90^{o}C and the formulation was stored at room temperature till use. It has been found to be stable at room temperature for several months.

Antimalarial efficacy

Antimalarial (Blood Schizontocidal) activity of DHA rectal formulation

DHA formulation was evaluated *in vivo* in Swiss mice (20-25 g). The mice were infected intraperitoneally with parasite (multi-resistant strain of *P. yoelii nigeriensis*) and the infected mice were treated for 5-7 consecutive days (day 0 onwards) with each mouse dose (0.2-0.3 ml oil by rectal route) of a DHA formulation prepared in neutralized groundnut oil. For higher doses (15 and 20 mg/kg) the drug was given in divided doses by rectal route. The tail blood smears of the mice were examined periodically after Giemsa staining up to 50 days post infections. DHA formulation has shown curative effect against multi-drug resistant rodent infection as shown by complete absence of parasitaemia till 50 days of observation (Table 103).

6. ARTEROLANE MALEATE

(OZ277 = RBx11160)

1. Discovery of a new synthetic peroxide antimalarial named OZ277 (RBx11160) was reported by Professor Verrenstrom *et al.* (2004). Arterolane maleate (also known as Dispiro 1,2,4-Trioxolane; OZ277; RBx11160) antimalarial was cited by Vennerstrom *et al.* (2004) in U.S. Patent: Dispiro 1,2,4-trioxolane (USPTO Patent Application 20080125411).
2. Arterolane is a **rapidly acting synthetic trioxolane** with a strong **blood schizontocidal activity** against asexual blood stages of both *P. falciparum* and *P. vivax.*
3. Arterolane (RBx11160) maleate is considered as rapidly acting blood schizontocide was developed by **Ranbaxy Research Laboratories (Haryana, India) (Gautam *et al.* 2011).**
4. Vennerstrom *et al.* (2010) reported that **OZ277 showed single dose curative activity** against *P. berghei* ANKA infection at 30 mg/kg in mice. This compound also shows prophylactic activity against *P. berghei* at 100 mg/kg single dose (Application Patent U.S. 20080125411).
5. Maerki *et al.* (2006) investigated the pharmacodynamic properties of Trioxolane OZ277/RBx11160 (Arterolane), the first fully synthetic antimalarial peroxide using synchronous *in vitro* cultures of *P. falciparum.* The uptake of OZ277 by *P. falciparum* infected red blood cells was measured by (^{3}H) hypoxanthine incorporation. The *P. falciparum* infected RBC containing mature parasites, were first exposed to Oz277 (100th IC_{50} dose).

The *P. falciparum* infected RBC containing mature stages of the parasite were exposed *in vitro* to OZ277 high drug concentration (100th IC_{50} drug dose for one hour). After one hour exposure to OZ277 at the highest concentration followed by removal of drug, the hypoxanthin assay showed that the growth of mature stages of the parasites was reduced to below 20%. Parallel studies with two reference drugs (artemether and chloroquine) and OZ277 showed that 6 hour or longer exposure of cultured *P. falciparum* infected RBC, showed strong inhibition (≤ 6% growth) of all the parasite stages was noticed when the parasites were exposed longer than 6 hours. The drug exposed *P.*

falciparum cultures were finally killed by *in vitro* exposure to OZ277 as well as by artemether or chloroquine drug exposure. Besides, the partitioning studies with OZ277 into *P. falciparum* infected red blood cells and uninfected RBC was also examined by measuring the radio-labeled [^{14}C] OZ277 incorporation *in vitro* between RBCs and the plasma. The authors observed a significant accumulation of the radiolabeled OZ277 in the *P. falciparum,* infected RBCs (R/P for OZ277 was reported to be **1.5 for uninfected RBCs, and up to 270 for infected RBCs.**

Maerki *et al.* (2006) have quoted the earlier studies by Vyas *et al.* (2002) as well as by Gu *et al.* (1984) which had established that [^{14}C] artemisinin and [^{3}H] dihydroartemisinin partition into *P. falciparum* infected RBCs and **their accumulation was up to 300-fold in the parasite**. Maerki *et al.* (2006) further reported that similar high degree of accumulation of [^{14}C] OZ277 was observed in *P. falciparum* infected RBC in incorporation experiments. The authors conclude that the **antimalarial action of OZ277 was comparable to high level of antimalarial profiles of known antimalarial dihydroartemisinin which is an exceptional highest level of drug in infected malaria parasites.**

Maerki *et al.* (2006) also provided experimental evidence that compound OZ277 radiolabeled in the **adamantine ring (named OZ277(L)** or in the right side chain (as in **compound OZ277** ®, showed that OZ277(L) molecules **accumulated the labeled drug continuously**, reaching a maximum level after 2 hrs incorporation, whereas OZ277® molecules incorporation **decreased steadily after 10 minutes**. Their findings have been reported to support the mechanism of **action of OZ277** as proposed by Professor Vennerstrom (2004) in that **carbon-centred radicals form predominantly in the adamantane ring**.

Mechanism of action of the synthetic Trioxolane RBx11160 (OZ277) a fully synthetic peroxidic antimalarial has been extensively investigated by Uhlemann *et al.* (2007. In view of its potential as **a new synthetic peroxide**, it can be **produced in bulk as fast acting blood schizontocide** for the treatment of **multidrug-resistant** *P. falciparum* at a very low treatment cost as compared to currently preferred drug **artenusate sponsored** by World Health Organization for emergency treatment of severe malaria as well as for uncomplicated/complicated ***P. falciparum*** malaria including

comatose cerebral malarias resulting from the pathogenesis caused by ***P. vivax*** and even zoonotic ***P. knowlesi*** infections (WHO, 2000).

CLINICAL TRIALS OF ARTEROLANE (RBx11160) AND PIPERAQUINE

Valecha *et al.* (2010) have carried out Phase II multicenter, randomized, dose-finding clinical trials with Arterolane (RBx11160) (a synthetic Trioxolane) for the control of acute uncomplicated *Plasmodium falciparum. P. falciparum* **multidrug-resistant 230 cases from Thailand, India and Tansania** (mainland and Zanzibar) were enrolled for clinical trials using three dose levels i.e. 50 mg (78 cases), 100 mg (76 cases), and 200 mg dose (76 cases) **once daily for 7 consecutive days**. Patients in age group 13-65 years were enrolled in the efficacy trial of Arterolane maleate. Following the above arterolane treatment, the parasite clearance time (PC_{50}) was 19.4h, 12.8h and 12.6h for groups treated with 50 mg, 100 mg and 200 mg dose levels. The **polymerase chain reaction corrected** adequate clinical and the parasitological responses of three groups were **63%, 71% and 72% after 28 days** post-treatment therapy.

In this phase II trials for safety and efficacy of Arterolane, **Valecha *et al.* (2010)** conclude that daily dosing of **100 and 200 mg** was found to be rapidly acting, effective and **safe synthetic Trioxolane** for the treatment of uncomplicated *P. falciparum* in multicetric clinical trials conducted at three centres namely (1) in Thailand where the malaria transmission is low and seasonal, and the first-line Act treatment is artesunate/mefloquine; (2) in Rourkela (India) where the transmission is high and perennial, and first-line treatment is artesunate/sulfadoxine-pyrimethamine and (3) in Tanzania and Zanzibar where the malaria is perennial and antimalarial treatment comprised of artemether/lumefantrine and artesunate/amodiaquine have been the treatment for multidrug resistant *P. falciparum*. During this trial the drug **Arterolane** (RBx11160 **produced by Ranbaxy Laboratories, New Delhi**) was used and the trials were **supported by Medicines for Malaria Venture, Geneva, Switzerland**.

Valecha *et al.* (2012) have extended the clinical trials with synthetic Trioxolane Arterolane (OZ277/RBx11160) in **combination with piperaquine** (a long-acting antimalarial) for the treatment of uncomplicated *P. falciparum* in **multicentric (Phase III)** trials conducted in Bangkok as well as in India, and another ACT comprising of

Coartem® (Artemether + Lumefantrine) was also evaluated for comparative efficacy and safety. In the earlier phase II trials Arterolane synthesized by Vennerstrom *et al.* (2004), was considered to be effective at both **100 and 200 mg dose levels x 7 day oral treatment,** but in the **phase III** trials drug combination of **150 mg of Arterolane x 3 days was combined with 750 mg x 3 days (as Arterolane-Piperaquine combination**) for safety and efficacy evaluation in multicentric trials against *P. falciparum.* For comparison a second ACT **Coartem** (artemether + lumefantrine) was administered as **six dose regimen** in patients with acute uncomplicated *P. falciparum*. **Coartem (FDC of artemether-lumefantrine**) as a single dose of 4 tablets was administered at the time of initial diagnosis, four tablets after approximately eight hours and then four tablets twice daily on each of the following two days. Follow-up of the treated cases was continued up to 2007-June 2008, comprised of 240 patients, out of which **160 cases were treated with arterolane + piperaquine, and 80 patients were treated with Coartem**. **Twenty-eight day cure rates were 94.4% with arterolane + piperaquine**, and 96.3% for Coartem. **PCR corrected ACPR** (adequate clinical and parasitological response) on day 28 was thus **94.4% for arterolane + piperaquine** combination in intent to treat population of *P. falciparum* cases. No treatment failure was noticed for arterolane + piperaquine therapy. Further studies have been proposed by the investigators.

The authors also reported **slightly higher gametocyte counts following arterolane-piperaquine therapy (181.4/µl) as compared to the Coartem treated *P. falciparum* cases (113/µl).** It is encouraging to note that **on day 28 of treatment, 98%** (148 patients) treated with **arterolane + piperaquie had achieved zero gametocytes count**, while 100% of the **78/78 cases treated with Coartem were free of gametocytaemia**. Overall assessment showed that arterolane + piperaquine treatment was **effective and safe** for control of **uncomplicated *P. falciparum***.

NOW, A SYNTHETIC ANTIMALARIA DRUG

India has developed a powerful new malaria drug – a combination of Arterolane and Piperaquine. “Clinical trials conducted by National Institute of Malaria Research (NIMR) with the Arterolane and Piperaquine combination, have shown that it is effective and as safe as artemisinin combinations like Artesunate and Sulphadoxine which is used in the national malaria programme”, said Dr. Neena Valecha, who was the Principal

Investigator of the trials. Arterolane – Piperquine combination will be an addition to combinations already recommended by WHO. Since this new drug is synthetic, its raw materials are created chemically in the lab. This will ensure constant supply of raw material and standize costs, said Dr. Valecha. WHO Director General Margret Chen explained that the “usefulness of these ACT therapies is now under threat because of possible emergence of resistance.

Table 37: Blood schizontocidal activity of artemisinin and arteether against *P. berghei* as reported by earlier investigators.

Treatment (doses/route)	Strain of *P. berghei*	ED_{50} mg/kg	ED_{90} mg/kg	Curative dose mg/kg	Reference
Artemisinin					
1 g/kg x 3 dose (Oral)	Sensitive	-	-	Cleared parasitaemia	(1)
50-60 mg/kg x 3 dose (Oral)	Sensitive	-	-	Cleared parasitaemia	(2)
3 dose (Oral)	Sensitive	130.7±21.2 To 138.8	-	-	(2)
3 dose (Oral)	Chloroquine Resistant	439.8±64.2	-	-	(2)
40 mg/kg x 3 dose (Subcutaneous)	Sensitive and chloroquine resistant	-	-	Cleared parasitaemia	(2)
50-100 mg/kg x 3 dose	Sensitive	-	-	Cleared parasitaemia	(1)
3 dose (on day 2,3,4)	Chloroquine resistant	-	6.20	-	(3)
3 dose (i/m on day 1,2,3)	Sensitive	0.77	2.15	-	(4)
3 dose (Oral on day 1,2,3)	-	176.6	-	-	(4)
3 dose (Subcutaneous on day 3,4,5)	Sensitive	-	-	160 mg/kg (Curative in 5/5 mice) 40 mg/kg (Curative in 2/5 mice)	(5)
Arteether (β)					
3 dose (i/m)	Chloroquine resistant	-	1.95	-	(3)

References: 1. Anon. (1982a); 2. Anon. (1979); 3. Anon. (1982b); 4. Anon. (1982c); 5. Lin *et al*. (1987)

Table 38 : Antimalarial activity of artemisinin against *P. berghei* infection in Swiss mice.

Treatment/dose	No. of mice	Parasitaemia %		Mean survival time (days)
		Day 4	Day 7	
Artemisinin				
100 mg/kg x 4	6	0.08 ± 0.204	8.62 ± 15.23	13.89 ± 3.125
50 mg/kg x 4	6	0.27 ± 0.392	24.01 ± 12.46	12.83 ± 3.18
25 mg/kg x 4	6	0.08 ± 0.129	29.45 ± 16.17	13.66 ± 2.33
12.5 mg/kg x 4	6	3.64 ± 2.876	25.83 ± 12.66	12.166 ± 3.31
6.25 mg/kg x 4	6	6.5 ± 6.14	45.3 ± 21.217	11.16 ± 4.26
3.12 mg/kg x 4	6	16.83 ± 17.62	23.00 ± 26.261	11.00 ± 2.10
Control	12	36.68 ± 19.56	27.83 ± 28.30	6.916 ± 2.10

Artemisinin injection for 4 days (day 0-3), i/m (in 0.2 ml distilled water).

Antimalarial activity of artemisinin against *P. berghei*.

Table 39: Antimalarial activity of artemisinin (suspension in neutralized ground nut oil) against *P.berghei* infection in Swiss mice

Treatment/ doses x days	No. of mice	Parasitaemia % (No. of mice)			Mean survival time (days)
		Day 4	Day 7	Day 21	
Artemisinin					
1000 mg/kg x 7	8	7 mice -ve (1 mouse + ve 0.01)	2 mice –ve	-	7.15 (Toxic)
316 mg/kg x 7	8	6 mice –ve	5 mice –ve	3 mice –ve	13.25 (cure 3/8)
100 mg/kg x 7	8	6 mice –ve (2 mice +ve 0.01, 0.02)	7 mice –ve	5 mice –ve	16.62 (cure 5/8)
31.6 mg/kg x 7	8	6 mice –ve (1 mouse +ve 0.02)	6 mice –ve	3 mice –ve	15.00 (cure 3/8)
Control	7	7 mice +ve 57.25±13.90	-	-	6.00

Artemisinin injection for 7 days (day 0-6), i/m (in 0.2 ml oil)

Table 40: Antimalarial activity of intramuscular injection of arteether (in neutralized ground nut oil) against *P. berghei* infection in Swiss mice.

Treatment/ dosexdays	No. of mice	Parasitaemia % (No. of mice)				
		Day 4	Day 7	Day 14	Day 21	Day 28
Arteether						
5 mg/kg x 1	10	Nil (10)	Nil (10)	Nil (8)	Nil (8)	Nil (8)
5 mg/kg x 2	10	Nil (10)	Nil (10)	Nil (9) (1 mouse +ve 0.001)	Nil (8)	Nil (8)
5 mg/kg x 8	5	Nil (5)	Nil (5)	Nil (5)	Nil (5)	Nil (5)
Control	10	2.06± 3.31	5.83± 4.45	8.50 ± 3.65	9.50 ± 4.79	-

Arteether was given as i/m injection (in 0.2 ml, oil) and treatment started from day of infection (day 0).

Table 41: Causal prophylactic activity of artemisinin and arteether against sporozoite induced *P. cynomolgi* B infection in rhesus monkey

Treatment group	Dose* (mg/kg xdays)	Route	Monkey No.	Result	Cure rate (No. protected/ No. treated)
Artemisinin	20 mg/kgx3	i/m	1 2	Patent on day 12 Patent on day 12	0/2
Arteether	20 mg/kgx3	i/m	1 2	Patent on day 9 Patent on day 12	0/2
Primaquine (Control)	1.78 mg(base)/ kgx3	Oral	1 2	No Patency till 60 days No Patency till 60 days	2/2
Control	-	-	1	Patent on day 10	0/1

*Drug administered for 3 days (on day-1, 0, and + 1).

Table 42: Radical curative (anti-relapse) activity of artemisinin and arteether against sporozoite induced *P. cynomolgi* B infection in rhesus monkey

Treatment group	Dose* (mg/kg xdays)	Route	Monkey No.	Result	Cure rate (No. protected/ No. treated)
Artemisinin*	20 mg/kgx7	i/m	1 2	Relapse on day 20 Relapse on day 19	0/2
Arteether*	20 mg/kgx7	i/m	1 2	Relapse on day 16 Relapse on day 15	0/2
Chloroquine (Control)	5 mg(base)/ kgx7	Oral	1	Relapse on day 16	0/1
Primaquine* (Control)	1 mg(base)/ kgx7	Oral	1 2	No relapse till 100 days No relapse till 100 days	2/2

*Companion blood schizontocide chloroquine is given orally at 5 mg(base)/kgx7 days with artemisinin/arteether/primaquine

Table 43: Antimalarial activity of intramuscular injection of arteether (in neutralized ground nut oil) against *P. berghei* infection in Swiss mice.

Treatment/ dosexdays	No. of mice	Parasitaemia % (No. of mice)				
		Day 4	Day 7	Day 14	Day 21	Day 28
Arteether						
5 mg/kg x 1	10	Nil (10)	Nil (10)	Nil (8)	Nil (8)	Nil (8)
5 mg/kg x 2	10	Nil (10)	Nil (10)	Nil (9) (1 mouse +ve 0.001)	Nil (8)	Nil (8)
5 mg/kg x 8	5	Nil (5)	Nil (5)	Nil (5)	Nil (5)	Nil (5)
Control	10	2.06± 3.31	5.83± 4.45	8.50 ± 3.65	9.50 ± 4.79	-

Arteether was given as i/m injection (in 0.2 ml, oil) and treatment started from day of infection (day 0).

Table 44: Blood schizontocidal activity of arteether (α/β) against multiple drug resistant strain of *P. yoelii nigeriensis* in Swiss mice

Treatment	Dose (mg/kg x days)	No. of mice	% Parasitaemia (mean±sp)				
			Day 4	Day 7	Day 14	Day 21	Day 40
Arteether (i.m. in oil)	5 x 3	10	-ve	-ve	-ve	-ve	-ve
	5 x 5	10	-ve	-ve	-ve	-ve	-ve
	5 x 7	10	-ve	-ve	-ve	-ve	-ve
	10 x 3	10	-ve	-ve	-ve	-ve	-ve
Control	-	8	24±10.66	Died	-	-	-
Control	0.2 ml x 3	7	16.66±9.07	Died	-	-	-

Inoculum 1 x 10 parasitized RB (ip).
Drug treatment was started on day 0 (i.e. day of infection).
Mice showing negative blood slide up to day 40 were considered as cured.
Dutta, Tripathi and Vishwakarma (1989b). *Pharmacol. Research* **21**, 415-418

Table 45: Blood schizontocidal activity of α/β-arteether against *P. knowlesi* in Rhesus monkeys

Monkey No.	Dose (im) Arteether Mg/kg x days	Parasitaemia (%)		Recrudescence
		Before treatment	After last dose	
4331	2 x 3	0.4	Cleared	Day 7
4332	2 x 3	0.2	Cleared	Day 7
4329	4 x 3	0.5	Cleared	None*
4330	4 x 3	0.2	Cleared	Day 14
5209	4 x 5	10	Cleared	Day 12
5205	4 x 5	10	Cleared	Day 12
4327	8 x 3	0.001	Cleared	Day 15
4328	8 x 3	0.2	Cleared	None*
5208	12 x 5	35	Cleared	None*
5210	12 x 5	35	Cleared	None*

*No recrudescence during the observation period of 60 days.

Bajpai, Dutta and Vishwakarma (1989b) *Trans. Roy. Soc. Trop. Med. Hyg*. **83**, 484

Table 46: Comparison of blood schizontocidal activity of α,β and α/β-arteether against *P. cynomolgi* B infection

Drug	Dose (im) Mg/kg x 3 days	Parasitaemia at the time of treatment (%)	Recrudescence on day	No. of monkeys cured/treated
α-Arteether	1. 2.5 mg	0.10	16	0/1
	1. 5.0 mg	0.06	15	
	2.	0.15	-	2/4
	3.	0.10	-	
	4.	0.25	17	
	1. 10.0 mg	0.50	-	2/2
	2.	1.10	-	
	1. 20.0 mg	0.70	-	2/2
	2.	0.60	-	
	1. 25.0 mg	0.60	-	2/2
	2.	0.80	-	
β-Arteether	1. 2.50 mg	0.80	10	0/2
	2.	0.90	10	
	1. 5.0 mg	1.2	-	
	2.	1.5	-	
	3.	1.3	-	5/5
	4.	2.5	-	
	5. 10.0 mg	4.5	-	
	1.	0.6	-	2/2
	2. 20.0 mg	0.7	-	
	1.	2.5	-	
	2.	4.5	-	3/3
	3.	5.8	-	
α/β-Arteether	1. 2.5 mg	0.30	14	
	2.	0.30	15	0/4
	3.	0.30	16	
	4.	0.02	13	
	1. 5.0 mg	0.04	-	2/2
	2.	0.30	-	
	1. 7.5 mg	0.10	-	2/2
	2.	0.30	-	
	1. 7.5 mg	0.10	-	4/4
	2.	0.30	-	
	3.	0.001	-	
	4.	0.20	-	
	1. 10.0 mg	0.02	-	3/3
	2.	0.20	-	
	3.	0.01	-	

Treated monkey was considered cured if there was no recrudescence during 60 days of observation

Tripathi, Dutta and Vishwakarma (1991) *Am. J. Trop. Med. Hyg*. **44**, 560-563.

Table 47: Blood schizontocidal activity of α/β-arteether against *P. fragile* infection in rhesus monkey

Dose of arteether (im)	Monkey No.	Initial parasitaemia %	Parasitaemia after last dose	Recrudescence (R) Cured[a]	No.cured/ No.treated	Cure rate
2.5 mg/kgx3 days	1	1.1	-ve	R on day 16	0/3	Nil
	2	0.5	-ve	R on day 15		
	3	1.5	-ve	R on day 12		
5.0 mg/kgx3 days	1	0.1	-ve	Cured	4/8	50%
	2	1.2	-ve	Cured		
	3	0.3	-ve	Cured		
	4	0.7	-ve	R on day 35		
	5	0.8	-ve	Cured		
	6	1.5	-ve	R on day 21		
	7	0.5	-ve	R on day 31		
	8	1.0	-ve	R on day 16		
7.5 mg/kgx3 days	1	0.1	-ve	Cured	6/8	75%
	2	0.2	-ve	Cured		
	3	0.3	-ve	Cured		
	4	0.5	-ve	Cured		
	5	0.5	-ve	R on day 31		
	6	1.1	-ve	Cured		
	7	0.3	-ve	R on day 38		
	8	1.0	-ve	Cured		
10 mg/kgx3 days	1	0.8	-ve	Cured	3/3	100%
	2	1.3	-ve	Cured		
	3	1.3	-ve	Cured		

[a]Observation recorded up to 60 days after treatment.

Tripathi, Vishwakarma and Dutta (1997) *Exptl. Parasitol*. **87**, 290-292

Table 48: Gametocytocidal activity of single dose α/β-arteether treatment against *P. cynomolgi* B infection in rhesus monkeys as shown by *An. stephensi* infectivity data

Monkey no.	Mosquito feeding	Treatment mg/kg (im)	Asexual parasitaemia	Gameto-cytaemia	No. of mosquitoes infected/ dissected	Infectivity rate	Oocyst count (mean±SD)
1	Pre-treatment						
	-1 hr	-	1.5%	0.05%	20/20	100%	44.14±21.27
	Post-treatment						
	5 hr	Arteether 5	-	-	21/26	78%	43.14±31.31
	18 hr	mg/kgx1	-	-	10/28	35.71%	2.6±1.95
	24 hr	-	-	-	0/30	Nil	Nil
	48 hr	-	Nil	Nil	/20	Nil	Nil
		-					
2	Pre-treatment						
	-1 hr	-	1.5%	0.1%	16/20	100%	59.94±29.42
	Post-treatment						
	4 hr	Arteether 5	-	-	19/20	95%	53.37±23.85
	18 hr	mg/kgx1	-	-	9/20	45%	4.44±3.57
	24 hr	-	Nil	0.01%	0/20	Nil	Nil
	48 hr	-	-	-	0/30	Nil	Nil
		-					
Control	Post-treatment						
	48 hr	2 ml oil x 2	2.5%	0.02%	12/20	60%	19.66±26.83
	72 hr	2 ml oil x 2	1.2%	0.02%	11/17	64.7%	14.9±23.33

Tripathi R. *et al. Am. J. Trop. Med. Hyg*. **43**(6) 1990 571-575

Table 49: Blood schizontocidal and gametocytocidal effect of α/β-arteether formulation based on gametocyte and asexual parasite clearance rate in *P. falciparum* cases

Treatment route	Dose	S.No.	Parasitaemia before treatment	%	Asexual parasitaemia/ gametocytaemia at	
					24 hr	48 hr
Arteether formulation (intramuscular)	150 mg x 3 days	1	Asexual	0.05%	Nil	
			Gametocyte	0.26%	Nil	
		2	Asexual	0.025%	Nil	
			Gametocyte	0.012%	Nil	
		3	Asexual	0.7%	0.016%	Nil
			Gametocyte	0.07%	0.01%	Nil
		4	Asexual	0.01%	Nil	
			Gametocyte	0.003%	Nil	
		5	Asexual	0.033%	Nil	
			Gametocyte	0.013%	Nil	
		6	Asexual	0.50%	Nil	
			Gametocyte	0.05%	Nil	
		7	Asexual	0.02%	Nil	
			Gametocyte	0.025%	Nil	
		8	Asexual	0.5%	0.01%	Nil
			Gametocyte	0.001%	Nil	

Dutta U.S. Patent 2000.

Table 50: Summary of the clinical trials data of *P. falciparum* cases treated with arteether α/β (150 mg/dayx3 dose)

Reference	Mishra et al. (1995) & Asthana et al. (1996)	Mohapatra et al. (1996)	Valecha et al. (1997)	Mohanty et al. (1997)	Singh et al. (1998)
Clinical Trials	Phase-II	Phase-Ill	Phase-Ill	Phase-Ill	Phase III
No. of cases	51 uncomplicated	30 uncomplicated	50 uncomplicated	50 (33 cerebral malaria) severe Pf	46 Uncomplicated
Mean parasitaema	0.6% (0.0-14.5)	-	0.65%	-	-
Mean body temperature	38.7±1.05°C	99-106°F	38.21±1.02°C	-	-
Mean PCT	48 hr (24-96)	37.6 ± 13.6	19.94±6.87	48 (24-96)	30.78± 10.92 hr
Mean FCT	52.04(±27.09)	42.4±17.5	37.81±21.67	72(12-120)	
Recrudescence rate / reinfection	14%	6.7%	6%	(7 patients) 20%	6.7%
Cure rate	86%	100%	94%	80%, recovery from coma 18 hr range (6-72).	93.3%
Gametocytocidal activity	-	Present	-	-	Present

Dutta and Tripathi (2003) *Proc. Indian Natn. Sci. Acad*. **B69**,861-870.

Table 51: Clinical presentation of the patients

	Arteether (n=29)	Quinine (n=22)	P
Male:Female	22:7	13:9	n.s.
Age mean±SD	31.86±12.9	32.4±15.8	n.s.
Duration of fever (in hours)	103.44±38.16	106.8±36.48	n.s.
Duration of coma (in hours)	24.4±16.6	27.0±11.3	n.s.
Seizures	3	3	n.s.
Glasgow Coma Score	6.59±2.41	8.4±2.7	<0.02
Renal failure	5	6	n.s.
Jaundice	14	6	<0.01
Severe anaemia	3	3	n.s.

Ns. = not significant

Mohanty *et al*. (2002) *J. Indian Acad. Clin. Med*. **5**, 119-123

Table 52: Outcome of therapy in cerebral malaria patients

	Arteether (n=29)	Quinine (n=22)	P
Fever clearance time (in hours) median (range)	72 (24-216)	72 (24-240)	n.s.
Parasite clearance time (in days) median (range)	2 (1-4)	2 (1-3)	n.s.
Coma recovery time (in hours)	24.33±14.33	38.87±22.45	<0.02
Death	2 (6.67%)	6 (27.27%)	<0.02

n.s. = not significant

Mohanty *et al*. (2002) *J. Indian Acad. Clin. Med*. **5**, 119-123

Table 53: Showing cure rate and parasite reappearance rate following arteether therapy in patients of uncomplicated *P. falciparum* malaria at different centres

Centres	Patients completing study (N)	Cure rate (%)	Parasite reappearance rate N (%)
Bhilai	48	100	-
Delhi	50	94	3 (6)
Dibrugarh	30	93.3	2 (6.7)
Guwahati	26	100	-
Jabalpur	25	93	3(7)
Jamshedpur	58	100	-
Rourkela	30	100	-

Asthana *et al.* (2001) *J. Assoc. Physicians India*, **49**, 692-696

Table 54: Showing fever clearance and parasite clearance time following arteether therapy in patients of uncomplicated *P. falciparum* malaria

Centres	Fever clearance time (hours)	Parasite clearance time (days)
Bhilai (n=48)	72.5 ± 54.3	1.4 ± 0.7
Delhi (n=50)	45.2 ± 23.5	1.2 ± 0.4
Dibrugarh (n=30)	43.8 ± 17.9	1.7 ± 0.6
Guwahati (n=26)	36.0 ± 15.5	1.0 ± 0.0
Jabalpur (n=25)	41.3 ± 22.5	1.2 ± 0.4
Jamshedpur (n=58)	38.9 ± 18.4	1.2 ± 0.4
Rourkela (n=30)	75.6 ± 39.8	1.3 ± 0.5

*(Values expressed as mean ± SD)

Asthana *et al.* (2001) *J. Assoc. Physicians India*, **49**, 692-696

Table 55: Showing pre- and post-drug haematological and biochemical parameters of patients participating in the study (n=267)

Parameters	Initial	Final
Haemoglobin (g%)	10.2 ± 1.9	11.0 ± 1.7
TLC (C.mm)	7130.4 ± 2238.7	7799.6 ± 1736.7
Serum urea (mg%)	33.3 ± 10.5	34.9 ± 54.5
Serum bilirubin (mg%)	1.2 ± 1.1	1.1 ± 0.6
Serum glucose (mg%)	100.6 ± 21.5	89.1 ± 23.6
Serum creatinine (mg%)	1.0 ± 0.2	0.9 ± 0.1

*(Values expressed as mean ± SD)

Asthana *et al.* (2001) *J. Assoc. Physicians India*, **49**, 692-696

Table 57: Blood schizontocidal activity of dihydroartemisinin against multidrug-resistant *P. yoelli nigeriensis* infection in Swiss mice (25±1 g)

Treatment (route)	Dose mg/kgxdays	Parasitaemia %						
		Day 5	Day 7	Day 9	Day 12	Day 16	Day 23	Day 30
Dihydro-artemisinin (Rectal)	5mg/kgx7	0.83±1.31 (8) 4-ve	1.12±1.55 (8) 4-ve	14.12±27.86 (8) 3-ve	0.0 (3)	0.0 (3)	1.33±2.30 (3) 2-ve	0.0 (2)
	10mg/kgx7	0.0 (7)	0.21±0.39 (7) 5-ve	0.42±0.78 (7) 5-ve	0.0 (6)	0.0 (3)	0.0 (3)	0.0 (3)
	15mg/kgx7	0.0 (8)	0.0 (8)	0.0 (8)	0.0 (8) 7-ve	0.0 (7)	0.0 (7)	0.0 (4)
	20mg/kgx7	0.0 (8)	0.0 (8)	0.0 (8)	0.0 (8)	0.0 (8)	0.0 (7)	0.0 (7)
Control	-	6±2.92 (8)	68.87±14.57 (8)	75 (1)	Died			

Note: No. of mice are given in parenthesis.
Drug is dissolved in sterile arachis oil for administration.

Table 58: Blood schizontocidal activity of dihydroartemisinin against multidrug-resistant *P. yoelii nigeriensis* infection in Swiss mice

Treatment (route)	Dose Mg/kgxdays	Wt. of mice	Parasitaemia %				
			6	9	14	20	30
Dihydroartemisinin (im)	2.5 mg/kgx5	25±1	0.0 (8)	0.012±0.03 (8) 7-ve	0.0 (7)	0.0 (7)	0.0 (7)
	5.0 mg/kgx5	25±1	0.0 (8)	0.0 (8)	0.0 (8)	0.0 (8)	0.0 (8)
	10 mg/kg x 5	25±1	0.0 (8)	0.0 (8)	0.0 (8)	0.0 (8)	0.0 (8)
	15 mg/kg x 5	25±1	0.0 (7)	0.0 (7)	0.0 (7)	0.0 (7)	0.0 (7)
	20 mg/kg x 5	25±1	0.0 (7)	0.0 (7)	0.0 (7)	0.0 (7)	0.0 (7)
	30 mg/kg x 5	25±1	0.0 (8)	0.0 (8)	0.0 (8)	0.0 (8)	0.0 (8)
Control		25±1	10.12±3.09 (8)	87.5±3.53 (2)	Died		

Note = No of mice are given in parenthesis.
Drug is dissolved in sterile neutral arachis oil for administration.

Table 59: Blood schizontocidal activity of dihydroartemisinin against multi-drug resistant *P. yoelii nigeriensis* infection in Swiss mice (20-25±1 g) by oral route

Treatment Route	Dose mg/kg x days	Wt. of mice	Parasitaemia (%) (Mean±SD)				Cure rate (%)
			Days	6	7	9	30
DHA (oral in oil)	30 mg/kg x 5	25±1 g		- (12)	- (12)	- (12)	100
Control 1	Vehicle oil	20±1 g		-	68.87±14.57 (8)	75 (1)	Nil
2	Vehicle oil	25±1 g		10.12±3.09 (8)	-	87.5±3.53 (2)	Nil

No. of mice are given in parenthesis.

Table 60: Evaluated studies of the antimalarial efficacy of dihydroartemisinin-piperaquine combinations (n = 2636)

Author & year published	Study site	Design[a]	Drug[b]	Malaria species[c]	No. of patients	Follow-up (days)	28-day cure rate (%)	PCR-adjusted cure rates (%)
Denis et al. (2002)	Cambodia	SAT	DHA-PIP	P.f	106	56	96.9	95.5
Wilairatana et al. (2002)	Thailand	RCT	DHA-PIP+ TMP MAS3	P.f	234 118	28	97 97	No genotyping
Ying et al. (2003)	China	RCT	DHA-PIP DHA-PIP+TMP	P.f	30 30	28	96.7 96.7	No genotyping
Hien et al. (2004)	Vietnam	Pilot RCT	DHA-PIP+TMP MAS3 DHA-PIP+TMP DHA-PIP MAS3	P.f or mixed	76 38 157 166 77	56	ND[d]	97.4 100 97.4 98.7 98.7
Karunajeewa et al. (2004)	Cambodia	SAT	DHA-PIP	P.f or P.v	62	28	100	No genotyping
Hung et al. (2004)	Cambodia	SAT	DHA-PIP	P.f or P.v	38 (adults) 47 (children)	28	97 98	No genotyping
Giao et al. (2004)	Vietnam	RTC	DHA-PIP+TMP+ Primaquine AP	P.f	82 79	28	94 95	No genotyping
Ashley et al. (2004)	Thailand	2 RCTs	DHA-PIP DHA-PIP+AS MAS3 DHA-PIP DHA-PIP+DHA MAS3	P.f or mixed	59 59 59 179 174 176	28 63	98 100 100 	No genotyping 96.1 98.3 94.9
Ashley et al.	Thailand	RCT	DHA-PIP (DP3)	P.f or	170	63	100	99.4

(2005)			DHA-PIP (DP40 MAS3	mixed	163 166		100 98.8	100 95.7
Tangpukdee et al.(2005)	Thailand	RCT	MAS3 DHA-PIP (DP3)	P.f	60 120	28	100 99	No genotyping
Smithuis et al. (2006)	Myanmar	RCT	DHA-PIP (Supervised) DHA-PIP (unsupervised) MAS3 (supervised) MAS3 (unsupervised)	P.f or mixed	156 171 162 163	42	99 99 100 100	99 99 99 100
Mayxay et al. (2006)	Lao PDR	RCT	MAS3 DHA-PIP (DP3)	P.f or mixed	110 110	42		99 100
Karema et al. (2006)	Rwanda	RCT	DHA-PIP (DP3) AS + AMO SP + AMO	P.f	252 252 258	28	90.4 82.1 74.1	95.2 92 84.7

[a]SAT: single-arm trial; RCT: randomized controlled trial.

[b]DHA-PIP: dihydroartemisinin-piperaquine; DHA-PIP + TMP: DHA-PIP+trimethoprim (Artecom); MAS3: artesunate + mefloquine; DHA-PIP + TMP + primaquine: DHA-PIP+TMP+primaquine (CV8); AP : atovaquone-proguanil (Maloarone); DHA-PIP+AS: DHA-PIP + artesunate; DHA-PIP+DHA: DHA-PIP+dihydroartemisinin; AS + AMO: artesunate + amodiaquine; SP + AMO: sulfadoxine-pyrimethamine + amodiaquine; DP3: three-dose regimen; DP4: four-dose regimen.

cP.f: *Plasmodium falciparum*; P.V: *Plasmodium vivax.*

[d]Not done.

Reproduce from Myint *et al.* (2007) *Trans. R. Soc. Trop. Med. Hyg*. **101**, 858-866.

Table 61: Clinical trials with dihydroartemisinin + piperaquine (DHA + Pipq) among the uncomplicated *Plasmodium falciparum* trials

Drug : Tablet (DHA 40 mg + Piperaquine 320 mg)			
1.	Denis *et al.* (2002) *P. falciparum* uncomplicated 106 cases Dose 0, 24, 32h	Total DHA = 9.1 mg/kg Pipq = 73.9 mg/kg	Cure 56 day 95.5% (Children=97.0%) (Adults = 90.9%)
2.	Tinh *et al.* (2004) Dose 0, 6, 24, 48h Uncomplicated *P. falciparum*	DHA 3.4 mg/kg=1.7 mg/kg Pipq 27 mg/kg =13.5 mg/kg	97.4-98.7%
3.	Mayxay *et al.* (2006) Acute uncomplicated (110)	DHA 2.1 mg/kg x 3 One death Pipq 16.8 mg/kgx3 Gametocyte 8%	Cure 42 day 100%
4.	Karema *et al.* (2006) Uncomplicated *P. falciparum* Children (252)	DHA 40 mg x 3 Pipq 320 mg x 3	Cure 28 day 95.2% PCR confirmed
5.	Smithus *et al.* (2006) 327 patients *P. falciparum*	DHA 6.3 mg/kgx3 Gametocyte appeared in 10% (One patient dies 22 days after drug) Pipq 50 mg/kg x 3	Cure 42 day 99.4%
6.	Hasngiun *et al.* (2007) Dose 0, 24, 48 h Piperaquine half-life = 28-35 day	DHA 40 (6.75 mg/kg) Gametocyte 2.5 case/1000 cases Pipq 320 (54 mg/kg)	87% cure rate
7.	Thanh *et al.* (2009) Uncomplicated *P. falciparum*	DHA 2.3 mg/kg x 3 Pipq 18.5 mg/kg	Cure 42 day 100%

Table 62: Blood schizontocidal activity of sodium α-artelinate intravenous formulation against *P. knowlesi* in rhesus monkeys

S.No. of monkey	Drug dose (mg/kg) x 3 days	% Parasitaemia		Recrudescence (R) after last dose	Cure
		Initial	After last dose		
1.	5.0	2.5	Nil	Day 5	0/2
2.	5.0	5.2	Nil	Day 4	(0%)
3.	10.0	2.7	Nil	Day 9	0/2
4.	10.0	5.5	Nil	Day 5	(0%)
5.	15.0	1.3	Nil	No	1/2
6.	15.0	5.7	Nil	Day 9	50%
7.	20.0	0.1	Nil	No	2/2
8.	20.0	0.2	Nil	No	(100%)
9.	20.0	0.02	Nil	No	2/2
10.	20.0	0.06	Nil	No	(100%)
11.	Control	4.2	75.0 on day 8	Died on day 8	0/1 (0%)

Tripathi,Vishwakarma and Dutta (2008) *Proc. Indian Natn. Sci. Acad*. **74**, 111-117

Table 63: Blood schizontocidal activity of sodium β-artelinate intravenous formulation against *P. knowlesi* in rhesus monkeys

S.No. of monkey	Drug dose (mg/kg) x 3 days	% Parasitaemia		Recrudescence (R) after last dose	Cure
		Initial	After last dose		
1.	15.0	0.05	Nil	Day 8	1/2 (50%)
2.	15.0	0.06	Nil	No	
3.	20.0	0.5	Nil	No	2/2 (100%)
4.	20.0	0.1	Nil	No	
5.	Control	0.03	55.0 on day 5	Died on day 6	0/1 (0%)

Tripathi,Vishwakarma and Dutta (2008) *Proc. Indian Natn. Sci. Acad*. **74**, 111-117

Table 64: Blood Schizontocidal Activity of Pyronaridine against *P. yoelii nigeriensis* (MDR Strain) in Swiss Mice

Treatment (oral)	Dose mg/kg (base)x day	Mean parasitaemia ± SD						Survival On day 28
		Day 4	7	10	14	24	28	
Pyronaridine	1.25 x7	0.08±0.17 (10)	1.27±2.66 (8)	4.1±5.81 (8)	8.4±15.9 (6)	0.0013±0.002 (3)	0.33±0.57 (3)	3/10
	2.5 x 7	0.03±0.09 (10)	0.4±1.26 (10)	4.66±14.4 (9)	0.62±1.76 (8)	0.001±0.002 (6)	3.66±8.98 (6)	6/10
	3.75 x 7	0.0±0 (10)	0.0±0.0 (10)	0.0±0 (10)	0.0±0 (9)	0.18±0.5 (8)	0.001±0.002 (8)	8/10
	5.0 x 7	0.0 (10)	0.0 (10)	0.0 (10)	0.001±0.003 (10)	0.001±0.003 (9)	0/.0 (9)	9/10
	10.0 x 7	0.0 (14)*	0.0 (14)	0.0 (14)	0.0 (14)	0.002±0.008 (14)	0.0 (14)	14/14
	15.0 x 7	0.0 (19)*	0.0 (19)	0.0 (19)	0.0 (19)	0.0 (19)	0.0 (19)	19/19
	20.0 x 7	0.0 (6)	0.0 (6)	0.0 (6)	0.0 (6)	0.0 (6)	0.0 (6)	6/6
	25.0 x 7	0.0 (6)	0.0 (6)	0.0 (6)	0.0 (6)	0.0 (6)	0.0 (6)	6/6
	30.9 x 7	0.0 (9)	0.0 (9)	0.0 (9)	0.0 (9)	0.0 (9)	0.0 (9)	9/9
Control		17.4±6.89 (10)	75.0±0.0 (1)		Died			

Note. No. of animals surviving is given in parentheses.
*Pooled data from three experiments.
Tripathi et al. (2000) *Exptl. Parasitol*. **94**, 190-193

Table 65: Blood schizontocidal activity of pyronaridine against multidrug-resistant *P. yoelii nigeriensis* following intramuscular 4 day treatment schedule

Treatment schedule	Dose mg base/kg	No. of mice	Parasitaemia % (Mean ± SD) day							No. of mice surviving
			4	7	11	15	19	21	28	
Pyronaridine (im) 4 days (at 0,24,48,72 hrs)	30 x 4	6	0.0 (6)	0.0 (6)	0.0 (6)	0.0 (6)	0.0 (6)	0.0 (6)	0.0 (6)	6/6
	25 x 4	6	0.0 (6)	0.0 (6)	0.0 (6)	0.0 (6)	0.0 (6)	0.0 (6)	0.0 (6)	6/6
	20 x 4	6	0.0 (6)	0.0 (6)	0.0 (6)	0.0 (6)	0.0 (6)	0.0 (6)	0.0 (6)	6/6
	15 x 4	6	0.0 (6)	0.0 (6)	0.0 (6)	0.0001±0.004 (6)	0.0 (6)	0.0 (6)	0.0 (6)	6/6
	10 x 4	6	0.0 (6)	0.0 (6)	0.0 (6)	0.0 (6)	0.0 (6)	0.0 (6)	0.0 (6)	6/6
	5 x 4	13	0.0 (13)	0.0 (13)	0.0 (13)	0.0007±0.002 * (13)	0.0 (13)	0.0 (13)	0.0 (13)	13/13
	2.5 x 4	7	0.0 (7)	0.0 (7)	0.0 (7)	0.0 (7)	0.0 (7)	0.0 (7)	0.0 (7)	7/7
	1.25 x 4	7	0.12±0.21 (7)	0.02±0.04 (7)	0.1±0.18 (7)	16.0±10.8 (6)	Died			0/7
	0.625 x 4	7	0.71±0.80 (7)	1.4±1.7 (7)	15.7±15.3 (4)	1.25±1.06 (2)	0.0 (2)	0.0 (2)	0.0 (2)	2/7
Control	-	6	6.65±3.5 (6)	48.75±11.8 (4)	Died					

Note: Number of mice surviving are given in parenthesis.
*One mouse in the group showed transient parasitaemia.
Dutta et al. (2000) *Life Sci*. **67**, 759-763

Table 66: Blood schizontocidal activity of pyronaridine against multidrug-resistant *P. yoelii nigeriensis* following intramuscular3 day treatment schedule

Treatment schedule	Dose mg base/kg	No. of mice	Parasitaemia % (Mean ± SD) Day						No. of mice surviving
			4	7	13	17	21	28	
Pyronaridine (i.m.) 3 days (at 0,8,24,48 hrs)	30 x 4	7	0.0 (7)	0.0 (7)	0.0 (7)	0.0 (7)	0.0 (7)	0.0 (6)	6/7
	25 x 4	7	0.0 (7)	0.0 (7)	0.0 (7)	0.0 (7)	0.0 (7)	0.0 (7)	7/7
	20 x 4	7	0.0 (7)	0.0 (7)	0.0 (7)	0.0 (7)	0.0 (7)	0.0 (7)	7/7
	10 x 4	6	0.0 (6)	0.0 (6)	0.0 (6)	0.0 (6)	0.0 (6)	0.0 (6)	6/6
	5 x 4	14	0.0 (14)	0.0 (14)	0.0 (14)	0.0 (14)	0.0 (14)	0.0 (14)	14/14
	2.5 x 4	13	0.0 (13)	0.0 (13)	0.0 (13)	0.0 (13)	0.0 (13)	0.0 (13)	13/13
Control	-	14	45.07±19.18 (14)	86.55±12.02 (2)	Died				

Note Number of mice surviving are given in parenthesis
*Non-specific death of one mouse.
Dutta et al. (2000) *Life Sci*. **67**, 759-763

Table 67: Blood schizontocidal activity of (Fenozans 7, 11 and 45) spirohydropyran derivatives (Fenozan 44,45,48 and 51) against multidrug resistant *P. yoelii nigeriensis* (MDR strain) by oral route of drug administration

Fenozan compounds (oral formulation)	Dose mg/kg/ Dayx5	No. of mice	Parasitaemia % (Mean ± S.D.)						No. of mice surviving on day 28	P value[a]
			Day 4	7	10	14	18	28		
Fenozen-7 (O)	30mg/kgx5	10	0.0 (10)	0.0 (10)	0.2±0.63 (10)	6.5±20.55 (10)	0.0 (9)	0.0 (9)	9/10	.000034
Fenozan-7 (D)	30mg/kgx5	10	0.0 (10)	0.0 (10)	14.6±31.0 (10)	0.62±1.76 (8)	0.0 (7)	0.0 (7)	7/10	.00103
Fenozan 11 (O)	30mg/kgx5	10	0.0 (10)	0.0 (10)	0.0 (10)	0.0 (10)	0.0 (10)	0.0 (10)	10/10	.0000028
Fenozan 11 (D)	30mg/kgx5	10	6.05±5.56 (8)	Died	-	-	-	-	0/10	1.0
Fenozan 45 (O)	30mg/kgx5	10	0.0 (10)	0.0 (10)	0.0 (10)	0.0 (10)	0.0 (10)	0.0 (10)	10/10	.0000028
Fenozan 45 (D)	30mg/kgx5	10	14.16±12.63 (10)	30.0 (1)	Died	-	-	-	0/10	1.0
Control	-	11	32.48±15.78 (11)	Died	-	-	-	-	0.11	-

[a]Derived by a Fischer TM exact test. O; oral route, oil vehicle, D; oral route, DMSO-Tween vehicle

Tripathi, Jefford and Dutta (2006) *Parasitology* **133,** 1-9

Table 68: Response of multi-drug resistant strain of *Plasmodium yoelii nigeriensis* (MDR) to antibiotics and antibiotic-chloroquine combinations in Swiss mice

Treatment	Dose (oral) mg/kg x days	No. of mice	% Parasitaemia (mean ± S.D.)						No. of mice surviving for 28 days
			Day 3	Day 5	Day 7	Day 9	Day 11	Day 14	
Tetracycline	500 x 4 (Day 0-3)	6	1.36±0.51	7.60±1.76	15.0±0.0	Died	-	-	Nil
Oxytetracycline	500 x 4 (Day 0-3)	6	1.30±0.94	6.95±3.0	8.70±0.28	8.70±0.28	11.0±0.0	Died	Nil
Doxycycline	25 x 4 (Day 0-3)	6	3.28±0.99	8.90±2.22	16.0±0.0	Died	-	-	Nil
Erythromycin	259 x 4 (Day 0-3)	6	5.86±0.14	15.55±4.0	16±0.0	Died	-	-	Nil
Ciprofloxacin	50 x 5 (Day 3-7)	8	11.75±5.1	64.2±28.0	Died	-	-	-	Nil
Norfloxacin	50 x 5 (Day 3-7)	8	11.75±5.1	74±18.27	Died	-	-	-	Nil
Tetracycline	50 x 5 (Day 3-7)	8	11.75±5.1	69.25±4.78	Died	-	-	-	Nil
Ciprofloxacin + Chloroquine	50 mg/kg x 5 32 mg/kg x 5 (Day 3-7)	10	11.75±5.1	9.8±3.25	8.6±4.12	-	-	1.5±4.9	Nil
Norfloxacin + Chloroquine	50 mg/kg x 5 32 mg/kg x 5 (Day 3-7)	10	11.75±5.1	8.7±3.3	10.3±12.8	-	-	40.0±36.0	3
Tetracycline + Chloroquine	50 mg/kg x 5 32 mg/kg x 5 (Day 3-7)	8	11.75±5.1	15±9.5	12.6±6.4	-	-	42.0±7.5	Nil
Chloroquine	32 mg/kg x 5 (Day 3-7)	8	11.75±5.1	12.5±4/17	25.1±33.7	-	-	44.5±15.1	Nil
Control	-	8	11.75±5.1	63.75±17.9	Died	-	-	-	Nil
Control	-	6	4.23±0.75	22.33±5.70	36.0±0	Died	-	-	Nil

Tripathi *et al*. (2005) *Parasitology* **131**, 295-301.

Table 69: Response of multi-drug resistant *Plasmodium yoelii nigeriensis* (MDR) to azithromycin treatment in Swiss mice

Treatment	Dose (oral) mg/kg x days	No. of mice	% Parasitaemia (mean ± S.D.)						No. of mice surviving for 28 days
			Day 5	Day 6	Day 8	Day 12	Day 16	Day 28	
Azithromycin	200 x 7	8	0.012±0.03 (8)	0.0±0.0 (8)	-ve (8)	-ve (8)	-ve (8)	-ve (8)	8
	100 x 7	8	0.01±0.03 (8)	0.0±0.0 (8)	-ve (8)	-ve (8)	-ve (8)	-ve (8)	8
	50 x 7	8	0.07±0.17 (8)	0.25±0.70 (8)	-ve (8)	-ve (8)	-ve (8)	-ve (8)	8
	50 x 7	8	N.D. (8)	0.02±0.04(8)	-ve (8)	-ve (8)	-ve (8)	-ve (8)	8
	25 x 7	8	N.D.	2.12±5.55 (8)	6.37±10.9 (7)	9.33±11.27 (4)	1.66±2.88 (4)	-ve (4)	4
	12.5 x 7	8	N.D.	17.14±5.55(8)	10.25±7.58(4)	5.0±3.8 (4)	9.59 (8)	-ve (3)	4
Control	-	8	11.25±2.86(8)	51.75±25.94(8)	65.52±34.39(4)	Died		-	Nil
	-	8	3.13±1.65 (8)	56.7±18.06 (8)	Died	Died		-	Nil

Tripathi *et al.* (2005) *Parasitology* **131**, 295-301.

Table 70: Chemotherapeutic response of azithromycin with α/β-arteether against MDR *Plasmodium yoelii nigeriensis* in Swiss mice

Sl. No.	Drugs/doses mg/kg x 4 days	% Mean parasitaemia ± S.D.					% Cure rate
		Day 4	Day 7	Day 14	Day 21	Day 28	
	Azithromycin						
1.	30.0	0.30±0.4 (5)	0.0 (5)	5.5±2.86 (5)	0.0 (4)	0.0 (4)	80.0
2.	15.0	1.22±0.97 (5)	8.0±7.77 (5)	4.3±2.8 (4)	0.0 (3)	0.0 (3)	60.0
3.	3.75	34.6±12.7 (5)	25.0±0.0 (1)	D			0.0
4.	Arteether						
5.	25.0	0.0 (5)	0.0 (5)	2.90±2.85 (5)	0.0 (2)	0.0 (2)	40.0
6.	16.6	1.0 0.0 (5)	0.0 (5)	0.0 (3)	0.0 (1)	0.0 (1)	20.0
7.	5.5	1.54±0.92 (5)	17.8±9.62 (4)	15.0±0.0 (1)	D		0.0
	Azithromycin + arteether						
8.	30.0 + 25.0	0.0 (5)	0.0 (5)	0.0 (5)	0.0 (5)	0.0 (5)	100.0
9.	15.0 + 16.6	0.0 (5)	0.0 (5)	0.0 (3)	0.0 (3)	0.0 (3)	60.0
10.	3.75 + 5.5	36.0±0.49 (5)	3.5±3.74 (5)	8.50±3.50 (2)	3.50±4.7 (2)	0.0 (2)	40.0
11.	Control	35.0±7.90 (5)	75.0±0.0 (1)	D			0.0

Tripathi *et al*. (2005) *Parasitology* **131,** 295-301

Table 71: Parasitologic-response data

Outcome	Monotherapy			Combination therapy	
	Azithromycin, Proportion (%)	Chloroquine, Proportion (%)	Difference, 95.1% Cl	Azithromycin and chloroquine proportion (%)	95% Cl
Day 3:eradication	3/16 (19)	9/16 (56)	-68 to 0	61/63 (97)	90 to 99
Day 7					
Eradication	10/16 (63)	14/16 (88)	-58 to 13	61/63 (97)	90 to 99
Failure: categories, RI, RII,RIII	0; 3; 3	0; 1; 1		0; 1; 1	
Day 28					
Eradication	5/14 (36)	4/15 (27)	-29 to 49	61/63 (97)	90 to 99
Failure: categories RI, RII,RIII	3; 3; 3	9; 1; 1		0; 1; 1	

Note: Cl, confidence interval.

Dunne, M.W. *et al.* (2005). *The J. Infectious Diseases* **191**, 1582-1588

Table 72: Mosquito infectivity rate and mean oocyst count of *A. stephensi* fed on *P. cynomolgi* B-infected rhesus monkeys before and after artemisinin treatment

Monkey No. and Mosquito feeding	Artemisinin Treatment	Mosquito infected/ dissected	Infectivity rate %	Oocyst count
4711				
Pretreatment				
-1 h	-	37/49	75.51	17.97 ± 17.83
Pretreatment				
24 h	20 mg/kg x 1	0/20	Nil	Nil
48 h	20 mg/kg x 2	0/59	Nil	Nil
72 h	20 mg/kg x 3	0/33	Nil	Nil
4712				
Pretreatment				
-1 h	-	38/46	82.60	51.52 ± 64.31
Posttreatment				
24 h	10 mg/kg x 1	0/25	Nil	Nil
48 h	10 mg/kg x 2	0/49	Nil	Nil
72 h	10 mg/kg x 3	0/9	Nil	Nil
4832				
Pretreatment				
-48 h	-	21/26	80/76	38.57 ± 28.97
-24 h	-	5/12	41.66	7.6 ± 4.82
-1 h	-	8/14	57.14	5.5 ± 5.5
Posttreatment				
24 h	10 mg/kg x 1	0/19	Nil	Nil
48 h	10 mg/kg x 2	0/16	Nil	Nil
72 h	10 mg/kg x 3	0/21	Nil	Nil
5131				
Pretreatment				
-1 h	-	19/22	86.36	154.05 ± 96.69
Posttreatment				
6 h	10 mg/kg x 1	19/26	73.07	14.73 ± 13.95
18 h	-	0/20	Nil	Nil
24 h	-	0/22	Nil	Nil
4903				
Pretreatment				
-1 h	-	16/32	50	3 ± 2.0
Posttreatment				
24 h	5 mg/kg x 1	0/51	Nil	Nil
48 h	5 mg/kg x 2	0/50	Nil	Nil

Table (continued)

Monkey No. and Mosquito feeding	Artemisinin Treatment	Mosquito infected/ dissected	Infectivity rate %	Oocyst count
4905				
Pretreatment				
-1 h	-	19/31	61/3	6.37±6.65
Posttreatment				
24 h	5 mg/kg x 1	0/72	Nil	Nil
48 h	-	0/47	Nil	Nil
5132				
Pretreatment				
-1 h	-	19/20	95/0	29.52±15.48
Posttreatment				
6 h	5 mg/kg x 1	14/20	70.0	18.05±16.92
18 h	-	0/25	Nil	Nil
24 h	-	0/18	Nil	Nil
5126				
Pretreatment				
-1 h	-	34/43	79.06	35.67±16.69
Posttreatment				
4 h	5 mg/kg x 1	1/15	6.66	1.00
10 h	-	0/27	Nil	Nil
24 h	-	0/27	Nil	Nil

Blood-induced infections were used in the study. Oocyst count values are given as means ± SD.

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Table 73: Sporontocidal action of artemisinin on the developing oocyst stages of *P. cynomolgi* B in *A. stephensi*

Age of infected mosquito used for feeding	Mosquito feeding on drug treated and control monkey[a]	Drug feeding	Mosquito infected/ dissected	Infectivity %	Oocyst count
1-day-old	Artemisinin	20 mg/kg x 2	19/20	82.60	42.60 ± 30.66
	control	-	19/20	95	31.15 ± 21.36
2-day-old	Artemisinin	20 mg/kg x 2	24/25	96	36.24 ± 31.12
	control	-	18/20	90	26.00 ± 24.83
3-day-old	Artemisinin	20 mg/kg x 2	24/25	96	25.6 ± 16.76
	control	-	18/20	90	6.15 ± 4.77
4-day-old	Artemisinin	20 mg/kg x 2	19/20	95	41.95 ± 30.50
	control	-	23/25	92	32.04 ± 16.01

Oocyst count values are given as means ± SD.

[a]Artemisinin was given as intramuscular injection in oil to healthy monkey and mosquitoes carrying 1- to 4-day-old infection were fed on artemisinin-treated healthy monkeys.

Dutta, Bajpai and Vishwakarma (1989) *Chemotherapy, Basel* **35**, 200-207

Table 74 : Effect of vehicle control (neutralized ground-nut oil intramuscular treatment) of the infectivity and oocyst count of *A. stephensi* fed on *P. cynomolgi* B-infected monkeys.

Monkey No. and Mosquito feeding	Vehicle (oil) treatment	Mosquito infected/ dissected	Infectivity rate %	Oocyst count
4836[a]				
Pretreatment				
-18 h	-	18/20	90	16.05 ± 11.21
-1 h	-	16/26	61.53	7.75 ± 4.29
Posttreatment				
48 h	2 ml oil x 2	3/5	60	10.0 ± 9.53
72 h	2 ml oil x 3	7/27	25.92	2.28 ± 1.38
4908[b]				
Pretreatment				
-1 h	-	16/17	94.11	15.0 ± 11.25
Posttreatment				
24 h	2 ml oil x 1	8/20	40	1.25 ± 0.46
48 h	2 ml oil x 2	12/20	60	19.66 ± 16.83
72 h	2 ml oil x 3	11/17	64.70	14.90 ± 22.33
4906[c]				
Pretreatment				
-1 h	-	20/20	100	61.25 ± 33.94
Posttreatment				
24 h	3 ml oil	36/36	100	19.88 ± 21.36
48 h	-	13/13	100	39/35 ± 0.78

Oocyst count values are given as means ± SD

[a]Blood-induced infection.

[b]Sporozoite-induced infection.

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Table 75: Gametocytocidal activity of single dose artemisinin (qinghaosu) against *Plasmodium cynomolgi* B

Batch of mosquitoes	Dose of drug	Time of mosquito feeding	*Anopheles stephensi* infectivity	
			Number infected/ number dissected	Oocyst count (mean ± SD)
Pretreatment				
Control	-	1 hr before drug	19/22 (86.36%)	154.05±96.69
Control	-	1 hr before drug	19/20 (95.00%)	25.52±15.48
Posttreatment				
Artemisinin	10 mg/kg x 1	18-24 hr after drug	0/78 (0)	0
Artemisinin	5 mg/kg x 1	18-24 hr after drug	0/246 (0)	0
Vehicle control	-	1 hr before oil	20/20 (100%)	61.25±33.94
Ground nut oil	3 ml x 1	24 hr after oil	36/36 (100%)	19.88±21/36
	-	48 hr after oil	13/13 (100%)	39.35±0.78

Dutta, Bajpai and Vishwakarma (1989) *Chemotherapy, Basel* **35**, 200-207

Table 76: Gametocytocidal activity of single dose α/β arteether treatment against *P. cynomolgi* B infection in rhesus monkeys as shown by *An. stephensi* infectivity data.

Monkey no.	Mosquito feeding	Treatment mg/kg (im)	Asexual parasitaemia	Gameto-cytaemia	No. of mosquitoes infected/ dissected	Infectivity rate	Oocyst count (mean±SD)
5128	Pre-treatment						
	-1 hr	-	1.2%	0.02%	18/26	69.23%	54.05±31.9
	Post-treatment						
	5 hr	Arteether 5 mg/kgx1	-	-	0/24	Nil	Nil
	8 hr	-	-	-	0/20	Nil	Nil
	24 hr	-	Nil	Nil	0/15	Nil	Nil
5384	Pre-treatment						
	-1 hr	-	1.5%	0.05%	20/20	100%	44.14±21.27
	Post-treatment						
	5 hr	Arteether 5 mg/kgx1	-	-	21/26	78%	43.14±31.31
	18 hr	-	-	-	10/28	35.71%	2.6±1.95
	24 hr	-	-	-	0/30	Nil	Nil
	48 hr	-	Nil	Nil	/20	Nil	Nil
5648	Pre-treatment						
	-1 hr	-	1.5%	0.1%	16/20	100%	59.94±29.42
	Post-treatment						
	4 hr	Arteether 5 mg/kgx1	-	-	19/20	95%	53.37±23.85
	18 hr	-	-	-	9/20	45%	4.44±3.57
	24 hr	-	Nil	0.01%	0/20	Nil	Nil
	48 hr	-	-	-	0/30	Nil	Nil
5971	Pre-treatment						
	-1 hr	-	1.6%	0.08%	12/16	75%	126.6±103.43
	Post-treatment						
	4 hr	Arteether 5 mg/kgx1	-	-	13/16	81.25%	98.92±81.93

	20 hr	-	-	-	0/30	Nil	Nil
	26 hr	-	Nil	0.002%	0/30	Nil	Nil
4541	Pre-treatment						
	-1 hr	-	1.3%	0.01%	49/54	98%	10.78±9.78
	Post-treatment						
	4 hr	Arteether 2.5mg/kgx1	-	-	24/40	60%	7.04±5.27
	18 hr	-	-	-	0/27	Nil	Nil
	24 hr	-	Nil	0.001%	0/32	Nil	Nil
	48 hr	-	-	-	0/26	Nil	Nil
5650	Pre-treatment						
	-1 hr	-	1.2%	0.1%	20/20	100%	46,8±24.5
	Post-treatment						
	4 hr	Arteether 2.5mg/kgx1	-	-	12/20	60%	26.5±35.72
	18 hr	-	-	-	0/35	Nil	Nil
	24 hr	-	Nil	0.01%	0/30	Nil	Nil
5758	Pre-treatment						
	-1 hr	-	0.67%	0.21%	20/24	83.33%	78,65±22,67
	Post-treatment						
	4 hr	Arteether 2.5mg/kgx1	-	-	9/22	40.9%	3.8±3.5
	8 hr	-	-	-	1/20	5%	1±0
	24 hr	-	Nil	0.05%	0/20	Nil	Nil
Oil	Pre-treatment						
(vehicle)	-1hr	-	3.5%	0.05%	16/17	94.11%	15.06±11.25
Control	Post-treatment						
4908	48 hr	2 ml oil x 2	2.5%	0.02%	12/20	60%	19.66±26.83
	72 hr	2 ml oil x 2	1.2%	0.02%	11/17	64.7%	14.9±23.33

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Table 77: Results of mosquito inoculation to determine the viability of sporozoites after gametocytocidal test

Time of mosquito feeding	Day 8 oocyst record		Viability of sporozoites on day 14	
	Percent infectivity	No. of oocysts	No. of mosquitoes used for infection in rhesus	Results
-1 hr	75	126.6±103.43	Not test	-
+4 hr	81.25	98.92±18.93	19	Patent on day 9
+20 hr	Nil	-	30	No patency till day 60
+26 hr	Nil	-	30	No patency till day 60

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Table 78: Sporontocidal action of α/β arteether on the developing oocysts of *P. cynomolgi* B in *An. stephensi*

	Age infection in Mosquitoes (days)	Mosquito feeding on drug-treated/control monkeys	Drug administration* (im)	No. of mosquitoes infected/ dissected	Infectivity rate	Oocyst count (Mean±SD)
Exp. 1	1	Arteether (2 hr after drug)	20 mg/kg x 1	33/24	97.05%	48.18±28.73
	1	Arteether (5 hr after drug)	20 mg/kg x 1	22/25	88%	56/09±34.59
	1	Control	-	20/20	100%	44.1±21.27
	2	Arteether (2 hr after drug)	20 mg/kg x 1	18/20	90%	89.77±71.62
	2	Arteether (5 hr after drug)	20 mg/kg x 1	10/13	76.92%	93.1±66.13
	2	Control	-	20/20	100%	46.1±23.68
Exp. 2	2	Arteether (5 hr after drug)	25 mg/kg x 1	13/13	100%	35.38±24.3
		Control		24/25	90%	26±24.83
Exp. 3	2	Arteether (2 hr after drug)	50 mg/kg x 1	16/20	80%	214.43±121.17
		Control	-	32/32	100%	284,96±105.23
Exp. 4	1	Arteether (5 hr after drug)	50 mg/kg x 1	9/16	56.25%	22.88±5.78
		Control	-	6/10	60%	23±5.69
	2	Arteether (5 hr after drug)	50 mg/kg x 1	8/11	72.72%	49.37±37.13
		Control	-	13/18	72.22%	29.77±23.38
Exp. 5	2	Arteether (3 hr after drug)	40 mg/kg x 1	10/12	83.36%	26.81±25.6
		Control	-	16/18	88.89%	147.87±70.59

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Table 79: Results of mosquito inoculation to determine the viability of sporozoites after sporontocidal test

Arteether dose to healthy monkeys	Age of infection in mosquitoes (days)	Day 8 oocyst record		Viability of sporozoite on day 14	
		Percent	No. of oocysts	No. of mosquitoes used for infection in rhesus	Results
50 mg/kg	1	56.25	22.8 ±5.78	15	Patent on day 12
at -5 hr	2	72.72	49.37 ±37.13	15	Patent on day 8
40 mg/kg at -3 hr	2	83.36	26.81±25.6	19	Patent on day 9
Control	-	88.89	147.87±70.59	19	Patent on day 9

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Table 80: Gametocytocidal action of orally administered α/β-arteether against *Plasmodium cynomolgi* B as shown by infection of *Anopheles stephensi*

Monkey No.	Feeding time	Dose	No. of mosquitoes infected/no. examined	% of mosquitoes infected	Mean±SEM oocyst count
1	Pretreatment (0 hr)	-	23/35	65.5	18.98±1.35
	Posttreatment	1.25 mg/kg			
	30 min	-	18/28	64.3	16.89±1.45
	4 hr	-	21/37	56.75	12.29±2.27
	20 hr	-	8/53	15.09	1.13±0.134
	24 hr	-	1/33	3.03	1±0.0
	48 hr	-	6/23	26.08	1.5±0.33
	72 hr	-	22/44	50.0	1.77±0.22
	96 hr	-	14/44	31.8	2.14±0.46
2	Pretreatment	-	14/20	69.8	16.52±2.35
	Posttreatment	2.5 mg/kg			
	4 hr	-	11/17	64.7	19.63±4.29
	8 hr	-	15/34	44.1	10.4±2.23
	20 hr	-	14/25	56.0	10.5±1.87
3	Pretreatment	-	29/40	72.5	43.72±4.81
	Posttreatment	5 mg/kg			
	4 hr	-	7/54	12.96	7.14±1.80
	9 hr	-	7/31	22.58	3.0±1.02
	20 hr	-	0/45	0	0
	24-48 hr	-	0/86	0	0
	96 hr		15/17	88.2	17.93±3.55
4	Pretreatment	-	22/22	100.0	42.59±7.44
	Posttreatment	10 mg/kg (2 divided doses)			
	1 hr	-	16/19	84.21	34.3±5.60
	4 hr	-	26/50	52.00	10.46±1.70
	8 hr	-	18/56	32.14	6.94±1.03
	19 hr	-	0/39	0	0
	26-96 hr	-	0/113	0	0
5	Pretreatment	-	20/22	90.9	55.75±5.37
	Posttreatment	10 mg/kg			
	8 hr	-	0/22	0	0
	24-96 hr	-	0/93	0	0

Table (continued)

Monkey No.	Feeding time	Dose	No. of mosquitoes infected/no. examined	% of mosquitoes infected	Mean±SEM oocyst count
6	Pretreatment Posttreatment 30 min 4 hr 8-96 hr	- 10 mg/kg - - -	18/20 12/24 0/20 0/136	90.00 50.00 0 0	52.33±6.31 47.66±5.45 0 0
7	Pretreatment Posttreatment 4 hr 18-48 hr	- 15 mg/kg - -	9/40 0/34 0/104	22.5 0 0	20.0±8.96 0 0
8	Pretreatment* Posttreatment 2 hr 24 hr 48 hr	- 5 ml of vehicle - 5 ml of vehicle -	20/23 6/6 2/9 9/15	86.96 100 22.22 60.0	45.15±9.62 189.33±24.42 23.5±11.53 20.88±5.88
9	Pretreatment* -24 hr† Posttreatment 24 hr 48 hr	- 5 ml of vehicle 5 ml of vehicle -	11/16 14/22 18/27	68.75 63.63 66.66	16.27±2.86 48.71±11.19 54.11±11.26
10	Control monkey 0 hr 18 hr 24 hr 48 hr 72 hr	 - - - - -	 10/10 9/9 9/9 8/9 12/14	 100 100 100 88.9 85.7	 54.2±8.22 51.8±8.45 58.11±11.4 50.12±8.75 55.08±6.40

*Monkeys 8 and 9 are vehicle control, 2 doses of the vehicle were given at 24 hr intervals.
†Control batch of mosquitoes fed on infected monkeys 24 hr prior to treatment with the vehicle.
Tripathi,Dutta and Vishwakarma (1996) *Am. J. Trop. Med. Hyg.* **54,** 652-654

Table 81: Results of mosquito homogenates inoculated into rhesus monkeys to determine the viability of sporozoites after testing the gametocytocidal action of α/β-arteether

Monkey No.	Doses of arteether	Posttreatment time (hr)	No. of mosquitoes	Results*
1	10 mg/kg in 2 divided doses	48-72	32	No patency
2	10 mg/kg	72-96	26	No patency
3	10 mg/kg	8-72	35	No patency
4	15 mg/kg	4	15	No patency
5	Control		15	Patency on day 8

*The follow-up period was more than 60 days.

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Table 82: Gametocytocidal activity of single dose arteether (α/β) against *Plasmodium cynomolgi* B

Dose	Batches of Mosquitoes	Time of mosquito feeding	*Anopheles stephensi* infectivity		
			Number infected / Number dissected	% Infectivity	Oocyst count (Mean±SE)
	Pre-treatment				
	Control	1 hr before drug	56/72	77.77	70.37±8.12
Arteether 5 mg/kgx1	Post-treatment	4-8 hrs after drug	53/106	50.00	60.49±7.04
		24 hrs after drug	0/95	Nil	Nil
	Pre-treatment				
	Control	1 hr before drug	89/98	89.00	23.60±2.89
Arteether 2.5 mg/kgx1	Post-treatment	4-8 hrs after drug	45/82	54.87	14.24±3.40
		24 hrs after drug	0/82	Nil	Nil
Ground nut oil*	Vehicle control	1 hr before oil	16/17	94.11	15.07±3.76
2 ml oil x 2		48 hrs after oil	12/20	60.00	19.66±7.75
2 ml oil x 3		72 hrs after oil	11/17	64.70	14.90±7.04

*2 ml oil was given 1 hr after control feeding and the dose was repeated after 24 and 48 hrs.

Tripathi,Dutta and Vishwakarma (1996) *Am. J. Trop. Med. Hyg.* **54,** 652-654

Table 83: Sporontocidal action of single dose arteether (α/β) against *P. cynomolgi* B

Batch of Mosquitoes	Dose of drug	Time of Mosquito Feeding	*Anopheles stephensi* infectivity		
			Number infected / Number dissected	Infectivity %	Oocyst count (Mean±SE)
Control	-	-	20/20	100%	46.1±5.29
Arteether	20 mg/kg	2 hrs	10/13	76.92%	93.1±20.92
Control	-	-	13/18	72.22%	29.77±6.49
Arteether	50 mg/kg	5 hrs	8/11	72.72%	49.37±13.16

Tripathi,Dutta and Vishwakarma (1996) *Am. J. Trop. Med. Hyg.* **54,** 652-654

Table 84: Gametocytocidal efficacy of sodium β-Artelinate against *P. cynomolgi* B in rhesus monkeys

Subject No.	Dose	Mosquito feeding time (hr)	Infectivity (%)	Mosquitoes (Infected/ tested)	Oocyst count (Mean±SE)
1.	25 mg/kg (Intravenous)	0 +4 +8 +24	78.13 1.32 Nil Nil	(50/64) (1/76) (0/40) 0/65)	185.44±56.06 1.0±0.0 Nil Nil
2.	20 mg/kg (Intravenous)	0 +4 +8 +24	100 30.4 Nil Nil	(6/6) (7/23) (0/26) (0/18)	8.0±1.78 1.43± 0.53 Nil Nil
3.	15 mg/kg (Intravenous)	0 +4 +24	72.2 Nil Nil	(13/18) (0/20) (0/5)	95.05±47.58 Nil Nil
4.	30 mg/kg (Oral)	0 +4 +18 +24 +48	91.66 50.0 Nil Nil Nil	(22/24) (15/30) (0/59) (0/26) (0/70)	95.91 ± 70.20 21.33 ± 17.62 Nil Nil Nil
5.	20 mg/kg (Oral)	0 +4 +8 +24 +48	82.19 25.9 2.17 Nil Nil	(60/73) (7/27) (2/92) (0/113) (0/92)	32.0 ± 2.94 4.0 ± 1.3 1.0 ± 0.0 Nil Nil
6.	10 mg/kg (Oral)	0 +4 +8 +18 +24 +48	93.02 7.29 Nil Nil Nil Nil	(40/43) (7/96) 0/82) (0/58) (0/72) (0/36)	32.95 ± 9.10 5.71 ± 2.55 Nil Nil Nil Nil
7.	Control	0 +8 +18 +24 +48	100 84.6 92.8 86.6 80.0	(15/15) (11/13) (13/14) (13/15) (12/15)	65.46 ± 27.96 60.27 ± 28.61 64.84 ± 28.83 54.53 ± 27.56 45.66 ± 28.06

Tripathi, Puri and Dutta (1996b) Sodium β-artelinate - A new potential gametocytocide. *Exptl. Parasitol*. **82** 251-254

Table 85: Sporontocidal efficacy of sodium β-Artelinate against *P. cynomolgi* B in *An. stephensi*

Dose	Infection age of mosquitoes (hr)	Oocyst count (Mean±SE)	Mosquitoes infectivity (%)	Viability of sporozoites	
				Mosquitoes used for sporozoite inoculation	Results
Sodium β-artelinate[a]	24	71.07 ± 13.3	86.66 (13/15)	30	Patent on Day 9
20 mg/kg (iv)	48	26.18 ± 3.15	82.78 (48/58)	20	Patent on Day 9

[a]Mosquitoes were allowed a blood/meal on a healthy monkey 15 min after drug administration.

Tripathi, Puri and Dutta (1996b) Sodium β-artelinate - A new potential gametocytocide. *Exptl. Parasitol*. **82** 251-254

Table 86: Effect of a single dose of compound CDRI 80/53 (elubaquine) on *Plasmodium cynomolgi* gametocytes as determined by their infectivity to *A. stephensi* mosquitoes

Dose mg/kg at 0 h	Time of mosquito feeding (h)	Parasitaemia (mm1)		Day 7 oocyst record	
		Asexual parasites	Gametocytes	Percent infectivity (mosquitoes infected/dissected)	Number of oocysts per gut (mean±SE)
1.25	-01	1,26,965	1895	89.5 (34/38)	22.40±2.0
	+05			21.1 (12/57)	2.17±1.4
	+24	1,03,846	1516	0 (0/36)	Nil
	+48	15,914	109	0 (0/24)	Nil
1.87	-01			83.3 (25/30)	28.20±3.8
	+05			15.0 (6/40)	1.17±0.2
	+24	18,020	530	0 (0/27)	Nil
	+48	7208	212	0 (0/24)	Nil
1.87	-01	61,560	1026	92.0 (23/25)	80.69±7.4[a]
	+05			58.1 (18/31)	13.00±0.6
	+24	42,180	798	0 (0/38)	Nil[b]
	+48	5130	228	0 (0/21)	Nil
2.50	-01	33,578	1442	78.3 (36/46)	13.72±1.5
	+05			60.6 (20/33)	2.90±0.4
	+24	45,320	927	0 (0/29)	Nil
	+48	18,025	206	0 (0/21)	Nil
3.75	-01	55,728	1296	96.3 (26/27)	22.35±3.0
	+05			0 (0/25)	Nil
	+24	56,808	540	0 (0/28)	Nil
5.00	-01	37,985	1090	93.6 (29/31)	78.17±13.1
	+05			0 (0/33)	Nil[b]
	+24	23,544	436	0 (0/26)	Nil[b]
5.00	-01	1,92,850	4060	82.1 (23/28)	68.74±10.6
	+05			0 (0/26)	Nil[b]
	+24	1,56,130	2039	0 (0/21)	
Primaquine 3.0	-01	29,680	1230	72.5 (37/51)	57.59±5.1
	+05			56.6 (30/53)	8.69±0.9
	+24	23,112	749	0 (0/33)	Nil
Primaquine 3.0	-01	16,824	1026	42.5 (20/47)	24.4±1.63
	+05			32.6 (15/46)	2.6±0.5
	+24	21,204	676	0 (0/43)	Nil

[a]Patent infection developed in 9-10 days in naïve monkeys upon inoculation of 10 mosquitoes' homogenate on day 15 post-infective blood meal

[b]No patency developed during 60 days observation period in naïve monkeys inoculated with 40-50 mosquitoes' homogenate on day 15 post-infective blood meal

Puri and Dutta (2005) *Exptl. Parasitol*. **111**,8-13

Table 88: Baseline parasitaemia vs enhanced post-treatment gametocytaemia in Gambian children

Antimalarial treatment		Pretreatment (%)		Post-treatment (day 28)	
		Asexual	Gametocytes	Asexual	Gametocytes on day 7
1.	Chloroquine	20.0	2.2	30.2 Lower efficacy	18
2.	Pyrimethamine/ Sulfadoxine (PSD)	21.7	5.5	3.7	56.3
3.	PSD + Artesunate x 1 dose	2.7	9.8	1.4	11.4
4.	PSD + Artesunate x 3 doses	5.4	12.7	0.0	8.7

Conclusion: First line PSD treatment in young Gambian children resulted in very high prevalence of gametocytaemia (56.3%) on day 7 post-treatment.

Von Seidlein *et al.* (2001).

Table 89: Radical curative activity of 6,7 and 8-aminoquinolines against *P. cynomolgi* B in rhesus model.

Chemical class and Code No.	Radical curative activity (primaquine index = PI)
6-Aminoquinolines WR188438 NI 147/36	 Active Active
7-Aminoquinolines WR 213640	 Active (PI 0.1)
5-Phenoxy-8-aminoquinolines WR 225374, 182232, 216100, 235720, 235724, 215295, 233878, 233881, 234738	 Active (PI 0.2-3.2)
2-Methyl-5-phenoxy-8-aminoquinolines WR 211532, 224097, 224486 2-Methyl-primaquine	 Active (PI 1.7-3.1) Active (PI 1.0)
3-Methyl-5-phenoxy-8-aminoquinolines WR 235485 3-Methyl-primaquine	 Active (PI 1.5) Active (PI 1.3)
4-Methyl-5-phenoxy-8-aminoquinolines WR 232956, 232584, 225448, 233195, 233078 4-Methyl-primaquine	 Active (PI 4.2-4.8) Active (PI 2.1)

From: Davidson *et al*. 1981. *Bull. Wld. Hlth. Org*. **59**: 463-479

Table 90: Comparison of antimalarial activity of CDRI 80/53 (Elubaquine, primaquine and WR 238605 (Tafenoquine) against *P. cynomolgi* B infection in rhesus monkeys.

Drugs (Oral)	CDRI 80/53	Primaquine	WR 238605
1. Radical curative (anti-relapse) dose (7 days test)	1.25 mg/kgx7 cure 9/9 monkeys 2.0-4.0 mg/kgx7 Cure 13/13 monkeys	1.0 mg (base)/kgx7 cure 12/12 monkeys	0.326 mg/kgx7 Cure 7/7 monkeys
2. Causal prophylactic dose (3 day test)	3.16 mg/kg x3 Cure 3/3 monkeys	1.78 mg (base) kgx3 Cure 7/7 monkeys	0.316 mg/kgx3 Cure 9/9 monkeys
3. Blood schizontocidal dose (7 day test)	Low activity	10 mg (base)/kgx7 (25% curative dose) monkeys	3.16 mg/kgx7 Cure 6/6 monkeys
4. Gametocytocidal dose	-	2.00 mg (base)/kgx1	2.00 mg/kgx1

CDRI 80/53 and primaquine data summarized from Dutta *et al.* (1989, 1994) and WR 238605 data from Puri *et al*. (1994).

Table 91: Pre-clinical and clinical efficacy antimalarial activity of new antirelapse drug CDRI 80/53 vs primaquine

Sl. No.		Curative doses	
		CDRI 80/53	Primaquine
I.	Antirelapse activity against sporozoite induced *P. cynomolgi* B in rhesus model (Test drug + chloroquine 5 mg base/kg as companion blood chizonticide	1.25 mg/kgx7 days Cure 9/9 rhesus	1.0 mg base/kgx7 days Cure 30/30 rhesus
		2.0 and 4.0 mg/kg x 7 days cure 13/13 monkeys	-
II.	Causal prophylactic activity *P. cynomolgi* sporozoite induced in rhesus	3.16 mg/kg x 3 days 10 mg/kgx1 day	1.78 mg base/kg x 3 days 10 mg/kg x 1day
III.	Blood schizontocidal *P. berghei*	ED_{50} 3.67+0.64 Mg/kg x 4 doses	-
IV.	Gametocytocidal *P. cynomolgi* B in rhesus – *Anopheles stephensi model*	1.25 and 2.0 mg/kg x 1 dose 100% gametocytocidal at 24 h	1 mg/kg x 1 not 100%
		3.75 mg/kg x 1 100% gametocytocidal at 5 h after treatment	3.16 mg/kg base x 1 100% action after 24-48 h
V.	LD_{50} toxic dose in mice Safety on LD_{50} basis	681 mg/kg 8.5 fold safer	80 mg base/kg -
VI.	Acute toxicity in baby rhesus (3-4 months) Drug powder administered i/p after laparotomy Safety level	Single doses 100, 200, 300, 500 and 1000 mg/kg – safe No death 10-fold safety as compared to primaquine oral	Single 50-300 mg/kg killed the monkey in 24 hr 100 mg/kg dose kills monkey in 1 h -
VII.	Subacute regulatory toxicity	Safe in 3 months toxicity in 2 animal species No adverse effects	-
VIII.	Methaemoglobin toxicity at x 3 times (antirelapse) dose in Beagle dogs Normal Met Hb (pretreatment value) 0.74+0.07 g%	3.75 mg/kgx7 days 2.14+0.89g% on day 7 3-4 times safer than primquine	3.0 mg/kg x 7 days 7.81+1.48g% on day 7

	Safety level		-
Sl. No.		Curative doses	
		CDRI 80/53	Primaquine
IX.	Teratogenic potential	-	-
X.	Phase I clinical trials (safety/tolerance)	Doe 25 mg ODx7 days	15mg OD x7
	Methaemoglobin valuses Pretreatment 2.29+0.94g% 3.97+1.33g%	3.02+1.31g%	16.32+1.41g%
	Safety level	5 time safer compared to primaquine	-

Table 92: Methaemoglobin levels in beagles after treatment with primaquine or compound 80/53

Group	Treatment (mg/kg x 7 days)	Methaemoglobin (g%)*			
		Day 0[+]	Day 3	Day 7[++]	Day 13[+++]
I	Primaquine 1.0 mg/kg	0.65 ± 0.03 (0.62-0.68)	0.85 ± 0.13 (0.75-1.0)	2.39 ± 0.23 (2.12-2.53)	1.98 ± 0.34 (1.69-2.36)
II	Primaquine 3.00 mg/kg	0.74 ± 0.07 (0.68-0.82)	1.94 ± 0.33 (1.56-2.17)	7.81 ± 1.48 (6.41-9.37)	5.51 ± 1.03 (4.32-6.20)
III	Compound 80/53 1.25 mg/kg	0.53 ± 0.11 (0.40-0.61)	0.87 ± 0.17 (0.67-0.99)	0.89 ± 0.29 (0.56-1.13)	1.04 ± 0.24 (0.77-1.21)
IV	Compound 80/53 3.75 mg/kg	0.66 ± 0.15 (0.62-0.82)	1.0 ± 0.19 (0.85-1.23)	2.14 ± 0.89 (1.23-3.01)	1.66 ± 0.52 (1.09-2.07)
V	Control	0.64 ± 0.09 (0.57-0.71)	0.46 ± 0.09 (0.40-0.53)	0.74 ± 0.01 (0.73-0.75)	0.65 ± 0.10 (0.58-0.73)

*Mean ± SD (range)

+Before start of treatment

++One day after last dose

+++Seven days after last dose

Puri *et al*. (1989) *Am. J. Trop. Med. Hyg.* **41**, 638-642.

Table 93: Haematological parameters in beagles after treatment with 3 mg/kg primaquine or 3.75 mg/kg compound 80/53

Parameter	Primaquine (3 mg/kg)*			CDRI 80/53 (3.75 mg/kg)*		
	Day 0	Day 7	Day 13	Day 0	Day 7	Day 13
Hemoglobin (g%)	13.97 ± 0.19	14.95 ± 0.08	14.25 ± 0.24	14.33 ± 0.87	14.06 ± 0.61	14.32 ± 0.39
RBC ($x10^6/mm^3$)	5.20 ± 0.10	5.00 ± 0.19	4.98 ± 0.17	5.19 ± 0.19	5.00 ± 0.38	5.03 ± 0.17
WBC (mm^3)	10355.3 ± 168.3	10712.6 ± 456.6	11185.9 ± 128.8	11157.6 ± 2224.3	10206.3±1980±7	11094.2±1371.3
Lymphocyte (%)	40.0 ± 2.0	26.66 ± 4.16$^+$	30.33 ± 0.57	33.66 ± 2.51	29.33 ± 1.52	31.66 ± 2.31
Neutrophils (%)	57.66 ± 2.08	71.66 ± 4.04$^+$	66.33 ± 2.30	62.33 ± 2.08	66.33 ± 2.08	63.00 ± 3.46
Mean corpuscular volume (II)	67.96 ± 6.14	61.03 ± 4.21	63.90 ± 2.32	60.96 ± 3.32	63.26 ± 0.25	65.10 ± 2.37

*Mean ± SD

+P<0.001

Puri *et al*. (1989) *Am. J. Trop. Med. Hyg.* **41**, 638-642.

Table 94: Changes in liver function test after treatment with 3 mg/kg primaquine or 3.75 mg/kg compound 80/53

Parameter	Primaquine (3 mg/kg)*			CDRI 80/53 (3.75 mg/kg)*		
	Day 0	Day 7	Day 13	Day 0	Day 7	Day 13
SGOT (IU/L)	35.66±1.15	39.66±3.51	34.66±1.52	30.66±3.78	29.66±2.08	30.88±3.21
SGPT (IU/L)	36.00±2.64	37.00±1.00	38.33±3.78	38.33±5.68	39.33±2.30	37.33±2.30
LDH (IU/L)	411.70±10.30	401.13±19.20	409.10±13.40	410.20±11.18	430.60±7.60	421.60±2.76
Bilirubin (mg%)	0.60±0.10	0.66±0.05	0.60±0.10	0.66±0.005	0.63±0.05	0.63±0.05

*Mean ± SD

Puri *et al.* (1989) *Am. J. Trop. Med. Hyg*. **41**, 638-642.

Table 95: Results of clinical trials for anti-relapse activity of CDRI 80/53 (Elubaquine), primaquine and WR 238605 (Tafenoquine) among *P. vivax* cases and methaemoglobin level produced in patients.

Treatment	Follow-up period long term	No. of *P. vivax* cases treated	Daily dose (x days)	Relapse rate	Met Hb values	
					Before treatment	After treatment
CDRI 80/53	1 year	219	25 mg x5	*29.6%	2.29%	3.02% (7 dose)
Primaquine	1 year	220	15mg x5	*26.8%	3.97%	16.32% (7 dose)
Placebo (chloroquine)	1 year	224	1500mg	*40.1%	-	-
Short terms (Non-transmission period)						
CDRI 80/53	Mid Nov-March	219	25 mgx5	4.9%		
Primaquine	Mid Nov-March	220	15 mgx5	3.05		
Placeboc chloroquine	Mid Nov-March	224	1500 mg	10.6%		
WR238605	2-6 months 2-6 months 2-6 months	7 9 7	300mgx7 500mgx3 (repeated After 1 week) 500mgx1	Nil 1 case 1 case	1-3% 1-3% 1-3%	13.5% 14.7% 6.4%
Total 23 cases 8.7%						
Placebo chloroquine	2-6 months	7	1500MG	4 case (57%)	1-3%	1-3%

CDRI 80/53 primaquine data summarized from Valecha *et al.* (2001), and WR 238605 from Walsh et al. (1999).

*Re-infections over one year period cannot be ruled out.

Table 96: Therapeutic responses

	Group A (n = 71)	Group B (n = 70)	P-value
No. (%) of patients withdrawal (day at withdrawal)	3 (4.3%) 22, 22, 22	4 (5.7%) 14, 14, 22, 28	-
No. (%) of patients with 28-day follow-up (evaluable patients)	68 (95.7%)	64 (94.3%)	-
No. (%) of patients with *P. vivax* reappearance	1 (1.5%)	0	-
Day of reappearance of parasitaemia	26	0	-
No. (%) of patient cured at day 28	67/68 (98.5%)	64/64 (100%)	0.565
Fever clearance time (hr) Mean (±SD) Range	 18.65 (13.65) 4~70	 22.74 (17.62) 4~90	 0.125
Parasite clearance time (hr) Mean (±SD) Range	 43.68 (17.29) 14~88	 44.07 (19.02) 20~106	 0.897

Krudsood et al. 2006 *Korean J. Parasitol*. 44 221-228

Table 97: Radical curative activity of compound WR 238605 against sporozoite induced *P. cynomolgi* B infection in rhesus monkeys

Daily dose mg(base)kg	Total course dose mg(base)/kg	No. of monkeys treated	Response to treatment	
			No. cured	No. not cured (day or patency)
0.10	0.70	2	0	2 (17, 24)
0.316	2.21	2	2	0
9.316	2.21	3	3	0
1.0	7.00	2	2	0
Reference drug primaquine				
1.00	7.00	3	3	0
Chloroquine control				
-	-	3	0	3 (15,17,20)

Puri, Dutta, Milhous, Schuster and Davidson (1994) Proc. Tropical Diseases: Mol. Biol. & Control Strategy. Ed. Kumar, S., Sen, A.K., Dutta, G.P. and Sharma, R.N. Publ. & Information Directorate, CSIR, New Delhi

Table 98: Causal prophylactic activity of compound 238605 against sporozoite induced *P. cynomolgi* B infection in rhesus monkeys

Daily dose mg(base)kg	Total course dose mg(base)/kg	No. of monkeys treated	Response to treatment	
			No. cured	No. not cured (day or patency)
0.03	0.09	4	0	4 (8,8,8,8)
0.10	0.30	3	0	3 (8,8,8)
0.10	0.30	4	1	3 (8,8,17)
0.316	0.95	3	3	0
0.316	0.95	3	3	0
0.316	0.95	3	3	0
1.00	3.00	2	2	0
Reference drug primaquine				
1.78	5.34	4	4	0
Vehicle control				
-	-	4	0	4 (8,8,8,9)

Puri, Dutta, Milhous, Schuster and Davidson (1994) Proc. Tropical Diseases: Mol. Biol. & Control Strategy. Ed. Kumar, S., Sen, A.K., Dutta, G.P. and Sharma, R.N. Publ. & Information Directorate, CSIR, New Delhi

Table 99: Blood schizontocidal activity of WR 238605 and primaquine against trophozoite induced *P. cynomolgi* B and *P. fragile* in rhesus monkeys

Dose Mg(base)/kg x 7 days	Total course dose Mg(base)/kg)	No. of monkeys treated	Response to treatment	
			No. of monkeys cured	No. of monkeys showing recur-descence (on day)
A.	*Plasmodium cynomolgi* B infection			
WR 238605				
0.316	2.21	4	0	4 (7,13,18,20)
1.00	7.00	12	10	2 (20,26)
3.16	22.12	6	6	0
Primaquine				
1,0	7.00	2	0	2 (10,12)
3.16	22.12	4	0	4 (13,15,16,19)
10.00	70.00	4	1	3 (15,24,28)
B.	*Plasmodium fragile* infection			
WR 238605				
0.316	2.21	4	0	4 (16,19,24,28)
1.00	7.0	11	10	1 (36)
3.16	22.12	4	4	0
Primaquine				
1.00	7.00	2	0	2 (13,16)
3.16	22.12	3	1	2 (17,20)
10.00	70.00	3	2	1 (18)

Puri, Dutta, Milhous, Schuster and Davidson (1994) Proc. Tropical Diseases: Mol. Biol. & Control Strategy. Ed. Kumar, S., Sen, A.K., Dutta, G.P. and Sharma, R.N. Publ. & Information Directorate, CSIR, New Delhi

Table 100: Gametocytocidal activity of compound WR 238605 in *P. cynomolgi – A. stephensi* – rhesus monkey model

Dose (mg/kg) at 0 hr	Time of mosquito feeding (hr)	Parasitaemia/mm^3		Day 7 oocyst record	
		Asexual	Gameto-cytaemia	No. of mosquito infected/dissected (% infectivity)	Oocyst number ((Mean±SD)
1	-1	23112	749	32/27	(86.49%)
	+6	-	-	31/36 (86.11%)	42.26±23.76
	+24	26215	533	31/39 (79.49)	25.77±17.63
	+48	7383	321	0/30 (Nil)	-
1	-1	39055	1391	32/40 (80.0)	17.13±10.01
	+5	-	-	32/44 (72.73%)	13.69±7.20
	+24	28248	321	0/31 (Nil)	-
	+48	5992	107	0/23 (Nil)	-
1	-1	48384	6832	25/34 (73.53%)	32.12±13.62
	+6	-	-	32/40 (80.0%)	31.06±12.73
	+24	42448	3256	36/48 (75.0%)	30.86±13.81
	+48	20832	1008	0/25 (Nil)	-
2	-1	54805	2938	43/53 (81.13%)	28.91±18.43
	+5	-	-	47/58 (81.03%)	27.13±16.30
	+24	26555	1243	0/38 (Nil)	-
2	-1	42619	2398	25/39 (64.10%)	18.40±10.19
	+5	-	-	30/48 (62.5%)	17.83±6.80
	+24	26487	1199	0/36 (Nil)	-
2	-1	42036	3503	22/27 (81.48%)	35.32±13.34
	+6	-	-	28/34 (82.35%)	26.89±11.19
	+24	21344	2668	0/31 (Nil)	-
4	-1	73902	3390	28/33 (84.85%)	29.75±12.41
	+6	-	-	31/36 (86.11%)	38.16±19.28
	+24	47008	1808	0/30 (Nil)	-

Puri, Dutta, Milhous, Schuster and Davidson (1994) Proc. Tropical Diseases: Mol. Biol. & Control Strategy. Ed. Kumar, S., Sen, A.K., Dutta, G.P. and Sharma, R.N. Publ. & Information Directorate, CSIR, New Delhi

Table 101: Instruction manual for 6 days treatment of malaria caused by *Plasmodium vivax*

	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
Chloroquine tablet	Start with 2 tablets (300 mgx2)	1 tablet 300 mg	1 tablet 300 mg	X	X	X
	1 tablet after 6 hours (300mgx1)	+	+			
Capsule of compound of formula (I) (Bulaquine)	X	1 capsule 25 mg	1 capsule 25 mg	1 capsule 25 mg	1 capsule 25 mg	1 capsule 25 mg

Aablaquin oral anti-relapse regimen for *P. vivax* comprising of Chloroquine (x 3 days) and Bulaquine (x 5 days)

CDRI Patent. 501/MUM/2000 Grant 31.05.2000 IN. Mumbai

Table 102: Blood schizontocidal action of arteether α/β-arteether formulation on multi-drug resistant *P. yoelii nigeriensis* (MDR II) infection in Swiss mice (25±1 g) by rectal route of administration

Treatment route	Dose mg/kg x days	Parasitaemia % (mean±SD)			
		Day 4	Day5	Day 11	Day 28
α/β-arteether (rectal	10x3	0.0002±0.001 (8) 6-ve	0.002±0.080 (8) 6-ve	0.0 (3)	0.0 (3)
	10x3*	0.0 (8)	0.0 (8)	10.25±10.0 (4)	0.0 (1)
	15x3	0.0 (8)	0.0 (8)	0.0 (4)	0.0 (4)
	15x3*	0.0 (8)	0.0 (8)	0.0 (4)	0.0 (4)
	20x3	0.0 (8)	0.0 (8)	0.0 (5)	0.0 (5)
	20x3*	0.0 (8)	0.0 (8)	0.0 (6)	0.0 (6)
Control	-	21.85±24.3 (7)	57.6±24.7 (5)	Died on day 7	

*The dose was given in 2 divided doses at 6 hrs intervals. Number of animals given in parenthesis.

(Data on Rectal Suppository using α/β-Arteether)

Table 103: Blood schizontocidal activity of dihydroartemisinin against multi-drug resistant *P. yoelii nigeriensis* infection in Swiss mice (25±1 g)

Treatment (route)	Dose mg/kg x days	Parasitaemia %						
		Day 5	Day 7	Day 9	Day 12	Day 16	Day 23	Day 30
Dihydro-artemisinin (Rectal route)	5mg/kgx5	0.025 ±0.07 (8) 7-ve	3.87 ±6.05 (8) 4-ve	26.75 ±30.33 (8) 3-ve	0.0 (3)	0.0 (3)	0.0 (3)	Died
	10mg/kgx5	0.012 ±0.035 (8) 7-ve	1.12 ±1.88 (8) 5-ve	31.55 ±36.50 (8) 4-ve	0.0 (4)	0.0 (3)	0.0 (3)	0.0 (1)
	20mg/kgx5	0.0 (8)	0.75 ±1.75 (8)6-ve	4.28 ±11.33 (7) 6-ve	13.5 ±33.0 (6) 5-ve	0.0 (5)	0.0 (3)	0.0 (3)
(Rectal Route)	5 mg/kgx7	0.83 ±1.31 (8) 4-ve	1.12 ±1.55 (8) 4-ve	14.12 ±27.86 (8) 3-ve	0.0 (3)	0.0 (3)	1.33 ±2.30 (3) 3-ve	0.0 (2)
	10mg/kgx7	0.0 (7)	0.21 ±0.39 (7) 5-ve	0.42 ±0.78 (7) 5-ve	0.0 (6)	0.0 (3)	0.0 (3)	0.0 (3)
	15mg/kgx7	0.0 (8)	0.0 (6)	0.0 (7)	0.012 ±0.03 (8)7-ve	0.0 (7)	0.0 (7)	0.0 (4)
	20mg/kgx7	0.0 (8)	0.0 (8)	0.0 (8)	0.0 (8)	0.0 (8)	0.0 (7)	0.0 (7)

CHAPTER-IV

DISCUSSION

ROLE OF INDIAN ANTI-MALARIAL DRUG DISCOVERY TO SUPPORT INTERNATIONAL AGENDA AND PROGRAMMES FOR ERADICATION OF MALARIA

CSIR-CENTRAL DRUG RESEARCH INSTITUTE: ANTIMALARIAL DRUG DISCOVERY INITIATIVE

HISTORICAL BACKGROUND

The Council of Scientific & Industrial Research was established by the Government of India, to formulate Science and Technology Policy for India. The Central Drug Research Institute was established at Lucknow in 1951, to undertake the Drug Discovery Programme in the areas of Tropical Diseases, Metabolic Diseases, Reproduction and Public Health. Sir Edward Mallanby, FRS (British Scientist) was appointed as the First Director of CDRI. The author of this status report on "New Antimalarial Drug Discovery in India" was awarded an International Post-Doctoral Fellowship of U.S. Public Health Service by the Indian Council of Medical Research for one year Research & Training Programme (July 1962-63) at U.S. Public Health Malaria Laboratory (Columbia, S.C.) and National Institutes of Health, Bethesda. This laboratory was engaged in experimental malaria transmission and antimalarial drug trials in humans against multidrug resistant malarias. The author joined the Central Drug Research Institute as Senior Scientific Officer on 29th September, 1964. I was appointed Principal Investigator of WHO sponsored project "Interaction of Hormonal Contraceptives on Malaria" in Primate Malaria Models (Dutta *et al.* 1984 *Bull. WHO* **62** 931-939).

The Research and Development work on antimalarials was initiated at CDRI in 1976 which was initially sponsored by Director General, Indian Council of Medical Research. CDRI was appointed Nodal Institute to develop projects on "Chemotherapy of Malaria". "Modified plan of operation for control of malaria" was coordinated by National Malaria Eradication Programme of Government of India with major focus on control of emerging chloroquine and MDR resistance by *P. falciparum*. Besides the emergence of parasite resistance to antimalarials, the malaria mosquito vectors had developed high level of insecticide (DDT/Malathione) resistance, which resulted in exponential rise of *P. falciparum* in the country. There was no safe

gametocytocidal/transmission blocking drug. To develop safe drugs for treatment of relapsing *P. vivax* was a challenging task to eradicate/kill the hypnozoites-the relapsing stage in liver. Primaquine discovered in 1934 continues to be the drug in clinical use to treat the relapses of *P. vivax* as well as transmission blocking drug to kill gametocyte (stage V) of *P. falciparum*. Toxicity of primaquine and related 8-aminoquinolines had been documented by WHO (1955) and this drug is highly toxic in G-6-PD deficient cases besides causing haemolytic effect on RBC and the drug is contraindicated for infants and pregnant women.

Drug Discovery Programme for new antimalarials at CDRI/CIMAP was started from 1976 backed by the introduction of **new technology upgradation at CDRI for antimalarial *in vivo* screening**, development of **highly multidrug resistant/fatal strain of rodent *Plasmodium yoelii nigeriensis*** (as Primary Screening Model) and *P. berghei*, establishment of **large scale mosquito *Anopheles stephensi* colony for transmission blocking studies** (**gametocytocidal activity)**, setting-up of large **primate/rhesus monkey colony** for evaluation of **new drugs and artemisinins** (**fast-acting blood schizontocides to stop malaria related mortality and screening of new radical curative agents against *P. cynomolgi* B – hypnozoites in simian malaria model).**

Baird (2009) has reviewed the problem related to "Drug Resistance of *P. vivax* malaria" and listed that the following priorities can lead to eradication of *P. vivax.*

1. New drugs (products) for clinical medicine.
2. Need for continuous *P. vivax* cultivation (blood stages, tissue stages, hypnozoites-relapsing stage in tissue cultivation).
3. *In vivo* tests for drug sensitivity of *P. vivax* blood stages/liver stages to drugs.
4. Suitable non-human primate facility and insectaries of *Anopheles* mosquitoes.
5. Establish clinical research centres for *P. vivax* sporozoite challenges.
6. Develop single, fixed dose combination of drugs which could be safe and eliminate all stages of *P. falciparum* and *P. vivax.*
7. Several references had also been cited by Baird (2009) to show that *P. vivax* could also cause cerebral complications like *P. falciparum* (Mishra and Singh, 1989; Verma and Magotra, 1976; Valecha *et al.*, 1992; Tjitra *et al.*, 2008). A new anti-relapse drug for *P. vivax* (compound CDRI 80/53 (WHO INN: Elubaquine,

Bulaquine) developed by CDRI has 3-4 times better safety than clinically used standard primaquine for **eradication of relapsing stage (hypnozoites) from liver** (Dutta *et al.* , 1989; Puri *et al.*, 1989; Patent U.S. Pratap *et al.*, 2005) and **as gametocytocide to stop transmission** (Patent: Pratap, R. *et al.,* 2006; Puri and Dutta, 2006). Besides, in **collaboration with U.S. WRAIR**, extensive studies led to the **discovery of tafenoquine (WR236085) (anti-relapse/blood schizontocidal/ gametocytocidal action** drug, which had been published (1988-1997). In addition, WRAIR-CDRI collaborative project with Walter Reed U.S., have identified several new anti-relapse/causal prophylactic 8-aminoquinoline compounds.

Besides bulaquine (elubaquine) another 8-aminoquinoline tafenoquine (WR238605) which possesses both blood schizontocidal, anti-relapse as well as gametocytocidal activity has been developed in close collaboration with Walter Reed Army Institute (WRAIR). Both bulaquine and tafenoquine can be used as safer primaquine substitutes. Bulaquine was marketed in India in 2000 and tafenoquine has been further developed by WRAIR and extensive clinical data is now available.

Since the discovery of artemisinin in 1970 by the Chinese Scientists, major advance has been made in the development of fast-acting blood schizont namely α/β-arteether (30:70) mixture of epimers which shows fast-acting blood schizontocidal activity against MDR *P. falciparum*. It is oil soluble artemisinin derivative with a half-life of more than 28 hrs. It is one of the most fast-acting drug for comatose cerebral malaria cases, besides having transmission blocking action against *P. falciparum.*

Fidock *et al.* (2004) while discussing the **problems of drug discovery** reported that malaria had been the most damaging disease of developing world, killing 1-3 million people and causing 300-500 million infections specially by *P. falciparum* which causes heavy mortality in children under 5 years. Fidock *et al.* (2004) also briefly report up-date on the progress of **Medicines for Malaria Venture** (a public-private partnership established in 1999 in Geneva) who claim to have identified 21 collaborative R&D projects. Besides, the MMV claims progress on several **development projects (to develop new drugs**) to cure uncomplicated MDR *P. falciparum* malaria within 3 days which specifically include (1) **rectal artesunate**, (2) **pyronaridine-artesunate**, (3)

mefloquine-artesunate, (4) **synthetic peroxides**, (5) azithromycin-chloroquine, (6) tafenoquine, (7) 8-aminoqunolines etc. It needs to be recorded that WHO sponsored ACT's (Mefloquine-AS and Rectal artesunate) are a part of MMV clinical projects. Several leads published/patented by CDRI group include – (1) Pyronaridine, (2) Azithromycin, (3) Dihydroartemisinin, (4) Tafenoquine, Bulaquine/Elubaquine (published as primaquine substitutes) have been co-opted as components of development projects by International MVV as their New Drug Development Programmes.

An Editorial entitled "**Is malaria eradication possible**" in Lancet (Vol. 370, Oct. 27, 2007) quote the statement of **Bill and Melinda Gates admit that malaria eradication**, **it is possible, is a long way off-years or more likely decades away** and they clearly argued that "Is eradication a realistic goal"? Bill and Melinda Gates emphasized that new Scientific advances made by Scientists, beside tremendous financial grants offered by International Agencies, have **made the goal of eradication realistic in decades to come.**

Another Editorial "**Malaria Eradication back on the table**", was published by Tanner and de Savingny (2008, Bulletin World Health Organization, Feb. 2008, 86(2), 82-83) reported that regional malaria elimination campaigns proposed in late 1940s, prepared the ground for Global Malaria Eradication Programme in 1955. This campaign achieved elimination of malaria from Europe, North America, the Caribbean and part of Asia and South Central Malaria. The global eradication plan was abandoned in 1969 because of wide-spread chloroquine resistance in *P. falciparum* and DDT resistance of malaria vector (*Anopheles* species). The **Roll Back Malaria Initiative launched by World Health Organization** in 1998 led to **Abuja Declaration** in 2000, which showed **commitment to eliminate malaria as a public health problem**. Increased **Global Fund to Fight AIDS, Tuberculosis and Malaria**, together with **World Bank's Booster Programme,** complimented with the **U.S. President's Malaria Initiative -** all these agenda finally showed **commitment for renewed global efforts for elimination/eradication of malaria**, including complete control of malaria from Africa and South-east Asian countries, together with **abolishing of malaria related mortality in children**. Aiming at ultimate elimination and eradication of malaria, would include

surveillance strategies to monitor the **better understanding of malaria transmission heterogeneity** in malaria endemic states.

Flannery *et al.* (2014) discussed the problem of antimalarial drug discovery directed to the ultimate **objective of complete elimination of global malaria.** Flannery *et al.* (2014) also admitted that the **current antimalarial drugs were inadequate to achieve complete elimination of malaria**. These investigators report that **artemisinins are fast-acting** and very potent against blood stage parasites as **schizontocides**, besides showing activity against early **sexual stages of the parasite**, and they also realized the **importance for blocking transmission of malaria**. **The author agrees** with the conclusions of Flannery *et al.* (2013) that **artemisinins can act as very effective gametocytocide to block transmission** of malaria and cite the **discovery of gametocytes from the Indian studies published internationally** during the **priod 1989-2003** in which **artemisinins were reported to completely sterilized the gametocytes** of human transmissible *P. cynomolgi* B in rhesus monkey model, eg. artemisinin (Dutta *et al.* , 1989), α/β-arteether (Tripathi *et al. ,* 1990 and 1996; Dutta and Tripathi, 2003; Dutta *et al. ,* 2000) as potent gametocytocides, dihydroartemisinin (Jain *et al.* 1999, 2000 Patents) and β-artelinate (Tripathi *et al.* , 1996) as gametocytocide and Dutta *et al.* (2001) U.S. Patent in which α/β-arteether was reported to be effective gametocytocidal for *P. falciparum* (in patients with early stages of gametocytes). Following the discovery of gametocytocidal action of artemisinin at CDRI in 1989, further analysis of retrospective investigations in Bangkok Hospital also indicated that artemisinin potentially reduced the gametocytaemia in *P. falciparum* clinical cases.

I wish to add to the comment of Flannery *et al.* (2013) that – "artemether and artesunate" are the only known artemisinins that works effectively against multidrug resistant parasites. Besides these two artemisinin derivatives, another compound α/β-arteether (30:70 mixture of epimers) 3-dose injectible formulation in neutralized groundnut oil has been developed by CDRI-CIMAP as a drug of choice for complicated/MDR *P. falciparum*/cerebral malaria cases, which had been published extensively since 1987-1996 by authors. α/β-arteether has also been recognized for control MDR malaria by WHO (2012) publication (see Dutta and Tripathi, 2003 for details and Indian and International Patents 1989, 2001). The drug was marketed in 1997

as **E.Mal**. The publication by Flannery *et al.* (2013) also records another **bottleneck in malaria (*P. vivax*) eradication strategy** i.e. the need to replace primaquine as hypnozoiticidal (antirelapse/radical curative drug) for safe treatment of *P. vivax*/*P. ovale*. Primaquine (an 8-aminoquinoline drug) is toxic to individuals with glucose-6-phosphate dehydrogenase deficiency. Nearly 2.85 billion people are at risk of *P. vivax* in Central and South-east Asia and Central and South America who need a safe anti-relapse drug for vivax control.

It may be recorded that compound **CDRI compound 80/53** (named **Bulaquine=Elubaquine** by WHO, 2000) which is 3-4 times safer than primaquine, is a potential 8-aminoquinoline which can replace primaquine as radical curative/anti-relapse drug. This drug **eradicates hypnozoite of *P. vivax*** at 25 mg/kg daily dose administered for 5 to 7 days. The drug shows much better safety margin compared to primaquine. Beside, **tafenoquine** (**WR 236805**) (another 8-aminoquinoline) has been **developed up to pre-clinical stage by CDRI in collaboration with WRAIR, Washington D.C**. (Milhous *et al.* 1988; Puri *et al.* , 1994; Puri and Dutta, 2003, Dutta and Puri, 2003).

A very comprehensive "**A Research Agenda for Malaria Eradication: Drugs**" was brought out by the MalERA Consultative Group on Drugs (2011) and the major thinking of the Group of International Participants was the "**Malaria Eradication is not possible with existing tools"** which include : (1) Artemisinin – based combination treatment (ACTs), (2) Long-acting insecticide-treated nets, and insecticide spraying. According to "View of malERA Drugs Consultative Group" "**the complete malaria eradication will not be accomplished within most of our lifetimes and that new tools, including new antimalarial drugs developed specifically for elimination indications"**, such as (1) **Transmission blocking safe drugs** to kill/sterilize immature and mature gametocytes of *P. falciparum*, (2) Successful **treatment of MDR complicated *P. falciparum* infection/fast-acting blood schizontocides for comatose malaria cases** (both *P. falciparum/P. vivax*), (3) **elimination of relapsing liver stages (hypnozoites)** of *P. vivax/P. malariae*, i.e. **safer antirelapse drugs for radical cure** of *P. vivax* (to relapse primaquine), (4) develop **safe causal prophylactic** agents and (5) **New ACTs** for treatment of uncomplicated *P. falciparum* infection e.g. **DHA-piperaquine combination** recently approved by WHO (2010), (6) Finally **zoonotic *P. knowlesi* human epidemics**

are fast spreading from monkeys to human subjects. It will be essential to develop effective therapy for *P. knowlesi* infections which are being transmitted to human population. Extensive chemotherapeutic investigations at **CSIR-CDRI Institute has already made major headway in tacking some of the listed obstacles in New Drug Discovery Programmes** and would also provide many new chemotherapeutic agents to tackle some of the bottle-beck to initiate programme for malaria eradication in collaboration with **National Antimalaria Programme of Govt. of India and International Malaria Control Agencies/WHO/U.S. Govt. programmes/WRAIR, Washington D.C. collaboration. The leads developed at CDRI** to tackle and support eradication plans **will be available to all International Agencies to progress towards global malERA Agenda.**

International Agenda For Antimalarial Drug Discovery And Malaria Eradication

International efforts to achieve malaria eradication has been the focus of Global Malaria Control Agencies and all the public health agencies in Malaria endemic countries including the World Health Organization, the World Bank and Global Fund for Malaria Eradication, the Tropical Diseases Programmes of WHO and numerous Public Health and Malaria Control Agencies in Africa, South-east Asia and Southern America including Australian Health Agencies and Chinese.

More than Thirty Two Research Agenda for Malaria Control and Global Eradication have been recently published with major objective to achieve the Malaria Eradication by **Promoting Antimalarial Drug Development and Drug Research, Antimalarial Vaccines,** Application of **Safe Insecticides, Mosquito nets, Mosquito Control technologies** involving new Insecticides Development, **Biocides** for Vector Control, **Indigenous Technologies** for repelling mosquitoes, so much so that nearly twenty-five Malaria Eradication Agenda are now published for International Cooperation through agencies like Medicines for Malaria Venture – a non-profit European Agencies with Intercontinental Collaboration spread over several Pharmaceutical Companies and **International Teams of Committed Malaria Research Scientists** have pooled their resources to protect African Children which suffer the most due to malaria epidemics. The CSIR-Central Drug Research Institute and collaborating laboratory (Central Institute of Medicinal & Aromatic Plants, Lucknow), The Malaria Research Centres (under Indian

Council of Medical Research, New Delhi), The National Institute of Malaria Research (ICMR), New Delhi, and nearly one dozen field Clinical Trial Centres and Medical Scientists involved in Clinical Trials of New Antimalarials generated by Central Drug Research Institute and CIMAP, have significantly contributed to New Antimalarial Drug Discovery and Drug Development at Central Drug Research Institute, together with International Collaboration with Walter Reed Army Institute, Washington, DC, USA (since 1982 to 1998) have given a great boost to develop and establish **Reliable Antimalarial Screening Programmes at CDRI.** The World Health Organization collaboration with CDRI was very fruitful to standardize the primate antimalaria screening as per WHO protocols and the **World Health Organization had recognized Central Drug Research Institute as WHO collaborating centre for screening new antimalarials (designated as CC59)** during 1985 to 1995 and the author had the privilege to submit the yearly progress reports to WHO, Geneva on the progress in developing new antimalarials developed at CDRI since 1986, particularly the research reports of the development of new blood schizontocides/antirelapse/causal prophylaxis, gametocytocidal/sporontocidal and safe gametocytocide to stop transmission of *Plasmodium cynomolgi* B to *Anopheles stephensi*.

Commendable efforts have been made by **Committed Scientists of MMV Group** (Anthony, Burrows, Duparc, Moehrle and Wells 2012) who have tried to report update on the current status of global pipeline of new antimalarials developed by their group which would be urgently required for the control and elimination of malaria. The investigators reviewed the world literature on global antimalarial drug discovery and development portfolios and identified initially over 50 drug discovery projects which were identified till **March, 2012**. **Twenty seven** of these were **regulatory studies.** In the past three years **five new medicines** were prequalified by World Health Organization as fixed dose, **artemisinin-combination therapy (ACTs)** to protect against emerging artemisinin resistance which included: (1) ACT comprising of **artemether-lumefantrine** (Coartem Tablets and Coartem Dispersable from Novartis), (2) **Artesunate-Amodiaquine** (Coarsucam^{TM}/artesunate amodiaquine (Winthrop^{R}, Sanofi), (3) **Pyronaridine-artesunate** (Pyramax^{R}) (Shin Poong Pharmaceuticals) and (4) **Mefloquine-artesunate** (Cipla/DNDi (prequalified by WHO). Besides four other types of ACTs are prescribed

for **uncomplicated malaria** (including (a) Dihydroartemisinin (DHA) – Piperaquine (Eurartesim^{R}-Sigma-Tau) and Duo-Cotecxin^{R} (Holley-Cotec), (b) Pyronaridine-Artesunate, (c) Artesunate-Mefloquine (ASMQ) (Cephalon/Mepha), (d) Artemisinin-naphthoquine (ARCO, Kunming, China) which is available as one day treatment (Not yet submitted for approval). According to this eradication agenda proposed by Anthony *et al.* (2012), there are still **serious gaps in development of new medicines** which would be essential to **achieve eradication of malaria** including (1) effective drugs to **prevent transmission** of malaria (i.e. need for developing **safe gametocytocides**, (2) **safe drugs to stop relapses of *P. vivax*** i.e. drugs to kill hypnozoites/dormant liver stages,(3) develop new powerful drug leads to provide **chemoprotection** against new malaria infections especially **multidrug resistant malarias**, which could be used as new artemisinin based combination drugs. The investigators have mentioned specifically to include clinical study of new ACT combinations under clinical trials particularly dihydroartemisinin (DHA)-piperaquine including development of Eurartesim^{R} (Sigma Tau)/Duo-Cotcxin^{R} (Holley-Cotec), pyronaridine-artesunate (ACT) e.g. Pyramax^{R} from Shin Poong Pharmaceuticals (approved by Korean FDA (in August 2011) and EMA (in February 2012) and prequalified ACT by WHO. Besides they recommend child friendly paediatric formulations specially (1) Coartem^{R} dispersible (artemether-lumefantrine dispersible combination) for treatment of child mortality under five years), (2) Paediatric formulation under development: a granule formulation of pyronaridine-artesunate was proposed (to be submitted early in 2013) and (3) a dispersible formulation of dihydroartemisinin-piperaquine had also been planned for submission in 2013 (according to Anthony *et al.* 2012 publication Malar. J. 2012, **11**, 316).

Burrows, van Huijsduijnen, Mohrle, Oeuvray and Wells (2013) have put forward the **malaria eradication agenda** with focus to design next generation of antimalarials to **target different stages of malaria parasite.** According to MMV concept for malaria control,(1) **New fast-acting medicines** would be an essential weapons for **control of emerging artemisinin resistant malaria**, (2) New medicines to **reduce transmission of malaria** (through their use to **eradicate early and late stages of gametocytocides,** (3) **Prevention of relapses due to dormant** (**hypnozoites stage**) of *P. vivax/P. malariae,* to eliminate relapsing stages of malaria, (4) For achieving **radical cure**, **a single exposure**

radical cure and prophylaxis therapy (**against sporozoite infection**) has been proposed together with **new drugs for elimination of asymptomatic sexual stages (interruption of transmission by the gametocytes)** needs to be developed in order to achieve malaria control, (5) Future drug development should also include **drugs to control zoonotic *P. knowlesi* infections** of rhesus monkeys, which are fast spreading as epidemics in human population in forest areas of South-east Asia. Chemotherapeutic control of ***P. knowlesi* epidemics in humans will be a high priority** in future drug-development strategy. Substantial progress made in the area of **New Drug Discovery and Development in India** during the last five decades (1963-2014) can provide some of the powerful new drugs and drug combinations to promote the Global Eradication Agenda.

This Indian National Science Academy sponsored document will briefly present new antimalarial drug developments strategy in India in the field of malaria. The CSIR-CDRI has developed very fast acting artemisinin derivatives for example **α/β-arteether, β-arteether, α/β-artelinate**, **dihydroartemisinin (as blood schizontocides)**, effective against highly multi-drug resistant malaria), two new **causal prophylactic agents/anti-relapse agents** to eliminate hypnozoites (**Tafenoquine, in collaboration with WRAIR, U.S.) and CDRI 80/53 compound** (**Bulaquine/Elubaquine**), discovery of several **new gametocytocidal drugs** to interrupt malaria transmission(**Artemisinin, α/β-Arteether, Artelinic acids, Artemether, DHA, Bulaquine/Elubaquine, Tafenoquine**), artemisinin derivative (**DHA - a blood schizontocide now patented in 16 countries** by CDRI/CIMAP (Jain *et al.* 1999, 2000) is now approved as **DHA-piperaquine ACT combination** (Eurartesim, Sigma-tau) for uncomplicated *P. falciparum* malaria and *P. vivax* (approved by WHO, 2010 Report), very fast-acting **pyronaridine** which can control multidrug-resistant *P. yoelii nigeriensis)*, new synthetic **1,2,4-Trioxanes** and **Fenozans** (**blood schizontocides**). **α/β-arteether** is one of the most fast-acting blood schizontocide and drug of choice to prevent mortality in severe malaria/cerebral malaria. This drug was marketed in 1997 and is manufactured by 18 pharmaceutical companies in India. α/β-Arteether is also life-saving drug for African countries where it has been used to control **multidrug-resistant falciparum and control heavy mortality** in 48 malaria endemic countries. The drug also has strong **gametocytocidal** action against *P.*

cynomolgi B as well as *Plasmodium falciparum* in clinical cases. Several of these new antimalarials have been **patented internationally** which include **α/β-arteether, bulaquine/elubaquine, dihydroartemisinin**. Azithromycin and Pyronaridine are blood schizontocides which as combination are new candidates for clinical trials in India (ICMR). Drug discovery research at CSIR-**CDRI/CIMAP** (Lucknow) and collaboration with Indian Institute of Integrative Medicine (Jammu) and the Indian Council of Medical Research/National Institute of Malaria Research (New Delhi) and multicentric clinical trials all over India have been finally approved by the Drugs Controller General of India for clinical use of some of these medicines for control of malaria. Besides large number of **8-aminoquinolines** including **Tafenoquine** have been developed in collaboration with Walter Reed Army Institute of Research, Washington DC. Bulaquine was marketed in the year 2000 and α/β-arteether in 1997.

Planning Global Malaria Eradication and Saving Lives

World Health Organization : Q + A on Malaria Mortality Estimates (February 2012)

1. According to World Malaria Report Dec. 2011) approximately 216 million cases occur every year (range 149 million – 274 million). Further malaria kills about 655,000 persons yearly (range 537,000 to 907,000). It is important to note that nearly 90% deaths due to malaria, are in Africa. According to WHO Experts the global mortality figure shows: **<u>One child dying from malaria every minute</u>**. It is hoped that this mortality figure will be of immense value for the International Community/Health Organizations and International Donors to assess the magnitude of the killer disease and will help to plan future strategy for **<u>Global Malaria Eradication Agenda of all Nations</u>**.
2. According to the Malaria Eradication Research Agenda (mal.ERA) statistics (2009) there are 3.28 billion people living in areas which have some risk of malaria transmission, and nearly 1.2 billion people ($1/5^{th}$ of the World Population) live in areas with high risk of malaria transmission, besides there were 225 million cases of clinical malaria, and 781,000 malaria related deaths. This report publish in PLOS Medicine further discusses ongoing malaria transmission in 106 countries (out of these 81 countries are attempting to control malaria while 25 countries are

in pre-transmission, elimination phase etc. It is encouraging to note that countries like **Morocco, the United Arab Emirates and Turkamenistan** have been certified as malaria free (WHO 2010a,b,c).

3. **The Malaria Eradication Research Agenda (malERA)** initiative sponsored by the **Bill and Melinda Gates Foundation/WHO/the Roll Back Malaria (RBM) partnership** and several **other International Organizations** was focused initially on Malaria Control and Elimination, with the ultimate Goal of Malaria Eradication (Roll Back Malaria Partnership, 2008; WHO, 2010a and Bill and Melinda Gates Foundation Malaria Forum 2007). There is special emphasis on the **Need for New Antimalarial drugs development** to make any progress to achieve Global Malaria Eradication.
4. **The Malaria Eradication Initiative to be accomplished would involve the development of new drugs**:
   i) To **interrupt malaria transmission** by killing stage V mature gametocytes of *P. falciparum* and sterilize the gametocytocytes of *P. falciparum*.
   ii) A new radical curative **(antirelapse) drug to eradicate hypnozoites (relapsing stage)** of *P. vivax*.
   iii) **A drug for prophylaxis against *P. falciparum/P. vivax***. According to above report 8-aminoquinolines can function both as radical curative and for prophylaxis. A safe drug of this category can also **sterilize the transmission stages** of *P. falciparum*, and should **replace currently available drug primaquine**. This drug produces methaemoglobinaemia, and haemolysis of RBC and is not recommended for infants, children, in pregnancy and G-6-PD deficient individuals. **We essentially need a safe primaquine substitute.** It seems that **since the discovery of primaquine in 1934**, the malERA Associates/Scientific Group have not been able to identify a safe primaquine substitute which should possess antirelapse (radical curative) activity/prophylactic activity for *P. vivax* and gametocytocidal action on stage V mature gametocytes of *P. falciparum*.

WHO (2000-2010) Global Report on Antimalarial Drug Efficacy and Drug-Resistance:

Artemisinin resistance and containment

Drug efficacy study along Cambodia-Thailand border shows emerging resistance to artemisinin among *P. falciparum*. Proportion of patients treated with artemisinin were still parasitaemic on day 3. Artemisinin resistance in *P. falciparum* cases has also appeared in Myanmar-Thailand. China-Myanmar borders in Greater Mekong subregion show artemisinin resistance.

Monitoring of ACTs should also evaluate the proportion of patients who still have parasites on day 3; this is the 1st sign of artemisinin resistance.

Global plan for containment of artemisinin resistance should be implemented

Note – The urgency is all the greater because no other antimalarials are available that offer the same efficacy and tolerability as ACTs, and few alternatives exist for the immediate future.

Artemisinin resistance has been observed along Cambodia-Thailand border (Noedl *et al.* 2008, Noedl 2005, Dondorp *et al.* 2009, Noedl *et al.* 2008).

Malaia 2012: Saving lives in the Asia-Pacific Conference, Sydney 31st Oct-2nd Nov. 2012 (Aus Aid).

Major objectives: Achieve goals set by the Global Malaria Saving lives in Asia-Pacific Region Community (22 countries).
Malaria control and elimination.
Long-term aim of malaria eradication.
Achieve 75% reduction in malaria deaths by 2015.
Assess the current and potential impact of artemisinin resistance and its containment.
Contain emerging resistance to antimalarials medicines and insecticides.
Reduce child mortality.
Reduce maternal deaths.
Control cerebral malaria which can cause long-term severe neurological problems.

In 2010, it was estimated that over 32 million malaria cases occurred in this Asia-Pacific Region.

According to this report (2011), the **problem of mono- and multidrug resistance** is increasing in South East Asia including India, particularly *P. falciparum* resistance to chloroquine, sulfadoxin-pyrimethamine, mefloquine and quinine. Besides emergence of *P. vivax* resistance to chloroquine is causing serious concern. Primaquine resistant gametocytes have been documented from Mumbai.

Bloland (2001) from World Health Organiation has emphasized on the problem of "Drug resistance in Malaria". According to this author, a **chlorproguanil/dapsone/ artesunate** fixed dose formulation should provide synergistic combination to control MDR malaria. Besides he has strongly supported "**New Drug Development**" focused specially **on transmission blocking drugs** which could be combined with blood schizontocides.

Caroline Lynch and Sean Hewitt (2012) **Malaria in the Asia-Pacific : Burden, Success and Challenges.** Malaria 2012 : Saving lives in the Asia-Pacific pp. 1-43

This document sponsored by **Australian Government entitled Malaria 2012**: is dedicated to **urgently tackle resistance to artemisinin.** This document is focused on the need to achieve: (1) Long-term aim of malaria eradication and (2) to achieve 75% reduction in malaria deaths by 2015 compared to 2000 baseline. According to above report, the **major challenges** to malaria control and elimination in Asia-Pacific include: 1) Emerging artemisinin resistance to drugs and insecticides, (2) Treatment of *P. vivax* malaria, (3) Risk of resurgent malaria, (4) Progress to achieve 75% reduction in malaria deaths by 2015,(5) Provide 2.3 billion RDTs, (6) 46.7 million doses of ACTs and (7) Provide 276 million LLINs for control of malaria between 2013 and 2015, (8) Elimination of drug resistant parasites and (9) To ensure no onward transmission in the area.

The ACT treatment recommended for Indian *P. falciparum* include AS + SP (in case of failure of treatment), the presecribed regimen should be Q + D or Q + T. For control of *P. vivax* CQ + PR (14 days) is recommended.

Dr. Samiee Plianbangchang from **WHO Regional Office for South-East Asia report** dated 25 April 2011, also report growing threat of **resistance to artemisinin**

(considered the most effective anti-malarial drugs) in South-East Asia Region. According to 2009 survey, there were 28-41 million malaria cases together with 49000 deaths due to malaria among the adult patients. Nearly 94% cases showed mortality due to malaria from three states including India, Indonesia and Myanmar. It was claimed that in Indian continent, besides Bhutan, DPR Korea, Nepal and Sri Lanka the **malaria incidence had reduced to fairly low level** and these states were **heading towards pre-elimination phase.** Besides improved detection of malaria by microscopy, the enhanced **use of artemisinin-based combination treatment courses** had increased from 260,000 in 2005 to 1.768 million in 2009. The use of insecticide treated bed nets (**ITNs**) and long last nets treated with insecticides (**LLINs**) from 4363 million in 2005 to 17, 387 million in 2009. The study has also cautioned the increased level of resistance of falciparum malaria infection to artemisinin based drugs in Thai-Myanmar border, which seriously threatens the malaria control efforts in this region.

In the face of emerging drug resistance the International Clinical Scientist from Tropical Malaria endemic countries had continued to assess **various drug combinations to cure multidrug resistant *P. falciparum*** in clinical cases and they had reported the clinical efficacy of selected drug combinations which could control *P. falciparum.*

Hien *et al.* (1992) reported **artemisinin + meflqouine** cured 100% patients of *P. falciparum*. Li *et al.* (1984) reported 100% cure with **artemisinin + mefloquine** + **sulfadoxine + pyrimethamine**. Shwe *et al.* (1989) and Winn *et al.* (1992) achieved 100% cure with **meflqouine + artemether**. Shwe *et al.* (1988) used **artemether + mefloquine + quinine**, while Win *et al.* (1992), Looareesuwan *et al.* (1992b,c) and Looareesuwan *et al.* (1996) were successful in achieving 100% cure rates against multidrug resistant *P. falciparum* in different countries/*P. falciparum* strains. Around 1999-2000, the World Health Organization/International Clinical Scientists in malaria endemic countries, realized the impending danger of emerging artemisinin resistance in clinical trials in which artemisinin/artesunate/artesunate/artemether/dihydroatemisinin etc. were used.

Malaria – RBM – The Malaria Problem in South-East Asia Region

(Success Stories in Malaria control from India and SEA Region)

The SEARO Office of WHO has reported that in India there is a strong linkage between Scientific Departments, NAMP, University system and now it has linked up with industry. According to the report, **India has emerged as the main contributor of important technologies in malaria control** which include:

(1) Development of α/β-arteether (30:70), an injectible artemisinin based drug developed by Central Drug Research Institute, Lucknow. Drug has been marketed since 1997.

(2) Bulaquine, a new anti-relapse drug CDRI 80/53 (Elubaquine) has also been developed by Central Drug Research Institute, Lucknow as a new safer anti-relapse antimalarial. This drug has been marketed after completing the clinical trials in India in year 2000.

(Last update: 09 January 2007) (South-East Asia Office).

CHAPTER-IV

SUMMARY AND CONCLUSIONS

HISTORICAL ACCOUNT OF GLOBAL MALARIA ERADICATION AGENDA AND CONTROL STRATEGY IN INDIA

The project aimed at 'Eradication of Malaria' was proposed by Hoffman (1917). By 1950, the World Health Organization Experts proposed the strategy that the malaria eradication could be achieved by **blocking malaria transmission** through the application of insecticide DDT, to end the further spread of malaria and through the **elimination of reservoirs of gametocyte carriers**. It was conceived that malaria control could be achieved through the **mass chloroquine treatment** of *Plasmodium falciparum* combined with **DDT insecticide sprays**. The National Malaria Eradication Programme (1958-65) was successful in preventing malaria deaths in India by 1965. From 1965 onwards there were reports of emergence of chloroquine resistant *P. falciparum* together with DDT resistance by anopheline malaria vectors, resulting in tremendous rise in *P. falciparum* cases which showed increasing trend of mortality due to malaria. The NMEP & ICMR in (1976-1977) decided to implement Modified Malaria Control Programme in the Country through ICMR sponsored "Modified Plan of Operation".

Extensive clinical trials of antimalarials were carried out and emergence of resistance to chloroquine and other antimalarials was recorded by NMEP. Even large foci of multiple drug resistance were identified in the extensive clinical trials. The World Health Organization recorded high level of mortality in African continent due to emerging drug resistant foci in large number of countries in South-East Asia, African continent and South America. *Plasmodium falciparum* showed development of resistance against most of the synthetic antimalarials. Following the discovery of artemisinins around 1972 by Chinese Scientists, clinical trials of drug combinations were initiated globally to stop malaria mortality. By 1998 the WHO proposed guidelines to use drug combinations comprising of an artemisinin based drug and one of the synthetic long-acting antimalarials (named as ACT combinations) which were clinically tested in different continents. In most areas, the ACT combinations (now numbering more than 20) were successful to control expanding foci of *P. falciparum*. Artemisinin based drug combinations were able to halt malaria mortality in most of the *P. falciparum* infected countries except Africa. By the year 2003-2010 the International Scientific Groups

started reporting emergence of resistance against artemisinins. WHO at this stage advised all the National Governments/Control Programmes to judiciously use the insecticides with various artemisinin based drug combinations (preferably having a long- acting drug in the ACT combination) in order to stop further emergence of drug resistance against various ACT's. All countries were advised by WHO (2000, 2002 and 2006) **not to use single artemisinins based drugs because there would be possible emergence of resistance against various artemisinins if used singly**. Although the mortality due to *P. falciparum* following ACT combination treatment has been significantly controlled, the high children mortality in African continent continues to pose serious concern. WHO now recommends that ACTs should be used as the first-line treatment as proposed for different countries depending on parasite susceptibility to various ACT combinations (WHO, 2010). WHO (2007) proposed that artemisinin-based monotherapies should not be used alone in order to prolong the effective use of ACT combinations. They have urged the Investigators **to develop** "**new antimalarials (blood schizontocides) to combat emerging multi-drug resistance of *P. falciparum*"** as well as for the control of emerging **drug resistant *Plasmodium vivax* strains** which could also be used to treat **zoonotic *P. knowlesi* (monkey) infections** which are now spreading in forest areas to human populations in South-East Asia jungles.

The **WHO** have also **strongly emphasized the global need of new antimalarials** particularly those **which possess gametocytocidal action against both *P.falciparum* and *P. vivax*.** There is **urgency to stop malaria transmission to contain and reduce the emergence of artemisinin resistance,** besides to **stop spread of multidrug resistant *P. falciparum***. This will be able to **prevent increasing trends of mortality due to malaria specially in the African Continent**. **WHO (2013)** have rightly warned all Nations that "there is, therefore, a **limited window of opportunity to avert a regional public health disaster"**. The WHO Report(Jan. 2011) on "The Global plan for artemisinin containment (GPARC)" was drawn: (1) To **protect the efficacy of ACTs and the emphasis should be** (2) **to invest in "Artemisinin related research",** and (3) **intensified malaria control should be focused in high transmission areas to limit the spread of malaria".** WHO (2013) have also warned all the malarious countries

that "**there is a real risk of parasites developing resistance to all available medicines**".

TECHNOLOGY FOR ANTIMALARIAL DRUG SCREENING AND DEVELOPMENT

HISTORICAL ACCOUNT OF DEVELOPMENT OF EXPERIMENTAL MODELS FOR ANTIMALARIAL SCREENING

Following the resurgence of malaria in India between 1965-75, a task force for chemotherapy of malaria was set-up at CDRI to develop new antimalarials to combat emerging multidrug resistant *P. falciparum*. Highest priority was given to establish antimalarial screening *in vivo* against *P. berghei* and *P. yoelii nigeriensis* rodent (mice) model and simian malaria models (*P. cynomolgi* B (a human transmissible *vivax* type rhesus monkey model), *P. knowlesi* W1, *P. coatneyi* and *P. fragile* etc.). Using Peters (1970) technique, a battery of *P. berghei* lines resistant to standard antimalarials were selected.

Like-wise *P. yoelii nigeriensis* strain was exposed to a battery of low oral doses of antimalarials, and finally **highly MDR strain resistant to a battery of drugs, antibiotics and drugs-antibiotic combinations was selected which was designated as MDR *P. yoelii nigeriensis***. **This was the only *in vivo* screen used** in random bred Swiss mice (20±2 g) for the **primary antimalarial evaluation and identification of most active blood schizontocidal** compounds. Control (**infected**) mice died in 6-7 days with **very high parasitaemia (80-100%) and mortality rate was also 100% in untreated mice.** There is no survival amongst control (infected) mice.

TECHNOLOGY DEVELOPED FOR ANTIMALARIAL DRUG SCREENING AND DISCOVERY

Primate Antimalarial Radical Curative Screening Model

Sporozoite-induced *P. cynomolgi* B strain which is human transmissible, was used to established radical curative (anti-relapse drug) screening model in Rhesus monkey,

Macaca mullata) as reported by Schmidt *et al.* (1966), using primaquine as standard anti-relapse antimalarial. Besides CDRI 80/53 (bulaquine=elubaquine) and nearly 10 more compounds synthesized in CDRI were screened for anti-relapse activity (S.K. Puri and G.P. Dutta, unpublished).

Primate Antimalarial Screening Models Established at CDRI

1. Blood schizontocidal screen
2. Antirelapse model
3. Causal prophylactic model
4. Gametocytocidal/sporontocidal screening model

Tafenoquine discovered in 1988 as primaquine substitute, was identified as broad-spectrum antimalarial **under CDRI-WRAIR collaboration**. Tafenoquine (WR 238605) possesses **blood schizontocidal, anti-relapse, causal prophylactic and gametocytocidal activity** (S.K. Puri and G.P. Dutta, unpublished)**.**

6. CDRI 80/53 (bulaquine/elubaquine): a primaquine substitute with **radical curative activity and gametocytocidal activity** was reported by Dutta *et al.* (1989) and Puri *et al.* (1989).

TECHNOLOGY AND WORKING PROTOCOLS USED FOR ANTI-MALARIALS SCREENING

Drug discovery protocols developed by the Authors have been incorporated in this document (Dutta, Puri and Tripathi, 1998 and Kar *et al.*, 1988, 1989; N. Sethi et al. 1988, 1993 and late Pandey V.C.).

(a) LD_{50} dose was determined in mice

(b) Primary antimalarial (blood schizontocidal, screening of new compounds was done against rodent *P. yoelii nigeriensis* MDR strain.

(c) Primate malaria, secondary blood schizontocidal screening, was done in Rhesus monkey

(d) Methaemoglobin toxicity tests of primaquine derivative was done in beagle dog model.

Protocols for Preclinical Studies on New Antimalarials

Tests for toxicity dose determination

A. LD_{50} in mice/rats: LD_{50} determination was done in Swiss mice/rats using primaquine or chloroquine as standard.

B. Acute toxicity of primaquine was done in rhesus monkey.

Standardization of Secondary Blood Schizontocidal Screen against Simian Malaria

A. *Plasmodium knowlesi* W1 infection

B. *Plasmodium cynomolgi* B

Causal Prophylaxis Screening against Simian Malaria

Technology for malaria transmission was standardized.

Technology Developed for Causal Prophylactic Screening

Technology Established for Radical Curative (anti-relapse) screen.

Technology for Gametocytocidal/Sporontocidal Screening

Assessment for Methaemoglobinaemia in beagle dogs

Animal Pharmacological Studies

Protocol for long-term sub-acute Toxicity

Conclusion: The document reports:

Selection of one of most active blood schizontocides which can be identified within 28 days, by **quick *in vivo* screening against *Plasmodium yoelii nigeriensis*** MDR strain *in vivo* model. This single mouse malaria primary *in vivo* screen can **select most active compound, the compound** e.g. α/β-Arteether (30:70), (one 150 mg IM injection daily x 3 day) is also effective/curative against uncomplicated/complicated/acute, comatose, cerebral malaria cases, specially quinine/mefloquine resistant cases,with very fast recovery/quick parasite clearance etc.

ANTIMALARIAL DRUG DISCOVERY AND DRUG DEVELOPMENT

A large number of blood schizontocides have been found to be curative against MDR malarias, both *Plasmodium falciparum* and blood stages of *P. vivax*.

1. α/β-Arteether injection (IM).

Criteria for Selection of α/β-Arteether 30:70)

The most important criteria for development of α/β-Arteether was the curative action against primary *P. yoelii nigeriensis* MDR screen, which is highly virulent rodent parasite, producing 100% mortality in control (untreated mice, and attaining 100% parasitaemia in 5-7 days. Arteether treated mice (5 mg/kg x 4 days, IM) completely cures all mice (Dutta *et al.* 1989b). Besides, the drug jointly developed by CIMAP and CDRI, is curative blood schizontocide against rodent and several simian malaria strains and against *P. falciparum*.

1. *P. berghei* (Dutta *et al.* 1987)
2. *P. knowlesi* (Bajpai *et al.* 1988; Tripathi *et al.* 1991)
3. *P. cynomolgi* B (Dutta *et al.* 1988;Tripathi *et al.* 1991)
4. *P. fragile* (Tripathi *et al.* 1996)
5. *P. coatneyi* (Dutta *et al.* 2003)
6. *P. falciparum* (Human malaria clinical trials, Dutta *et al.* 1990 & U.S. Patent2001.

Clinical trials in ***P. falciparum*** **cases have shown 95-100 cure rates in 3 dose IM injection schedule**. The drug is reserved for acute MDR resistant malarias and emergency drug for comatose cerebral malaria.

1. β-Arteether (formulation in neutral ground nut oil)
2. α/β-Arteether
3. Dihydroartemisinin (DHA) (Artenimol/Dihydroquinghaosu)
4. An oral combination of DHA + Piperaquine has been approved by WHO (2010) for uncomplicated MDR *P. falciparum* (as a **new first line ACT**). (**MMV have incorporated Piperaquine (as a long-acting) partner drug with dihydro-artemisinin (patented by author in 1999/2000)** (CDRI/CIMAP collaboration).

Although, there is no formal agreement between Council of Scientific & Inustrial Research (CSIR), New Delhi (CDRI/CIMAP Patent Assignee), and Medicines for Malaria Venture (Switzerland) – a non-profit European Agency, to cooperate/collaborate in promoting "New Antimalarial Drug Development Internationally", it is encouraging to report that **at least five antimalarials patented or published by CSIR-CDRI/CIMAP, Lucknow (Azithromycine, 2005), Tafenoquine (WR 238605) (1988, 1994, 2003), Pyronaridine (2000a,b), dihydroartemisinin (DHA) (Patent 1999/2000) and technology transfer for Artemether (Process 2000) have been incorporated in the**

MMV Drug Discovery Candidates for Clinical Development (2014) (see MMV Chart). It is heartening to note that clinical trials with some of these drugs have been carried out on Indian Malaria Infected Clinical cases also to ensure efficacy against malaria infected *P. falciparum* cases in India. All the five drug candidates have **shown curative efficacy against highly MDR strain of *P. yoelii nigeriensis*** developed by the Author. Discovery and development of **tafenoquine (WR 238605) a broad spectrum antimalarial (synthesized by Walter Reed) was tested at CDRI under WRAIR (Walter Reed Army Institute of Research (WRAIR)–CSIR-Central Drug Research Institute collaboration and was published in 1988, 1994, 2003 & 2005) jointly by WRAIR-CDRI groups**. This compound (WR 238605=**Tafenoquine**) underwent extensive studies at CDRI for its **blood schizontocidal**, as **anti-relapse (hypnozoiticidal** against dormant liver stages), as **causal prophylaxis drug**, **as gametocytocidal drug** against *P. cynomolgi* B infection in rhesus monkey model. The drug has **moderate toxicity** like **methaemoglobin toxicity** of primaquine, but it can be **administered at considerably high doses of the drug, which are**

MMV CHART: SHOWING CDRI IDENTIFIED ANTIMALARIAL LEADS (PUBLISHED/ PATENTED) BECOME CANDIDATES FOR CLINICAL DEVELOPMENT IN MMV-2014 REPORT

DEVELOPMENT			ACCESS
Patient Exploratory	**Patient Confirmatory**	**Under Review**	**Post Approval**
OZ439	**Azithromycin CQ**	**Rectal Artesunate**	**Artemether Lumefantrine Dispersible**
KAE 609	**Tafenoquine**	**SP-AQ**	**Artesunate for Injection**
KAF 156	**Pyronaridine Artesunate Paediatric**		**DHA-Piperaquine**

	DHA-Piperaquine Paediatric		**Pyronaridine-Artesunate**
			AS + Amod.
			AS+MFQ

Note: **Council of Scientific & Industrial Research, New Delhi (Central Drug Research Institute, Lucknow) Scientists have already published preclinical research results/patents on five drugs listed in MMV-2014 report under clinical development.**

1. **Azithromycine – 2005**
2. **Tafenoquine – 1988, 1994, 2003**
3. **Pyronaridine-2000a, b**
4. **Dihydroartemisinin (DHA). Patent 1999/2000 (Patented internationally in 16 countries)/DHA-Piperaquine formulations**
5. **Artemether – Bulk Production Technology Transfer in 2000 to Pharmaceutical Industry**

These drugs had been claimed by CDRI to be highly *Curative against Multidrug Resistant P. yoelii nigeriensis*

Author: recommends that MMV Executives may approach Director General, Council of Scientific & Industrial Research, New Delhi/Professor S.K. Puri, Director, Central Drug Research Institute, Lucknow for signing MOU/Collaborating Agreements with CSIR for utilizing the Antimalarial (IPR) Protected Drug Discoveries of CDRI/CIMAP, Lucknow

considered safe. International Investigators are continuing clinical trials in U.S. Defense Department as well as Australia. US Agency (WRAIR) had also **agreed that tafenoquine (WR 238605) could be developed by CDRI** for use in the Indian Malaria Control but WRAIR would hold US and International Rights, to develop this broad spectrum antimalarial, initially developed as long-acting antimalarial for use by the US. In the **2014 MMV Report, tafenoquine (WR 238605) has also been included for joint collaboration with Glaxo-Smith Kaline**. The **first joint report published by Milhous, W.K**., Theoharides, A.D., Schuster, B.G., **Puri, S.K., Dutta G.P**., Heisey G.B., Kyle D E, Oduola A.M.J., **Dhar M.M**., Neiffer M.H., Reid W.A. and Davidson D.E. JR **New possibilities in the development of malaria drugs-new alternatives to primaquine. XIIth International Congress for Tropical Medicine and Malaria 18-20th Sept., 1988, Amsterdam**, Abstract Fr-S-12-4, was **jointly co-authored by WRAIR and CDRI collaborative programme** had reported in the abstract that WR 238605 anti-relapse

activity calculated on the basis of CD50 dose was **7.4 times more active as anti-relapse agent when compared to primaquine** in rhesus monkey *P. cynomolgi* B radical curative test. It was further reported that new 8-aminoquinoline WR 238605 combined with its reduced toxicity, good oral bioavailability and much longer half-life, makes this anti-relapse drug an **excellent potential candidate to replace primaquine**.

New Anti-relapse compounds (WRAIR)

Besides tafenoquine (WR 238605) two more 8-non-aminoquinolines (Quinoline Esters)

1. WR 197236
2. WR 194905

had been received from Walter Reed Army Institute (under WRAIR-CDRI collaboration programme (1982-83) which are **non-primaquine compounds** (S.K. Puri & G.P. Dutta, unpublished)**.**

α/β-ARTELINATE

α/β-Artelinate is a **fast acting blood schizontocide for the control of multidrug resistant malarias**. This is a water soluble artemisinin derivative and can be given intravenously for **emergency treatment** of **complicated, comatose/cerebral malaria *P. falciparum* patients** as well as a blood schizontocide for **drug resistant *P. vivax* infection**. The compound is safe with LD_{50} dose of 670-1000 mg/kg in mice model. Besides, the α/β-artelinate shows **high gametocytocidal activity** and **possesses transmission blocking activity**. Both α and β annomers of artelinate showed **curative activity against *Plasmodium knowlesi*** infection in rhesus monkey, which is a model for cerebral malaria. α, β or α/β-Artelinate have been **identified as Drug Development Candidates at CDRI** (Tripathi, Dutta and Klayman, 1992; Tripathi, Vishwakarma and Dutta 2008; Vishwakarma *et al.* 1989, 1992; Tripathi, Puri and Dutta, 1996).

PYRONARIDINE

Pyronaridine is one of the **most effective blood schizontocide** effective against MDR *Plasmodium yoelii nigeriensis* (discovered at CDRI, Dutta *et al.* 2000, Tripathi *et al.* 2000). The compound is water soluble and **produces 100% curative effect against MDR infection by both oral route and intramuscular injection**. Following oral administration the aqueous solution of pyronaridine was curative against MDR *P. yoelii nigeriensis* at 15 mg/kg x 7 days treatment schedule. Pyronaridine showed very high blood schizontocidal action by intramuscular route of drug administration in Swiss mice (20±2 g) and the IM dose of 2.5-3 mg/kg x 4 days was 100% curative against MDR *P. yoelii nigeriensis* (**Dutta *et al.* 2000; Tripathi *et al.* 2000**). **A combination of artesunate with pyronaridine has been included in the MMV (2014) Drug Development Portfolio in which pyronaridine** has the potential to control MDR malaria. **Artesunate is a fast-acting drug, but it may not cure highly MDR *P. falciparum* infection in complicated cases**, unless it is given with a longer acting partner like **mefloquine (WHO proposed ACT).** In view of **Author's earlier publications (2000), MMV have incorporated pyronaridine as effective longer acting drug partner** which can control highly MDR infection. MMV (2014) are also developing oral **paedriatic formulation** of artesunate + pyronaridine (ACT) for the children malarias. Pyronaridine has dual role for its ability to control MDR malaria and a **much longer half-life** than conventionally used artesunate in WHO ACT formulations. **CSIR-CDRI is committed to promote global malaria control programmes.**

Besides MMV, Shin Poong Pharmaceuticals has also marketed pyronaridine combination with artesunate (oral formulation) designated **(Pyramax®) for MDR *P. falciparum* treatment.**

SYNTHETIC 1,2,4-TRIOXANES/FENOZANS

Among the synthetic endoperoxides the Fenozans compounds 11 and 45 received through the courtesy of **Professor C.W. Jefford (Switzerland)** have been found to be **curative against *Plasmodium yoelii nigeriensis* MDR screening rodent malaria model**. The compound Fenozan 11 is designated as 3,3-spirocyclopentane and Fenozan 45 as 3,3-spirohydropyran. Both Fenozans 11 and **45 formulations in neutral**

groundnut oil were **100% curative at 30 mg/kg x 5 doses (by oral route)** administered to mice infected with MDR *P. yoelii nigeriensis*. Both these synthetic trioxanes can be considered for drug development as blood schizonts (Tripathi, Jefford and Dutta, 2006).

SYNTHETIC SUBSTITUTED 1,2,4-TRIOXANES

Extensive synthesis and preclinical antimalarial investigation of very promising synthetic trioxanes had been carried out at CDRI by Singh and Puri (2001) and patented (U.S. 6316,493). Extensive *in vivo* screening had been carried out **against MDR *P. yoelii nigeriensis*** rodent parasite in Swiss mice. Nearly 10 compounds (25a,27a, 27b, 27c, 27f, 28a,31a, 35a1, 41a, 41b) were curative against MDR parasite by im route. Three compounds (27b, 41a and 41b) were curative by oral route against *P. yoelii nigeriensis*. Further tests against *P. cynomolgi* B in rhesus monkey blood schizontocidal test confirmed activity of 27a, 27b, 41a, 41b which were considered for further studies against *P. knowlesi* in rhesus monkey model. Finally compound **27a also cured *P. knowlesi* infection (U.S. 6316, 493).** Two of these **compounds 27a and 41a** were also tested for **gametocytocidal activity against *P. cynomolgi* B and *P. yoelii nigeriensis* and their gametocytocidal action was confirmed.**

Two fully synthetic 1,2,4-trioxane derivatives are under preclinical development. Compound **CDRI 99-411 had been identified for drug development**. Activity of 99-411 against *P. yoelii nigeriensis* MDR and against *P. cynomolgi* had been confirmed and this was **handed over to IPCA Pharmaceuticals for further Phase I evaluation**.

Additive effects of CDRI 97-78 and 99-411 with piperaquine and lumfantrine have been planned. **Gupta and Srivastava (2014)** reported that **pharmacokinetic studies** with **97/78 showed very rapid metabolism to CDRI 97/63**, resulting in low bioavailability of 97/78.

ARTEROLANE MALEATE (OZ277=RBx11160)

Compound OZ277 (RBx11160) a new fully synthetic peroxide antimalarial (also known as **Dispiro 1,2,4-Trioxolane** antimalarial series was synthesized and patented by **Professor Vennerstrom *et al.* (2004) in U.S. (Patent Application 20080125411),** as a fast acting **blood schizontocide**. Vennerstrom *et al.* (2010) had discovered that OZ277 (Arterolane) showed **single dose curative activity** against *P.*

berghei ANKA infection, with a curative dose of 30 mg/kg in mice. Besides, this compound possesses **prophylactic activity** against *P. berghei* at 100 mg/kg single dose (U.S. 20080125411). It is interesting to note that the **antimalarial profiles of this 1,2,4-Trioxolane (OZ277) is considered by the authors comparable to that of dihydroartemisinin** which had been **patented the author (Dutta *et al.* 1999,2000, U.S. Patents)** as one of the most active artemisinin derivative. **Our DHA patent** jointly file by CSIR-CDRI and CIMAP (Lucknow) in 1998, has been now **granted internationally by 16 countries**. Recently **Professor Valecha *et al.* (2010) from India** have carried out successfully **Phase II multicentric trials** with Arterolane (RBx11160) for acute uncomplicated MDR *P. falciparum* in Thailand, India and Tanzania. Recently Professor Valecha *et al.* (2012) have extended the clinical trials with synthetic Trioxolane **Arterolane in combination with piperaquine (in Phase III) in Bangkok and India.** Arterolane-piperaquine combination is considered safe and effective new drug combination for the control of **uncomplicated *P. falciparum***. It is a **promising future addition to antimalarials for malaria eradication.**

According to Professor Neena Valecha the combination therapy is a deliberate strategy to delay the development of drug resistance. ACTs deliver a two-punch attack on the malaria parasite. By combining two drugs with different mechanisms of action and different time spans of activity; such combinations can increase the likelihood that any parasites not killed by one drug will be killed by the second one. The **usefulness of these therapies is now under threat", said WHO Director General Margaret Chan.**

LIFE SAVING RECTAL ANTIMALARIAL SUPPOSITORIES

ARTEMISININ SUPPOSITORIES

Rectal suppositories of artemisinin (Qinghaosu) had been used to control critically sick cases of *Plasmodium falciparum* including cerebral malaria complications by large number of workers including: Li *et al.* (1985), Li *et al.* (1994), Arnold (1990), Hien *et al.* (1991), Hien *et al.* (1992), Cao *et al.* (1997). **A very high dose of artemisinin had been used (2800 mg drug in 48-56 h)** to achieve complete parasite clearance in blood smears.

This high dose may approach neurotoxicity level together with frequent problems of low cure rates.

ARTESUNATE SUPPOSITORIES

Artesunate suppositories given to *P. falciparum* critical cases **required a very high dose (1600 mg** in 8 doses over 3 days), but **in order to prevent recrudescence of malaria, a second long-acting drug was always prescribed (1250 mg mefloquine to achieve 92% cure rate** (Looareesuwan *et al.* 1995). Looareesuwan *et al.* (1995) used 1600 mg artesunate suppository + 1250 mg mefloquine (96% cure rate), Bhatt *et al.* (1996) used 1600 mg artesunate + 1000 mg mefloquine. In spite of **double antimalarial use there was 13.6% mortality (86.4% cure rates). Gomez Landires (1996) used 14 00 mg artesunate suppository** followed by **750 mg mefloquine**, Thwe *et al.* (1996) gave 1200 mg artesunate suppository followed by 1250 mg mefloquine for treatment of severe falciparum malaria. Artemisinin or artesunate or artemether based suppositories are not able to prevent mortality in children, infants and in pregnant women.

α/β-ARTEETHER SUPPOSITORIES

α/β-Arteether suppositories as shown by results in experimental severe MDR malaria model (*P. yoelii nigeriensis* MDR) are **very fast acting, and highly efficient in controlling** acute infections in **mice model and 20 mg/kg dose x 3 days, has shown 100% suppression** of virulent MDR infection, leading to survival of treated mice. Suppository containing 50 mg/kg α/β-arteether (x3 days) gives 100 percent survival of mice up to 28 days and no recrudescence of parasitaemia was observed. There is **no need to give a second antimalarial e.g. mefloquine as reported with artesunate suppositories.**

DIHYDROARTEMISININ (DHA) SUPPOSITORIES

DHA suppositories developed in the present study is **most active antimalarial** amongst the artemisinin derivatives. The conversion of artesunate, arteether, artemether, artelinate etc. to DHA is unique example in pharmacology because **all these artemisinin derivatives are metabolized into DHA which is the most active antimalarial** as shown

by several investigators. **DHA suppositories are considered as life saving** in acute malaria. DHA suppositories can revive comatose cases of malaria, can be used in rural areas where hospital facilities are not available. **DHA requires much lower dose** which is safe. DHA formulation are known to **exhibit gametocytocidal activity** and **give additional benefit to interrupt malaria transmission.** DHA suppositories **can save lives** in malaria endemic countries (Patent 2000).

World Health Organization report (2010a) has approved DHA-piperaquine oral tablets (40 mg + 320 mg combination) treatment for 3 days as **fifth ACT for the control of uncomplicated *falciparum* malaria cases**. MMV has already used this combination in more than dozen clinical trials against *P. falciparum*. **DHA has strong blood schizontocidal action against highly MDR *P. yoelii nigeriensis* (Patented 1999/2000).** This information regarding DHA antimalarial profile (IM, Oral, Rectal use) **patented by Dutta *et al.* Internationally in sixteen countries** has made this molecule as one of the important component of antimalarial control.

DHA like other molecules such as α/β-arteether exhibits exceptionally high activity against MDR malarias

- Fast acting by all the three routes of administration
- Very high blood schizontocides activity
- **Very safe gametocytocidal drug** capable of stopping malaria transmission
- Fast **blood schizontocidal action can save lives**.

Planning Global Malaria Eradication and Saving Lives

WHO (2012) global assessment of malaria casualty based on World Malaria Report (2012) Document recorded:

- 216 Million malaria cases (range 149 million-274 million)
- Malaria kills around 655,000 cases (range 537,000-907,000)
- 90% Deaths due to malaria recorded in Africa
- Global mortality figures (one child dying from malaria every minute)

An Editorial entitled "**Is malaria eradication possible**" in Lancet (Vol. 370, Oct. 27, 2007) quotes the statement: **Bill and Melinda Gates admit that "malaria eradication**, **it is possible, is a long way off-years or more likely decades away"** and

they clearly argued that "**Is eradication a realistic goal**"? Bill and Melinda Gates emphasized that "new Scientific advances have **made the goal of eradication realistic in decades to come".**

The **Roll Back Malaria Initiative launched by World Health Organization** in 1998 led to **Abuja Declaration** in 2000, which showed **commitment to eliminate malaria as a public health problem**. Increased **Global Fund to Fight AIDS, Tuberculosis and Malaria**, together with **World Bank's Booster Programme,** complimented with the **U.S. President's Malaria Initiative -** all these agenda finally showed **commitment for renewed global efforts for elimination/eradication of malaria.**

INTERNATIONAL AGENDA FOR ANTIMALARIAL DRUG DISCOVERY AND MALARIA ERADICATION

More than Thirty Two Research Agenda for Malaria Control and Global Eradication have been recently published with major objective to **achieve the Malaria Eradication** by **Promoting Antimalarial Drug Development and Drug Research, Antimalarial Vaccines,** Application of **Safe Insecticides, Mosquito nets, Mosquito Control technologies** involving new Insecticides Development, **Biocides** for Vector Control, **Indigenous Technologies** for repelling mosquitoes, so much so that nearly twenty-five Malaria Eradication Agenda (see Appendix) are now published for International Cooperation through agencies like Medicines for Malaria Venture – a non-profit European Agencies with Intercontinental Collaboration spread over several Pharmaceutical Companies and **International Teams of Committed Malaria Research Scientists** have pooled their resources to protect African Children which suffer the most due to malaria epidemics.

A very comprehensive "**A Research Agenda for Malaria Eradication: Drugs**" was brought out by the MalERA Consultative Group on Drugs (2011) and the major thinking of the Group of International Participants was the "**Malaria Eradication is not possible with existing tools"** which include : (1) Artemisinin – based combination treatment (ACTs), (2) Long-acting insecticide-treated nets, and insecticide spraying. According to "View of malERA Drugs Consultative Group" "**the complete malaria eradication will not be accomplished within most of our lifetimes and that new tools,**

including new antimalarial drugs developed specifically for elimination indications", such as (1) **Transmission blocking safe drugs** to kill/sterilize immature and mature gametocytes of *P. falciparum*, (2) Successful **treatment of MDR complicated *P. falciparum* infection/fast-acting blood schizontocides for comatose malaria cases** (both *P. falciparum/P. vivax*), (3) **elimination of relapsing liver stages (hypnozoites)** of *P. vivax/P. malariae*, i.e. **safer antirelapse drugs for radical cure** of *P. vivax* (to relapse primaquine), (4) develop **safe causal prophylactic** agents and (5) **New ACTs** for treatment of uncomplicated *P. falciparum* infection e.g. **DHA-piperaquine combination** recently approved by WHO (2010). Extensive chemotherapeutic investigations at **CSIR-CDRI Institute has already made major headway in tackling some of the listed obstacles in New Drug Discovery Programmes** and would also provide many new chemotherapeutic agents to tackle some of the bottle-becks to initiate programme for malaria eradication in collaboration with **National Antimalaria Programme of Govt. of India and International Malaria Control Agencies/WHO/U.S. Govt. programmes/WRAIR, Washington D.C. collaboration. The leads developed at CDRI** to tackle and support eradication plans **will be available to all International Agencies to progress towards global malERA Agenda.**

Commendable efforts have been made by **Committed Scientists of MMV Group** (Anthony, Burrows, Duparc, Moehrle and Wells 2012) who have tried to report update on the current status of global pipeline of new antimalarials. In the past three years **five new medicines** were prequalified by World Health Organization as fixed dose, **artemisinin-combination therapy (ACTs)** to protect against emerging artemisinin resistance which included: (1) ACT comprising of **artemether-lumefantrine** (Coartem Tablets and Coartem Dispersable from Novartis), (2) **Artesunate-Amodiaquine** (Coarsucam^{TM}/artesunate amodiaquine (Winthrop^{R}, Sanofi), (3) **Pyronaridine-artesunate** (Pyramax^{R}) (Shin Poong Pharmaceuticals) and (4) **Mefloquine-artesunate** (Cipla/DNDi (prequalified by WHO). Besides four other types of ACTs are prescribed for **uncomplicated malaria** (including (a) Dihydroartemisinin (DHA) – Piperaquine (Eurartesim^{R}-Sigma-Tau) and Duo-Cotecxin^{R} (Holley-Cotec), (b) Pyronaridine-Artesunate, (c) Artesunate-Mefloquine (ASMQ) (Cephalon/Mepha) and (d) **Artemisinin-naphthoquine (ARCO, Kunming, China) which is available as one day treatment**.

According to this eradication agenda proposed by Anthony *et al.* (2012), there are still **serious gaps in development of new medicines** which would be essential to **achieve eradication of malaria** including (1) effective drugs to **prevent transmission** of malaria (i.e. need for developing **safe gametocytocides**, (2) **safe drugs to stop relapses of *P. vivax*** i.e. drugs to kill hypnozoites/dormant liver stages.

The Malaria Eradication Initiative to be accomplished would involve the development of new drugs:

iv) To **interrupt malaria transmission** by killing stage V mature gametocytes of *P. falciparum* and sterilize the gametocytocytes of *P. falciparum*.

v) A new radical curative **(antirelapse) drug to eradicate hypnozoites (relapsing stage)** of *P. vivax*.

vi) **A drug for prophylaxis against *P. falciparum/P. vivax***. According to above report 8-aminoquinolines can function both as radical curative and for prophylaxis. A safe drug of this category can also **sterilize the transmission stages** of *P. falciparum*, and should **replace currently available drug primaquine**. This drug produces methaemoglobinaemia, and haemolysis of RBC and is not recommended for infants, children, in pregnancy and G-6-PD deficient individuals. **We essentially need a safe primaquine substitute.**

Burrows, van Huijsduijnen, Mohrle, Oeuvray and Wells (2013) have put forward the **malaria eradication agenda** (see Appendix) with focus to design next generation of antimalarials to **target different stages of malaria parasite.** According to MMV concept for malaria control,(1) **New fast-acting medicines** would be an essential weapons for **control of emerging artemisinin resistant malaria**, (2) New medicines to **reduce transmission of malaria** (through their use to **eradicate early and late stages of gametocytocides,** (3) **Prevention of relapses due to dormant** (**hypnozoites stage**) of *P. vivax/P. ovale,* to eliminate relapsing stages of malaria, (4) For achieving **radical cure**, **a single exposure radical cure and prophylaxis therapy** (**against sporozoite infection**) has been proposed together with **new drugs for elimination of asymptomatic sexual stages (interruption of transmission by the gametocytes)** needs to be developed in order to achieve malaria control. Substantial progress made in the area of **New Drug Discovery and Development in India** during the last five decades (1963-2014) can

provide some of the powerful new drugs and drug combinations to promote the Global Eradication Agenda.

Flannery *et al.* (2013-2014) discussed the problem of antimalarial drug discovery directed to the ultimate **objective of complete elimination of global malaria.** Flannery *et al.* (2014) also admitted that the **current antimalarial drugs were inadequate to achieve complete elimination of malaria**. These investigators report that **artemisinins are fast-acting** and very potent against blood stage parasites as **schizontocides**, besides showing activity against early **sexual stages of the parasite**, and they also realized the **importance for blocking transmission of malaria**. **The author agrees** with the conclusions of Flannery *et al.* (2013) that **artemisinins can act as very effective gametocytocide to block transmission** of malaria and cite the **discovery of gametocytes from the Indian studies published internationally** during the **priod 1989-2003** in which **artemisinins were reported to completely sterilized the gametocytes** of human transmissible *P. cynomolgi* B in rhesus monkey model, eg. artemisinin (Dutta *et al.* , 1989), α/β-arteether (Tripathi *et al. ,* 1990 and 1996; Dutta and Tripathi, 2003; Dutta *et al. ,* 2000) as potent gametocytocides, dihydroartemisinin (Jain *et al.* 1999, 2000 Patents) and β-artelinate (Tripathi *et al.* , 1996) as gametocytocide and Dutta *et al.* (2001) U.S. Patent in which α/β-arteether was reported to be effective gametocytocidal for *P. falciparum* (in patients with early stages of gametocytes).

It may be recorded that compound **CDRI compound 80/53** (named **Bulaquine=Elubaquine** by WHO, 2000) was marketed as ABLAQUINE (2000) which is 3-4 times safer than primaquine, is a potential 8-aminoquinoline which can replace primaquine as radical curative/anti-relapse drug. This drug Bulaquine **eradicates hypnozoite of *P. vivax*** at 25 mg/kg daily dose administered for 5 to 7 days. The drug shows much better safety margin compared to primaquine (Dutta *et al.* 1989; Dutta, Puri, Tripathi and Gupta, 1998; Puri *et al.* 1989; Dutta and Puri,2003). Beside, **tafenoquine** (**WR 236805**) (another 8-aminoquinoline) has been **developed up to pre-clinical stage by CDRI in collaboration with WRAIR, Washington D.C**. (Milhous *et al.* 1988; Puri *et al.*1994; Puri and Dutta, 2003, Dutta and Puri, 2003).

BRIEF REPORT ON ANTIMALARIAL DRUG DEVELOPMENT AT CDRI

Success Stories in Malarial Control from the SEA Region

Malaria: RBM – The Malaria Problem in South-East Asia Region – Success Stories in Malaria Control from the SEA Region

Stories in Malaria Control from the SEA Region

(iv) α/β-Arteether (30:70 ratio) a racemic mixture of ethyl derivative of Artemisinin developed by the Central Drug Research Institute and CIMAP, Lucknow, India. Multi-centric studies in over 500 patients have confirmed the efficacy of drug in uncomplicated and complicated *falciparum* malaria with recrudescence rate of about 8%. The drug is produced by Themis Chemicals, Mumbai, India. It is available throughout the country and **has been introduced in the treatment of severe malaria by the NAMP** (Marketed by CDRI/CIMAP, 1997).

(v) **Azithromycin in Malaria**: Azithromycin, a macrolide antibiotic has shown to have antimalarial activity *in vitro* and in animal models. Prophylactic efficacy 97-98% and 49-83% has been reported in *vivax* and *falciparum* malaria in Kenya, Thailand and Indonesia. Multi-centric studies to evaluate efficacy in treatment of *vivax* and falciparum malaria are in progress at the Malaria Research Centre, New Delhi, India.

(vi) **Bulaquine – a new anti-relapse (CDRI-80/53):** Central Drug Research Institute, Lucknow, India has synthesized **a new safer anti-relapse antimalarial Bulaquine** which has successfully completed Phase I, II/III clinical trials and soon will be marketed in India (Marketed by CDRI, 2000 and – Ablaquine 2000).

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APPENDIX-I

GLOBAL MALARIA ERADICATION INTERNATIONAL AGENDA-UPDATE

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