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New Antimalarial Drug Development in India: Radical Curative Agents CDRI 80/53 (Elubaquine) and WR 238605 (Tafenoquine)

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Global efforts had been continued for the last 50 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 250 potential tissue schizontocides had been synthesized and screened, and two new primaquine derivatives had been identified in primate malaria radical curative tests which could be the candidate drugs to replace primaquine:

Key Words: Malaria, Anti-relapse drugs, CDRI 80/53/Bulaquine/Elubaquine, WR 238605/ Tafenoquine, Antimalarials, Plasmodium, Gametocytocide, Aablaquin

Introduction

The only 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

1. CDRI 80/53 (ELUBAQUINE=BULAQUINE)

In our search for new 8- aminoquinoline analogues with potential radical curative activity against relapsing simian malaria 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/day x 5 days) dose schedule is comparable to primaquine (15mg/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. A combination kit of anti-relapse drug CDRI 80/53 (25 mg/day x 5 days) and blood schizontocide chloroquine has been successfully marketed as Aablaquin since 2000.

2. WR 238605 (TAFENOQUINE):

Definitive radical curative (anti-relapse) activity of WR 238605 has been established in simian malaria *P. cynomolgi* B screening model, under WRAIR – CDRI collaborative malaria programme. The compound has very high anti-relapse, causal prophylactic and blood schizontocidal activity as compared to primaquine. 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 draw back of WR 238605 is that it raises methaemoglobin 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. vivax* cases. Finally its safety in G-6 PD deficient cases has not been established till todate. 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.

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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 five decades to discover a safer anti-relapse drug for replacing primaquine, which would not produce significant levels of methaemoglobinaemia and haemolytic 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) of *P. cynomolgi* 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 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 et al. 1998).

Search for New Radical Curative (Anti-relapse) Anti-malarials

Major efforts had been made by the Walter Reed Army Institute of Research (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 by Davidson et al. (1981), Schmidt (1983 a,b), and Sweeney (1984). Davidson et al. (1981) listed 24 new compounds (table 1) 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 1, 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 activity of these very compounds was also reported by Schmidt (1983 a,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 (Elubaquine) for further development as radical curative agent.

Table 1. *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	Active
NI 147/36	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	Active (PI 1.7-3.1)
2-Methyl-primaquine	Active (PI 1.0)
3-Methyl-5-phenoxy-8-aminoquinolines	
WR 235485	Active (PI 1.5)
3-Methyl-primaquine	Active (PI 1.3)
4-Methyl-5-phenoxy-8-aminoquinolines	
WR 232956, 232584, 225448, 233195, 233078	Active (PI 4.2-4.8)
4-Methyl-primaquine	Active (PI 2.1)

From Davidson et al. 1981. *Bull Wld. Hlth. Org.* **59** 463-479

CDRI 80/53 (Elubaquine) – A New Oral Radical Curative (Anti-relapse) Drug

It must be emphasized that although we had identified in 1987 several 8- aminoquinolines as potenial 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, 8-fold safety 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 2).

(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%. Likewise higher doses (2.0 to 4.0 mg/kg x7 days) were also 100% curative in 13/13 monkeys (table 2) (Dutta et al. 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 et al. 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 2).

3. Blood Schizontocidal Activity Against P. berghei (Sensitive Strain)

Compound 80/53 shows weak blood schizontocidal action 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.

Table 2. *Comparison of anti-malarial 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 2.0-4.0mg/kgx7 cure 13/13 monkeys	1.00 mg (base)/ kgx7 cure 12/12 monkeys	0.316 mg/kg x7 cure 7/7 monkeys
2. Causal prophylactic dose (3 day test)	3.16 mg/kg x3 cure 3/3 monkeys	1.78 mg (base)/kg x3 cure 7/7 monkeys	0.316 mg/kg x3 cure 9/9 monkeys
3. Blood schizontocidal dose (7 day test)	Low activity	10 mg (base)/kgx7 (25% curative dose) 1/4 monkeys	3.16 mg/kg x7 cure 6/6 monkeys
4. Gametocytocidal dose	—	2.00 mg (base)/kg x1	2.00 mg/kg x1

CDRI 80/53 and primaquine data summarized from Dutta et al. (1989, 1994) and WR 238605 data from Puri et al. (1994).

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).

Test Compound CDRI 80/53 was screened for any observable side- effects or toxicity in animal models using a battery of pharmacological tests to detect any adverse drug reaction etc. (Kar et al. 1988). The compound was found to be safe and no drug related effect was observed. On the basis of LD_{50}, 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 behaviour 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 escalated 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 CDRI 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 ODX7 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 comparsion 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 3) (Dutta et al. 1994).

Phase II/III Clinical Trials with CDRI 80/53

Phase II/III clinical trails 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 followup 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-3) (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 can not 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 for 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.

Table 3. *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	25mg 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	3.0%		
Placebo chloroquine	Mid Nov-March	224	1500mg	10.6%		
WR238605	2-6 months	7	300mgx7	Nil	1-3%	13.5%
	2-6months	9	500mgx3 (repeated after 1 week)	1 case	1-3%	14.7%
	2-6months	7	500mgx1	1case	1-3%	6.4%
Total 23 cases				**8.7%**		
Placebo chloroquine	2-6months	7	1500mg	4 cases (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.

WR 238605 (Tafenoquine) – Potential Anti-relapse Agent

WR 238605 succinate is an 8 – aminoquinoline which was orginally 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 WR238605 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 2).

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 2). *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 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 x 7 days (table-2) (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 preerythrocytic 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 2) (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 trails for gametocytocidal activity have not been under taken 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

Methaemoglobin Toxicity

Anders et al. (1988) had reported increased methaemoglobin toxicity of WR 238605 compared to primaquine in dogs (MetHb 16.0% 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 guineapigs 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 guineapigs (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 upto 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 (170 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 4-600 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 dizzyness (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 3. 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 –3 for comparison with WR 238605 efficacy/met Hb toxicity data.

Overall relapse rate within 2-6 months of followup with three regimens of WR 238605 was 2/23 cases (8.7%).

Regimen 1: 300 mg x 7 days – relapse Nil/7. (metHb 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 (metHb 6.4%)

Placebo (chloroquine 1500 mg)–relapse 4/7 (57%).

Corresponding relapse rate with one year followup with CDRI 80/53 (25 mg x 5 days) was 29.6% among 219 total cases treated, and with primaquine (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 (table 3).

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 women 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 is still 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 WR 238605 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 followup, 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.

Conclusions

Two new anti-relapse drugs had been identified under primate anti-malarial screening at CDRI:

1. **CDRI 80/53 (Elubaquine=Bulaquine)**
2. **WR 238605 (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 3.0%
   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), 300mg x 7 days)-relapse rate 8.7%
2. CDRI 80/53 has cleared long term (3 months) subacute 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 3.02%) 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 2100mg 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 x 5days as a standard radical curative treatment for *P. vivax.*
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).

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