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Review Article

Chemical Radioprotection : Thiols

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In spite of extensive research during the last four decades, no ideal radioprotective drug for radiotherapy of cancer has emerged. The radioprotectors tried so far have shown either low dose modification factor (DMF) or high toxicity. Sulphydryl compounds are very good radioprotectors but their application in radiotherapy is limited because they lack the twin properties of low toxicity and high DMF. Various classes of radioprotective compounds, theories of chemical protection, review of radioprotective effects of various drugs especially WR 2721 and MPG have been discussed. The drawbacks of drugs to differentially protect the normal tissues especially the side effects have been reviewed and indications for further research work have been given to improve the efficacy of each method. Possibilities of other drug delivery system using much lesser amount of radioprotective drugs and hence reducing the toxic effects of the drugs such as trapping them with liposomes and then targetting them to respective sites have been shown. Further work in these areas is necessary.

Key Words: Chemical radioprotection, Thiols, Radiotherapy

Introduction

Radiotherapy has greatly rested on the observation of Bergonie and Tribondeau (1906) that the sensitivity of cells to irradiation is in direct proportion to their reproductive activity and inversely proportional to their degree of differentiation. However, in the clinical trial of radiotherapy on some cancer patients, normal cells are invariably found to be more radiosensitive than the cancer cells. The reason for this has been traced to the hypoxic cells in the inner core of the cancer tissue. Considerable research has shown that presence of oxygen during irradiation generally enhances the sensitivity of cells and organisms. This has instigated an influx of more scientific activity to develop the best modality to tackle the greater radioresistance of hypoxic cells.

Radiotherapy has achieved a boost with the finding that new radiation sources yielding heavy particles have advantages which are physical and biological. The goal of any radiotherapy is to increase linear energy transfer and to have low oxygen enhancement ratio. The simultaneous use of low and high energy radiation seems to be very promising. Further, the introduction of chemical sensitizers and protectors possibly for tilting the balance in favour of killing more cancer cells than normal cells has opened newer avenues in cancer radiotherapy. There is still, however, need for greater basic research in this area.

The following chart shows some of the possible strategies to modify differentially the radiation response of a tumour cell population relative to that of a normal tissue population (Becker 1977).

(A) INCREASED YIELD OF IRREVERSIBLE RADIOCHEMICAL LESIONS
1. Oxygen
2. Nitric oxide; organic nitroxides
3. Metronidazole, nitrofurans, Ro-07-0582
4. High-LET particulate radiations

(B) INCREASED INTRINSIC SENSITIVITY OF TARGET DNA
1. Halogenated pyrimidine analogues (BUdR, BCdR, IUdR)
2. Possible potentiation of BCdR by tetrahydrouridine
3. Purine Starvation

(C) INHIBITION OF REPAIR
1. Hyperthermia
2. Chemical inhibitors of single-strand-break repair (actinomycin D)
3. High LET particulate radiations.

(D) PARTIAL SYNCHRONIZATION IN CYCLE-DEPENDENT SENSITIVE STATES
1. Fractionated radiotherapy-timed mitotic delay
2. Colchicine, Vinca alkaloid mitotic spindle poisons.

(E) DIFFERENTIAL RADIOPROTECTION OF NORMAL TISSUES

Though there are some drawbacks in each of these methods, especially the side-effects, research is being carried out to develop and improve the efficiency of each method. Use of radioprotective and sensitizing drug either alone or in combination in case of conventional radiation such as photons, gamma-rays and electrons may be more advantageous in all aspects particularly in terms of cost/benefit ratio (tables 1 & 2).

Chemical Radioprotection

Chemical radioprotection by sulphur compounds is a vast field. New facts appear fast from work on micro-organisms, mammalian cells in culture and on animals. Chemical protective agents or radioprotective agents are substances which, administered to an animal or added to a culture medium shortly before exposure to ionizing radiation, significantly decrease the effects of this radiation; administration after irradiation does not have this favourable effect.

Four main steps must be earmarked in the development of our knowledge of chemical protection against ionizing radiations.

In 1942, W M Dale of Manchester University, showed that addition of certain substances (colloidal, sulphur, thiourea, formate, etc.) to an aqueous solution of carboxy peptidase and D-aminoacid oxidase decreases the inactivation of these enzymes by X-rays (Dale et al. 1949).

In 1948, Latarjet and Ephrati tested certain substances on bacteriophage which, logically, on the basis of the theory of indirect action, might act as chemical protective

Table 1 *Choice of equipment for a radiotherapy department*

	Capital cost (in thousands of US $ including installation)	Annual fixed costs (in thousands of US $ including source and maintenance)	Number of patients per year	Cost per patient (in US $)
Two Cobalt-60 units, both with vertical supports, outputs 0.774 and 1.55 C.kg$^{-1/hr}$ at 1 m (3000 and 6000 R/h at 1 m)	189	25.3	730	35
Two cobalt-60 units, one with vertical support and output 0.774 C. kg$^{-1/hr}$ at 1 m and one with isocentric support and output 1.55 C.kg$^{-1/h}$ at 1 m	249	31.0	795	39
Two cobalt-60 units, both with isocentric supports, outputs 1.55 and 3.10 C. kg$^{-1/hr}$ at 1 m	439	53.6	970	55
One isocentric cobalt-60 unit with output 1.55 C.kg$^{-1/hr}$ at 1 mm and one isocentric 10-MV	667	75.2	1015	74

Note: Reproduced from Optimization of Radiotherapy (1980)

Table 2 *Cost of chemotherapy per course of treatment*

		Cost per patient (US $)
Chlormethine + vincristine + prednisone + procarbazine	6 cycles	777
Bleomycin + chlormethine + vincristine + prednisone + procarbazine	6 cycles	716
Doxorubicin + bleomycin + vinblastine + dacarbazine	6 cycles	2119
Vinblastine	Weekly for 1 year	1030

Note: Reproduced from Optimization of Radiotherapy (1980).

agents. They obtained positive results with thioglycolic acid, tryptophane, glutathione, cystine and cysteine, even in the absence of oxygen. They also pointed out the importance of certain active groups (SH and NH_2). This fact was emphasized a few years later by Bacq and Herve (1952) when discussing their results in mice.

Later in 1949, Patt and his associates from the Argonne Laboratory in Chicago, put to clear-cut experimental test a general hypothesis built by G Barron and his associates (1949) that pure crystallized thiol enzyme are much more sensitive to ionizing radiation in aqueous solution than non-SH enzymes and had succeeded in reactivation of radiation-altered SH enzymes by adding an excess of cysteine or reduced glutathione (GSH). For Barron, the main mechanism of action of ionizing radiation is the oxidation of $-SH$ functions to form $S-S$ bridges with inactivation of enzyme or coenzyme activity depending on these functions. These findings probably formed a basis for the more sophisticated mixed disulphide hypothesis (Eldjarn & Pihl 1958).

At that time, the work of Patt et al. (1949) was centered around the role of the $-SH$ functions. Bacq was interested in the NH_2 group also because amines had been known to inhibit the action of mustard (Bacq & Alexander 1961a). The simplest amine, methylamine, has a slight but consistant radioprotective activity (Bacq & Herve 1951). Bacq tried to increase the effectiveness of cysteine by applying a well known rule in pharmacology, of liberating the NH_2 function of this amino acid by removal of the carboxyl group (Bacq & Alexander 1961a). Thus, cysteamine was synthesized and immediately proved to be much more active than cysteine. Cystamine was found to be as active as MEA, a surprising fact at that time since it was known that cystine, the $S-S$ form of cysteine, is totally inactive (Patt et al. 1949). In 1952, it was already known that not all $-SH$ substances are radioprotective and that many amines (including histamine, tryptamine, 5-hydroxytryptamine, norepinephrine, tyramine) were also good protective agents (Bacq 1954, Bacq & Herve 1952).

Since 1952, research has taken many directions:

(a) During 1951-52, Bacq had predicted that in the future years, compounds much more powerful than MEA would be synthesized and tested, not many have been found to emerge as potent alternatives to MEA.
(b) The metabolism of the radioprotective agents, their distribution in the body, and their pharmacological and biochemical effects have been actively investigted.
(c) The detailed knowledge of what happens in the cells and tissues of an animal or cells irradiated under chemical protection has engaged the attention of many radiobiologists.
(d) Finally, much progress has been made on the elucidation of the mechanisms of action of various important protective compounds.

Classes of Radioprotective Compounds

The following is a discussion of some important drugs from each of the several classes of protective compounds.

Aminoalkyl-thiols and their disulphides: Since the discovery of the protective effect of cysteine, a large number of these compounds have been tested. Among these the best agents are cysteine, cysteamine (mercaptoethylamine or MEA), cystamine (the disulphide of MEA), mercaptoethyl-glutanidine (MEG), and guanidoethyl disulfide (GED), MEG is usually obtained from S-2(-aminoethyl) isothiouronium bromide hydrobromide (AET) by dissolving AET in aqueous solutions at neutral *p*H.

An extension of these drugs is another class of radioprotectors called aminoalkyl-

phosphorothioates. The simplest of these is the aminoalkylphosphorothioates. The simplest of these is the aminoethyl phosphorothioate (MEAP). These compounds have been synthesized and found to be as effective as their free sulphydryl or disulphide counterparts. They are, in actuality, potential sulphydryl compounds since the phosphate group can be removed by hydrolysis or by enzymatic action and thereby creating an aminoalkylthiol. The advantage of some of the phosphorothioates is their moderate toxicity compared to the thiols.

The other sulphur compounds: Thiols other than aminoalkylthiols show small protective ability. Thiourea and its derivatives give some protection. Dimethylsulfoxide has moderate protective effect. Perhaps the best group in this category is the dithiocarbamates. Dimethylammonium diethyldithiocarbamates was reported to be particularly successful, approaching the activity of the aminoalkyl-thiols.

Cyanide and Organic Nitriles: Cyanide in toxic doses has been reported to be protective in mice. Detoxification is extremely rapid and parallels the protective activity; therefore, protection disappears in a few minutes. Hydroxyacetonitrile has been reported to be effective in mice against fast neutron irradiation.

Amines: A number of amines have been shown to be protective in mice. Histamine and other amines such as epinephrine, tyramine, and hydroxyphenylethylamine are fair protective agents. The most promising one of this group is 5-hydroxytryptamine. Its antagonists have been reported to block protective action. Serotonin has been successful not only in mice but in other mammals such as monkeys. Several of its derivatives have been reported to have the same protective effect. Another amine, which has shown significant protection, is p-aminopropiophenone.

Chelating agents: Some chelating agents have been tested, the best known of which is EDTA (ethylenediamine-tetraacetate sodium salt). They have shown some protection in rodents.

Other compounds: There is a large list of additional compounds that have shown some protection in rodents but do not show particular promise for the future. Some protection is provided by drugs that protect by altering the physiological state of the organism. Compounds that induce hibernation and various hormones may be included here. ACTH, oxytocin and putressin are somewhat protective. Various adenine nucleotides have been shown to give some protection.

Mixture of protective agents: There has been a great deal of interest in testing mixtures of protective agents. It is done with the hope that the protective action of the different agents will be additive.

Theories of Chemical Radioprotection

Theories of radiation protection can be considered at both the molecular and biochemical-physiological levels. Four molecular level protection hypotheses; radical scavenging, hydrogen donation, mixed disulphide formation and endogenous non-protein sulphydryl increase, probably describe different aspects of the actual protection mechanism, although each has inconsistencies. At the biochemical-physiological level, hypothermia induction, hypoxia induction and biochemical shock may be involved in protection of the organism against radiation-induced damage and death. It is certain that no single mechanism can account for protection afforded *in vivo* by radioprotective drugs. Certain compounds may operate mainly by means of physiological effects resulting in hypoxia or hypothermia induction in critical tissues. Others may operate primarily by influencing the intrinsic radiosensitivity of the target molecules by causing localized

radical scavenging or by donating a hydrogen atom. Metabolic effects such as biochemical shock, release of endogenous non-proteins sulphydryls, induction of structural changes in target molecules or delay in DNA synthesis and cell division are also posssible . mechanisms of radio-protection. The difficulty in giving a plausible mechanism of radiation protection arises because of the complexity of the process of damage; the direct and indirect effect and host of ultrafast chemical reactions that initiate them. The sequence of events sparked off within 10-16 seconds continue till generations.

Theories of chemical radioprotection are now an integral part of all standard textbooks on radiobiology (Modig 1976, Copeland 1978).

Radical Scavenging

Free radicals are highly reactive, electrically neutral atoms or molecules which are characterized by the presence of an unpaired electron. A number of short lived free radicals are generated during irradiation of water e.g.; H′, OH′, e_{aq}. The H′ and OH′ free radicals may react with molecular oxygen to give a more stable free radical HO_2 (hydroperoxyl). The majority of the radicals will react with the biologically important macromolecules (target molecules) and account for the indirect effect of radiation. Such interaction may eventually lead to structural defects and ultimately to cellular death.

Using ESR and pulse radiolysis studies, it has been demonstrated that thiols and disulphide groups readily interact with water radicals like OH.

RH(Target)	*Radiation*	*R*
R′ + R′	———	R-R (cross linking)
R′ + O_2	———	ROO′ (peroxidation)
R′ + R′SH	———	RH + R′S. (Repair)

The hydrogen donation repair mechanism was proposed by Alexander and Charlesby (1955). ESR studies shown that it is effective with macromolecules in solution and with bacteria in the dry state. No data is, however, available for its applicability *in vivo*. In any case, like free radical scavenging, this mechanism also requires concentration of the protector in the vicinity of the critical target molecule.

The Mixed Disulphide Hypothesis

Pihl and Eldjarn have shown that several aminothiols and their oxidation products form mixed disulphides with protein −SH and −SS-groups (Eldjarn & Pihl 1956, 1958, Pihl & Eldjarn 1958). They found a good correlation between the ability of thiols and disulphides to form mixed disulphides and the radioprotective ability of these substances. When the mixed disulphide is attacked by radicals formed by the radiolysis of water, one of the sulphur atom will be oxidised to sulphinic or sulphonic acid, whereas the other would be reduced to a free −SH group, the probability of both being equal. Thus mixed disulphides could result in restoration of the target in 50% of the cases. It is interesting to note that the binding between protector and protein is reversible since an enzymatic mechanism exists for the removal of the attached protector (Mannervik & Axelsson 1975).

A significant weakness of the mixed disulphide hypotheses is its apparent failure to explain protection of the nucleic acids which are the important cellular target for ionizing radiation. It also does not explain why several biomolecules which are not dependent on −SH groups are equally well protected by thiol compounds.

Endogenous Non-protein Sulphydryl Compound Release

Revesz and Modig in mid-1960's suggested that when exogenous sulphydryl radioprotective compounds reached the

cell, they displaced glutathione and other NPSH from natural mixed disulphide structures and it was this released endogenous NPSH compounds which actually scavenged water radicals (Revesz & Bergstrand 1963, Revesz & Modig 1965, Modig & Revesz 1967). It is based on the observation that the 'Sulphydryl increase factor' of a series of compounds correlated well with their radioprotecive ability. Unfortunately, one of the main endogenous NPSH compound, glutathione, is not a good radical scavenger.

Hypothermia Induction

A reduction in body temperature has been noted after administration of various sulphydryl compounds. It is suggested (Copeland 1978) that during the period of lowered temperature a reduced metabolic rate could permit repair of crucial radiation damage before the stress of normal metabolism returned. Although hypothermia induction may be the principal mechanism for protection by chloropromazine, it is usually only a side effect particularly for the sulphur containing protectants.

Hypoxia Induction

Prevention of radiosensitization by oxygen is apparently the mechanism by which a number of compounds, primarily those related to histamine, protect. Such drugs can reduce oxygen tension by blocking haemoglobin function or reducing blood flow.

Thiols and aminothiols oxidize readily at physiological *p*H and consume oxygen present in the medium, thus resulting in hypoxia.

There is, however, much evidence that speaks against the idea of induction of cellular hypoxia/anoxia as the sole mechanism of action. It has been reported that cells irradiated under anoxic conditions can also be well protected by thiols (Vergroesen et al. 1967). Cysteamine has been found (Littbrand & Revesz 1971) to modify the survival of oxically as well as anoxically irradiated cells in tissue culture. The aminothiol cysteine which is more readily oxidized than cysteamine at neutral *p*H, affords much less protection *in vitro* and several other easily oxidizable-SH substances are without protective effect (Modig 1976). All these reports suggest that reduction in oxygen tension cannot be the sole mechanism of radioprotection.

Biochemical Shock

Bacq and his associates (1965, 1966, 1969) suggested a generalized mechanism of protection for the sulphur compounds. It has been proposed that binding of the sulphur compounds primarily to mitochondrial membranes induces transitory lesions which alter mitochondrial metabolism in such a way that the cellular systems become temporarily radioresistant. The biochemical shock hypothesis suggests that a compound's ability to form mixed disulphides correlates with protection because such binding is the first step in the chain of events leading to characteristic biochemical lesions in mitochondria. The hypothesis contains no explicit mechanism of increased radioresistance.

Sulphydryl Radioprotectors with Special Reference to MPG and WR-2721

From the accumulated data on chemical radioprotectors, it has become abundantly clear that sulphur compounds are at the best radioprotectors both from the point of view of direct effect and indirect effect.

Excellent books, reviews and general discussions are numerous and are included in some of the recent publications (Ayene & Srivastava 1985, Ayene et al. 1988, Ayene & Srivastava 1989, Bacq 1975b, Sugahara & Srivastava 1976, Modig 1976, Locker &

Flemming 1977, Mishra et al. Communicated Yuhas 1981, 1982). Over 3,000 compounds were reported to have been tested for their radioprotective capacities by the year 1975 (Bacq 1975a).

The search, however, for an ideal radioprotector is still not over. Many radioprotectors tried have shown either low DMF or high toxicity; most of them have failed to show differential protection of the tumour and normal cells—a condition so very vital to obtain therapeutic gain during radiotherapy of cancer. To find out more effective and less toxic radioprotective drugs, a chemical protector screening program has been initiated at the Fox Chase Cancer Centre under a contract with the National Cancer Institute in USA (Brown et al. 1982).

The explanation for the excellent protection exhibited by sulphur compounds has been suggested on the basis of their electronic structure by Nagata and Yamaguchi (1978). They have suggested that because of the significantly low energy of the LUMO (low unoccupied molecular orbital) and the relatively high energy of HOMO (high occupied molecular orbital) in comparison to others, sulphur compounds show: (i) high reactivity towards OH radical, (ii) both electron-donating and electron-accepted properties for easy reaction with radical reagents, and (iii) both intra- and inter-molecular energy transfers, as a result of which free radicals formed in other parts of a molecule eventually become transferred to sulphur groups which presumably act as the sinks of the radiation energy.

In spite of the fact that sulphydryl compounds are very good radioprotectors, their application in radiotherapy is very much limited because of nonavailability of such compound with twin properties of low toxicity and high DMF. However, two compounds MPG and WR-2721, have been put to clinical tests. While MPG has already undergone clinical trials in Japan (Sugahara & Srivastava 1976), the clinical trials of WR-2721 are currently in progress and results of phase I trials are now being released (First conference on Radioprotectors and Anticarcinogens, June 21-24, 1982, the National Bureau of Standards, Gaithersburg, Maryland, USA., Symposium on perspective in Radioprotection, March 13-14, 1987, National Bureau of Standards, Bethesda, Maryland, USA).

Following is the brief review of the radioprotective effects of MPG and WR-2721.

Mercaptopropionylglycine (MPG)

The first report on radiation protection by MPG was published by Nagata et al. (1972). They estimated the $LD_{50/30}$ for mice injected with 0.5 mg/mouse, 15 min before irradiation to be 8.8 Gy as compared to 6.20 Gy in case of untreated controls. Thus a DRF of 1.4 was obtained with only 1% of the toxic dose of MPG. Concentration of MPG less or more than 0.5 mg/mouse gave DMF less than 1.4.

Ever since then, MPG which was used as a detoxicating agent in Japan under the trade name Thiola (proceedings of the second International Symposium on Thiols, 29-30 December 1972, Montego Bay, Jamaica) has undergone extensive research for its radioprotective effectiveness both *in vivo* and *in vitro*.

The radioprotective effects of MPG against various kinds of radiation damages have been compared with other sulphydryl compounds in cultured mouse L cells. They have classified the sulphydryl compounds into three classes according to their toxicity and radioprotective action. Most effective protection was obtained by cysteamine and cysteine, followed by AET and MPG-amide in that order. Toxicity of these sulphydryl compounds were generally observed in the range 0.1-2.0 mM, while they are much less

toxic and effectively radioprotective in higher concentration, especially in case of cysteamine and cysteine. On the other hand, MPG, MPPA, MPPG and 3-MPG were all found to be non-toxic and generally ineffective in protecting irradiated cells, except that MPG in concentrations around 0.02mM and 15 mM and MPPA and 3-MPG around 15 mM had a slight but significant protection.

While working with cultured mammalian L-5 cells, Kawasaki (1977) has shown that MEA, AET and cysteine protected G2 phase but GSH, MPG and thiourea did not. All these compounds, however, have a protective effect on mitotic delay of S phase cells at irradiation. He has further suggested that the difference in action of these drugs is presumably because of different interaction with radiation-induced free radicals.

Radiation-induced DNA damage (Single Strand Breaks) have been shown (Modig 1976) to be protected by both cysteamine and MPG under oxic and anoxic conditions. Protection was larger in the presence of oxygen than in anoxia and MPG was not as good as cysteamine however, the latter was ten times as toxic as the former.

The differential radioprotective effect of MPG in normal and tumour tissues was studied by Urano and Tsukiyama in mammary carcinoma in C3H/He mice (Sugahara & Srivastava 1976). A DMF of 1.19 in air was obtained when the tumour was irradiated locally with 40 Gy X-ray with or without MPG (20 mg/Kg) pretreatment.

Extensive work on radioprotection by MPG against gamma-radiation induced damage to liver (Saini et al. 1977), bone marrow lymphocytes (Saini et al. 1978), thyroid (Uma Devi & Jagetia 1979), testes (Uma Devi & Sahran 1978, Sharan & Uma Devi 1977), small intestine and Jejunum (Uma Devi et al. 1978, Uma Devi 1977, Sharan et al. 1978), thymus (Saini & Uma Devi 1979a, Saini & Uma Devi 1979b, Uma Devi & Saini 1977) and growth inhibiting effects of in utero irradiation in mice (Dev et al. 1982) has been done. In all these cases an enhanced recovery and accelerated restoration of normal structures, counts, etc. were observed in case of MPG-pretreated animals as compared to the controls. It is interesting to mention here that MPG has been also found to protect mouse liver against weak beta radiations from injected tritiated water (Gupta et al. 1979).

It has been demonstrated that MPG releases lesser quantity of glutathione (Revesz et al. 1972) Monstantinova and Revesz (1977) have demonstrated, using mice and Ehrlich ascites tumour cells, that the protective action of MPG is due to increase in glutathione content in cells rather than the presence in cells of the protector itself.

Clinical observations on the radioprotective action of MPG have been made by Tanaka (1972) in cervical carcinoma patients receiving routine radiotherapy. It was observed that 250 mg/patient in 20% glucose given in 15-30 min before irradiation was enough to produce radioprotection. Higher doses did not increase the protective effect. In the MPG treatment, per cent leucocyte count of the patients recovered earlier than in the non-treated group. When radiation was given beyond 20 Gy, the chromosomal aberration was significantly lower. MPG also prevented radiation sickness.

S-2-(3-aminopropylamino) thio phosphoric acid (WR-2721)

WR-2721 was developed by the U S Army Anti-radiation Drug Development Program and supplied by David P Jacobus, Walter Reed Army Institute of Research (USA) to John M Yuhas and John B Storer, who in 1969 reported the diferential protection afforded to normal and malignant tissues by this drug (DRF = 2.6 at 500 mg/kg for haematopoietic death). It was found that injection of WR-2721 (500 g/Kg)

15 min before X-ray exposures increased the $LD_{50/30}$ by 150-170%, increased the X-ray dose required to induce skin ulceration in 50% of the mice by 140%, but increased the dose required to inhibit tumour transplantability by only 15% (Yuhas & Storer 1969). They also reported that the DRF increased with increasing concentrations of WR-2721 starting from 1.59 ± 0.04 for 100 mg/Kg to 2.72 ± 0.04 for 500 mg/Kg. Ever since then, the drug has undergone extensive research as regards its differential protection of normal and malignant tissues, preferential absorption by normal tissues and the mechanism of radioprotection.

With development of the lung tumour-radiotherapy system where both normal and tumour tissues could be assayed in the same animal, it was observed that injection of WR-2721 just before exposures as high as 30 Gy at 0.68 Gy/min, offered no protection to the tumours but increased the radioresistance of the skin and those tissues, which determined survival by factors of 1.6-2.1 (DRF) (Yuhas 1973). However, tumour protection in the same system was observed if the drug was given 1-2 hr before irradiation indicating a time-dependent differential protection by WR-2721. The results supported an earlier report (Yuhas 1972) where WR-2721 doubled the radioresistance of the normal tissues in the radiation fied but did not protect lung tumours.

Echols and Yuhas (1976) reported the ability of WR-2721 to protect mice against hair loss induced by multiple exposures to radiation. In two mouse strains tested, RFM and BALB/C, the relatively low drug dose used (200 mg/Kg) protected the mice against hair loss from single exposures by factors of 1.67 and 1.73 respectively.

Working on rat parotid glands, Sodicoff et al. (1978) reported DMFs of 2.5 for gland weight, 1.7 for amylase concentration and 1.8 for total gland amylase. Extensive work on the normal tissue protection by WR-2721 has been reviewed (Yuhas 1981, 1977) and it has been clearly shown that the drug protects almost all kinds of tissues of the body except the brain and spinal cord. However, the DRF varies from 1.2 to 1.5 for lung, oesophagus and kidney to 2.4-3.0 for bone marrow (tables 3 & 4).

Table 3 *DRFs for WR-2721 in various radiation-injury assay systems*

Radiation-injury endpoint	DRF
Hematopoietic death	2.7
	2.2
Gastrointestinal death	1.8
Pulmonary death	1.7
	1.2
Renal death	1.5
Esophageal death	1.4
CNS death	1.0
Skin injury:	
Epilation	2.0
Dry desquamation	2.4
	2.0
Moist desquamation	2.0
Cell survival:	
Immunocompetent cells	3.5
Colony-forming units	3.0
Tumour injury:	
Transplantability	1.15
Regression	1.0
Control	1.2

All experiments involve the injection of maximum tolerated drug dose 15 min before exposure. (Yuhas 1977)

Table 4

Alkylating Agent	Assay[a]	Factor Increase[b] in Resistance
Nitrogen Mustard	Lethality	2.0
	CFU	2.5
Cyclophosphamide	Lethality	1.4
	CFU	2.6
BCNU	CFU	2.3
Cis-platinum	Nephro-toxicity	1.7-4.6[b]

[a] CFU = bone marrow colony forming units
[b] factor varies with drug doses and number of treatment Table reporduced from Yuhas (1981).

To widen the scope of WR-2721 as a clinical agent for modifying radiosensitivity of biological tissue, studies on protection of normal tissues against toxicity induced by different cancer chemotherapeutic agent were carried out. It was found that injection of WR-2721 for 30 min before injection of nitrogen mustard (HN_2 increased the resistance of mice to HN_2 induced mortality by a factor of 2, but did not alter the sensitivity of the Line 1 lung carcinoma, they bear, to HN_2 induced delay (Yuhas 1979). Similar results have been obtained with other alkylating agents like cyclophosphamide and cis-platinum (Yuhas 1981). It has, therefore, been suggested that WR-2721 could prove to be an effective adjunct to alkylating-agent chemotherapy (Yuhas 1980a). Glover et al. (1986) demonstrated in the Phase I/II trials of WR-2721 and cis-platinum that WR-2721 does not protect against the antitumour efficacy of cis-platinum in man while providing protection against platinum-induced nephrotoxicity and neurotoxicity.

In view of the differential protection by WR-2721, work was done on distribution of WR-2721 in normal and malignant tissues of mice and rats (Washburn et al. 1974) demonstrating that the drug was readily absorbed by most normal tissues and could increase their radioresistance by factors of up to 3. By contrast, solid tumours have been shown to absorb barely detectable quantities of WR-2721 and are, therefore, not radioprotected (Yuhas 1980b). Work was then initiated on the mechanism of selective absorption of WR-2721 by normal tissues. It was initially suggested that normal tissues actively concentrated WR-2721 against a concentration gradient. Whereas solid tumours passively absorb it both *in vivo* (mice and rabbits) and in vitro (1.0 mm^3 of normal and tumour tissue in HBBS or PBS) (Yuhas 1980c). The idea that little or no absorption takes place was earlier assumed (Yuhas 1980b) to be because of poor vasculature of tumours.

Further work on selective absorption of WR-2721 indicated that both tumour cells and RBCs restrict the passage of WR-2721 across their membranes while normal tissues like the liver, allow it to pass freely and to concentrate intracellularly via binding of the drug to endogenous macromolecules (Yuhas 1981).

It has been demonstrated (Yuhas 1982) that WR-2721 is a very hydrophilic drug, and as such it has difficulty crossing the membranes of most solid tumour cells and RBC. He has further found that protective drugs which are less hydrophilic than WR-2721 can pass through both. Since most standard radioprotectors are less hydrophilic, this explains why WR-2721 is the exception to the rule in terms of its selective action. Yuhas et al. (1982) have reported that when the highly dydrophilic WR-2721 was dephosphorylated (WR-1065), the drug became less hydrophilic and could readily cross tumour cell membranes. In addition conventional radioprotectants, such as cysteine and MEA, were shown to be less hydrophilic than WR-2721 and also to cross tumour membranes readily.

While comparing the phosphorylated and nonphosphorylated forms of WR-2721, Yuhas (1981) has shown that the addition of the phosphate group reduced the toxicity of the drug, thereby allowing the administration of larger drug doses and the attainment of higher levels of protection.

Ten years after the first report on radioprotection (Yuhas & Storer 1969), WR-2721 entered clinical trials in USA (Kligerman et al. 1980) and Japan (Sugahara & Tanaka 1980). Kligerman et al. (1980) failed to detect any toxicity following single injections of WR-2721 as high as 500 mg per patient. Further, Sugahara and Tanaka (1980) have observed a 1.6 fold increase in the resistance of head and neck cancer patients to radiation induced stomatitis when they were given 50 mg of WR-2721 30 min prior to each daily irradiation; they also observed

protection against radiation pneumonitis under same conditions. The human pharmacokinetics of WR-2721 were also investigated in 13 cancer patients and was found that WR-2721 is rapidly taken up by tissues and converted to metabolites. (Shaw et al. 1986). Their results suggested that the drug exits the bloodstream rapidly and enters normal tissues where it presumably exerts its protective effects and is rapidly converted to metabolites. Further, studies to reduce the toxicity has been carried out by Fatome et al. (1987). They have demonstrated in mice that the oral administration of WR-2721 in microspheres as compared with that of WR-2721 in suspension in vaseline oil led to a lowering of toxicity and an enhancement of radioprotective activity. Such studies may help in diminishing the side-effects described during clinical studies.

The results of the clinical trials started in USA are currently being discussed. Details of experimental data from phase I trials of WR-2721 involving cancer patients (Glick 1982, Kligerman 1982) were being discussed in the First Conference on Radioprotectors and Anti-carcinogens in Maryland, USA.

Some other results of the phase I trials have been published after a conference on Chemical Modification: Radiation and Cytotoxic Drugs, September 17-20, 1981, Key Biscayne, Florida, USA. Blumberg et al. (1982) have reported that 740 mg/m^2 WR-2721 was well tolerated in a trial involving 65 patients. Side effects included hypotension, hypertension, emesis, somnolence, allergic reactions, nausea and vomiting. Protection by WR-2721 of bone marrow from depression following hemibody irradiation was assessed in patients receiving palliative therapy for widespread symptomatic metastasis on a Phase I/II radiation Therapy Oncology group protocol (Constine et al. 1986). Their data demonstrated a protective effect by WR-2721 on radiation-induced bone marrow depression in humans, which may become more apparent with the use of higher radiation and WR-2721 doses (Constine et al. 1986). Final report of the phase I protocol for the initial clinical study of multiple dose WR-2721 with radiotherapy has already been published by Kligerman et al. (1988). They found that the maximum tolerated dose established by this study is 340 mg/m^2 given 4 days a week for 5 weeks. The drug is delivered intravenously in 7 min. There were no long term blood chemistry changes. There were no deaths due to the administration of the radioprotector. Preliminary results of phase I trials (Glick et al. 1982) also indicated that (1) WR-2721 may provide protection to normal tissue when given before cyclophosphamide or nitrogen mustard although not of clinical benefit, (2) there is no protection against anti tumour activity of alkylating agents nor does there appear to be increased acute toxicity whenit is combined with alkylating agent chemotherapy, (3) no protection against platinum induced toxicity.

As regards the mode of action of WR-2721, it has been suggested that it acts by inducing hypothermia (Pittock et al. 1982), enhancing the DNA repair process (Riklis 1980), binding with those regions of DNA which are left uncovered by histones (Brown 1967, Modig 1976, Riklis et al. 1982) and by binding/shielding the enzyme active site (Hahn et al. 1975).

Thus considering the toxicity and DMF values, it is obvious that only MPG and WR-2721 have been found suitable for clinical testing. A comparative account of some important natural and synthetic radioprotectors has been shown in table 5. It has been reported that for a DRF of 1.4, roughly 50% of the toxic dose of cysteamine, 14% of WR-2721 and 1% of MPG has to be used (Sugahara & Srivastava 1976). This indicates that MPG is better from the point of view of toxicity and WR-2721 from the point of view of DRF.

In spite of the extensive literature on radioprotection by WR-2721 and MPG,

Table 5 *Chemical radiation protection in mouse and man*

		MOUSE		MAN			
Compound	Biological features	Toxicity LD_{50} mg/kg	Protective dose	DRF based on $LD_{50}(30)$	Administered dose (mg)	Protection Leucopenia	Against Chromosome aberration
Cysteine	Natural	1500	1200 I.V.	1.42	Notrial		
Glutathione	Natural	4000	4000 I.P.	1.28	200	Yes	—
			1600 I.P.	1.12			
Cysteamine	Toxic	275	150 I.P.	1.45	200-400	Yes	—
AET	Toxic	690	400 I.P.	2.15	100-200	Yes	—
WR-2721	Toxic	A/J 554	80 I.P.	1.47			
			400 I.P.	2.78			
		BALB/cJ 784	56 I.P.	1.35	Notrial		
			120 I.P.	1.55			
			400 I.P.	2.44			
MPG	Non-toxic	2100	20 I.P.	1.40	250	Yes	Yes

(Sugahara & Srivastava 1976)

doubts have been cast on their capacities as radioprotectors, in isolated reports. It has been reported that whereas AET at half the recommended dose for radioprotection increased the survival of mice after 10.60 Gy of gamma irradiation, MPG treatment either alone or in combination with AET failed to do so (Ghose & Srinivasan 1980). Pant and Ghose (1981) have also shown that not only MPG does not protect the depression in RBC level in peripheral blood after gamma-ray exposure, it also hindered partially the protection rendered by AET. Failure of MPG to protect mice against gamma-ray induced mortality has also been reported (Uma Devi et al. 1979).

The less protective effect of MPG could be due to the after effect of radiation. Ayene and Srivastava (1985) have demonstrated the radio-sensitization by MPG/Fe complex formation in microsomes. Enhancement of lipid peroxidation was observed at 0.1 mg/ml of MPG instead of protection (figure 1). However, MPG in the presence of EDTA gave significantly more protection at all doses of radiation (figure 1). The complex formation was further confirmed by the exogenous supply of ferrous sulphate during irradiation of microsomes. The addition of Fe ions enhanced the formation of lipid peroxides. A further increase in lipid

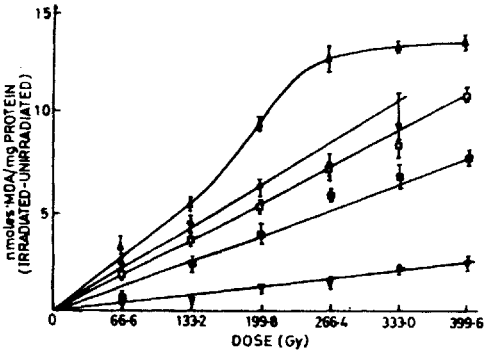

Figure 1 Effect of EDTA (0.2 mg/ml) and MPG on the radiation-induced lipid peroxidation of microsomes (0.3 mg protein/ml) at various doses of γ-rays. Lipid peroxidation of microsomes without MPG (○——○), lipid peroxidation of microsomes with 0.1 mg/ml of MPG (△——△) and 0.03 mg/ml of MPG (■——■), lipid peroxidation of microsomes with EDTA (0.2 mg/ml) (□——□), lipid peroxidation of microsomes with both EDTA and MPG (△——△). (from Ayene & Srivastava 1895)

peroxides was observed in the presence of MPG with an abrupt increase up to a dose of 133.2 Gy and a slight increase at higher doses of radiation (figure 2). Similarly greater protective effect of MPG was observed in erythrocytes in absence of MPG/Fe complex

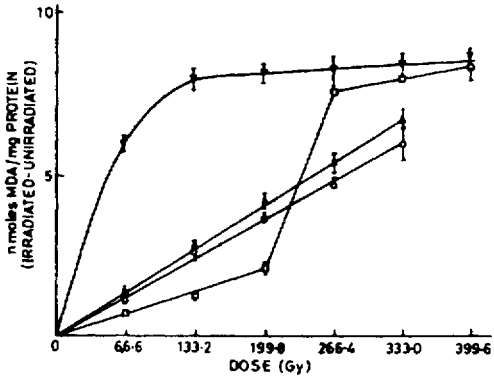

Figure 2 Effect of $FeSO_4$ (0.1 μg/ml) and MPG (0.1 mg/ml) on the radiation-induced lipid peroxidation of microsomes (0.5 mg protein/ml) at various doses of γ-rays. Lipid peroxidation of microsomes without MPG (○——○), lipid peroxidation of microsomes with 0.1 mg/ml of MPG alone (□——□), lipid peroxidation with $FeSO_4$ (0.1 μg/ml) alone (△——△), lipid peroxidation of microsomes with both $FeSO_4$ and MPG (△——△) (from Ayene & Srivastava 1985)

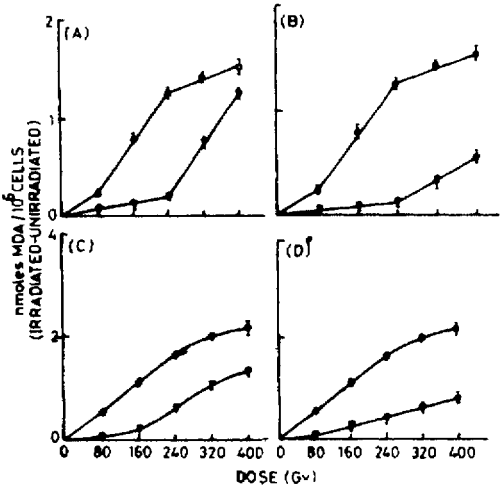

Figure 3 Effect of MPG (**A** and **C**: 0.2 mg/ml; **B** and **D**: 0.5 mg/ml) on radiation-induced lipid peroxidation of erythrocytes (**A** and **B**: 8.6 × 10^5 cells/ml; **C** and **D**: 4.3 × 10^5 cells/ml) at various doses of γ-rays (Erythrocytes only, ○——○; + MPG ▼——▼) (from Ayene et al. 1988)

formation (Ayene et al. 1988). Figure 3 shows that MPG rendered protection to erythrocytes of high concentration at both concentrations used. It also exhibited a similar effect in erythrocytes of low concentration but with a variation in the degree of protection. Further, the data on the effect of $FeSO_4$ provided a clear picture about the role of Fe^2 in the enhancement of lipid peroxidation in presence and absence of MPG. The data in figure 4 demonstrated that both the spontaneous and radiation-induced lipid peroxidation of erythrocytes were increased by $FeSO_4$. The results also showed the enhancement of radiation-induced lipid peroxidation by MPG in the presence of $FeSO_4$. Such complex formation may be responsible for the less protective effect of MPG (DMF = 1.4) in mice.

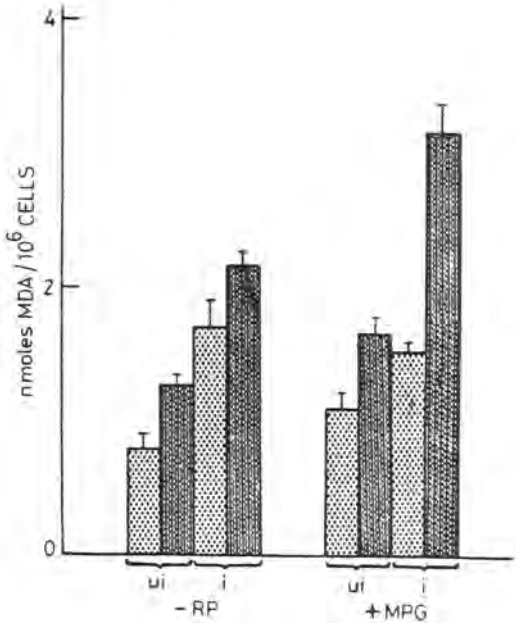

Figure 4 Effect of MPG (0.5 mg ml) on lipid peroxidation of erythrocytes (4.3 × 10^5 cells ml) in presence (wavy lines) and absence (dotted panels) of $FeSO_4$ (0.02 mg/ml) (Ui = unirradiated; i = irradiated at 135 Gy; RP radioprotective drug). (from Ayene et al. 1988)

However, experimental verification of this point is yet to be done in in vivo systems.

In our recent study it has been shown that if MPG is delivered by entrapping it with liposome then liposomes can be targeted to a particular tissue by linking specific antibodies on to the surface of the liposome. Because MPG may act as radioprotector as well as sensitizer, liposome mediated delivery system may after several advantages over the conventional mode administration (Sharan et al. 1992). These results open the possibility to develop a more effective MPG or any other drug delivery system using much lesser amount of the drug and hence reducing the toxic effects of toxic drugs which have a high DMF. Further work in this area is being continued.

Some discrepancies on WR-2721 also indicated that caution be adopted in the understanding of conditions in which thiol compounds might protect or fail to protect, tumours relative to normal tissue (Lunec et al. 1981). Reports on failure of WR-2721 to exhibit differential radioprotection of normal and tumour tissues have also appeared (Rojas & Stewart 1980, Rojas et al. 1982, Clement & Johnson 1982, Milas et al. 1982). Phillips et al. (1973) had also failed to substantiate the very low protective effect on tumours by WR-2721 reported by Yuhas and had shown a wider variation in protection afforded to normal tissues than was previously reported. The importance of dephosphorylation of WR-2721 in the protective efficiency of WR-2721 has been demonstrated in erythrocytes (Ayene & Srivastava 1989). Figure 5 & 6 indicated the protective and non-protective effect of WR-2721 in microsomes and erythrocytes. The results in erythrocytes was suggested due to the inadequate amount of dephosphorylating enzymes. This was confirmed by the addition of microsomes to erythrocytes that reduced the radiation damage of the erythrocytes (table 6).

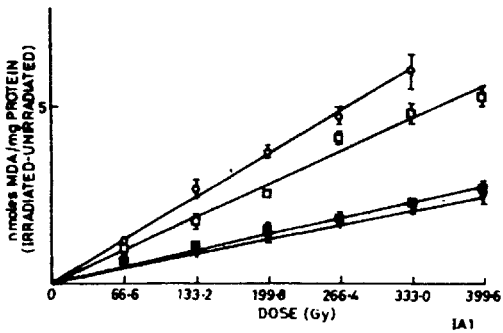

Figure 5 Effect of EDTA (0.7 mmol/dm^{-3}) and WR-2721 on the radiation-induced lipid peroxidation of microsomes (0.5 mg protein/ml) at various doses of γ-rays. Microsomes only (○); + 2.2 mmol dm^{-3} of WR-2721 (■); + EDTA (0.7 mmol dm^{-3}) (□); + EDTA + WR-2721 (△). (from Ayene & Srivastava, 1989)

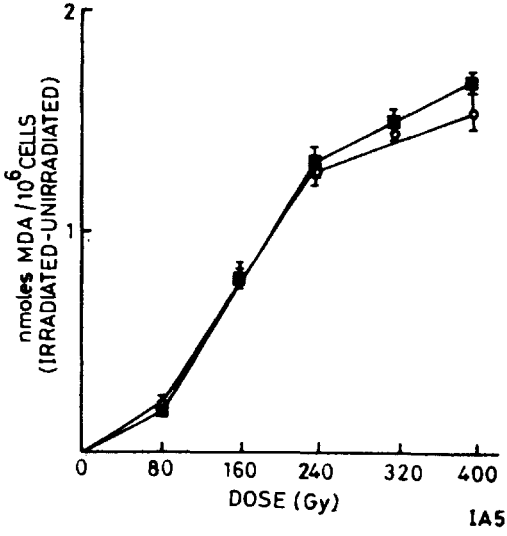

Figure 6 Effect of WR-2721 (0.9 mmol dm^{-3}) on the radiation-induced lipid peroxidation of erythrocytes (8.6 × 10^5 cells/ml) at various doses of γ-rays. Erythrocytes only (○); + WR-2721 (■). (from Ayene & Srivastava 1989)

Such dephosphorylation was demonstrated in the presence of dephosphorylating enzyme-Alkaline Phosphatase (Collobro Jones et al. 1985, Mishra et al. 1988, 1989). Research has also been carried out to study and regulate the

Table 6 *Effect of WR-2721 (0.5 mg/ml) on the lipid peroxidation of erythrocytes (4.3 × 10^5 cells/ml) in presence or absence of microsomes (0.3 mg protein/ml)*

Lipid peroxidation	−WR−2721	+WR-2721	−WR−2721	+WR-2721
	Unirradiated		Irradiated (135 Gy)	
Erythrocytes nmoles/10^6 cells	0.76±0.24	1.9±0.18	2.66±0.45	3.42±0.46
Microsomes nmoles/mg protein	2.34±0.16	3.72±0.38	8.32±0.78	9.36±0.78
* Erythrocytes + microsomes nmoles/10^6	2.04±0.22	2.85±0.25	11.78±0.84	5.70±0.74

* In tables VIII, the data was expressed on the erythrocytes basis and the correction was done for the peroxidation in microsomes alone by substracting mirosomes alone from erythrocytes + microsomes. (Ayene & Srivastava, 1989)

metabolism, toxicity and radioprotection of WR-2721 by an inhibitor of alkaline phosphatase-levamisole (Brown et al. 1986). Research in the direction of inhibiting the alkaline phosphataese activity in tumour cells may further enhance the chances of using WR-2721 in the differential protection of normal against the cancer cells.

In any case the scope of the use of radioprotective drugs in clinical radiotherapy has widened by reports (in tissue culture, animal and human systems) on the combined use of radioprotectors and radiosensitizers to improve the efficiency of cancer therapy, some of which have shown promising results (Yuhas et al. 1977, Yuhas & Li 1978, Koch & Howell 1981, Grigsby & Maruyama 1982).

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