Interactions between drugs and chemicals in industrial societies.
G.L. Plaa, P. du Souich, S. Erill, editors.

RADIOPROTECTIVE DRUGS

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INTRODUCTION

Radioprotection

The term radioprotection is generally used in connection with protection by means of a drug or chemical against the damaging effects of radiation exposure. The use of a similar term, radiological protection, is confined to matters to do with avoiding or minimizing radiation exposure of personnel. Radioprotective compounds have long been the subject of research both as an important topic in the study of biological effects of radiation and with a view to their possible military application in the protection of personnel against exposure to nuclear weapons attack. It is the latter application that has resulted in the development of many new radioprotective compounds with potential or actual application to man. These developments have in turn aroused interest in their possible clinical uses for improving the efficacy of radiotherapy in certain situations. Before discussing radioprotective compounds further, there follow a few general remarks about radiation effects.

Biological effects of radiation

Ionizing radiations, for example X-rays, γ-rays, alpha particles and neutrons, are highly efficient at producing certain biological effects (1). The most sensitive cellular effects involve damage to the genome and these are cell kill and mutation. Cell kill is important in relation to certain short- and medium-term risks of exposure of individuals to radiation and in the use of radiation in the treatment of cancer. Mutations are important in relation to longer-term risks such as the induction of cancer or of genetic defects by radiation exposure. All these effects arise from damage at the cellular level and are thought to involve lesions induced in, or close to, the DNA.

Free-radical nature of radiation damage.

The critical lesions are known to be produced by a chain of free-radical reactions induced locally by the radiation-induced ionizations, each of which is in itself an initiating free-radical event. An important aspect of the involvement of these free-radical processes is that the amount of damage produced by a given dose of radiation is strongly dependent on the presence within the cell of various agents which have a high degree of reactivity with free radicals.

Chemical modification of radiation damage

In general, chemically reducing agents that are present within the cell, for example thiol compounds, have a terminating effect on the damage-inducing reactions and therefore act as radioprotectors. Conversely, oxidizing agents, especially oxygen itself, act as propagators of these reactions and have a radiosensitizing effect (2). By manipulating the levels of such agents, it is possible to alter the effectiveness of radiation over a range of approximately 3 to 1. Thus about two thirds of the overall damage occurs through radiation-induced free-radical reactions which are susceptible to chemical modification. The remaining one third of the damage appears not to be modifiable by these means and it is this that sets a limit to the degree of radioprotection that can be obtained.

THEORIES OF RADIOPROTECTIVE ACTION

Various theories have been advanced to explain the actions of radioprotectors. It is generally agreed that any understanding of radioprotective phenomena and their association with chemical reduction has to be viewed together with the association between radiosensitization and oxidation. Thus chemical modification of radiation response is achieved by shifting the balance between oxidizing and reducing equivalents within the cell (2, 3).

Hydrogen donation

It was postulated in the early 1950's that a radical formed on a target molecule could be chemically repaired, or restituted, by H transfer from another molecule (4). These ideas recognized that the repair could be blocked if sufficient oxygen were present to have a high probability of reacting with the target radical before its repair. It was soon demonstrated that this concept did appear to explain the radiosensitizing action of oxygen in bacteria (5). Later, the kinetics of the repair reaction were measured (6).

The competing repair and fixation reactions can be represented as

$$T^{\bullet} + RSH \text{ --- } TH + RS^{\bullet} \qquad (1)$$

$$T^{\bullet} + O_2 \text{ --- } TO_2^{\bullet} \qquad (2)$$

Here $T^{\bullet}$ represents a radical site formed by radiation on an important target molecule, T. RSH represents free sulphydryls present within the cell which are capable of chemically reducing or repairing $T^{\bullet}$ to a less damaging or even to its undamaged form, TH. Such a reaction is energetically favoured by the relative weakness of the S-H bond compared with the C-H bond. In addition to free sulphydryls, other reducing compounds, such as vitamins C and E and reduced nicotinamide adeninine dinucleotide (NADH) may participate in these repair processes. Although written as hydrogen transfer in equation (1), repair could equally be by electron transfer to the target radical. The competing damage

fixation reaction, equation (2), is shown as a peroxidation, although this could also be an electron transfer from the target radical to form $O_2^{-\bullet}$ and T^+. Oxidizing molecules other than O_2, for example electron-affinic compounds and nitroxyls can also fix damage as in equation (2) (1-4). Kinetic evidence supporting reactions (1) and (2) has been obtained by the pulse radiolysis of model chemical systems (7). Evidence that these reactions do indeed apply at the cellular and DNA levels has been obtained kinetically using fast mixing and irradiation techniques (8, 9).

Induction of hypoxia

In addition to thiols and oxygen competing for radical damage sites as described above, thiols react with oxygen itself. This leads to the formation of disulphides and to the consumption of dissolved oxygen. In situations where there is little oxygen available the addition of thiols could cause hypoxia and in this way produce radioprotection. This concept has long been recognized (10) and more recent work shows that it could be relevant to the actions of thiol-based radioprotective drugs (11). There is evidence, at least in rodents, that the levels of oxygen present in many tissues, although just high enough for respiration, are sufficiently low that a state of mild hypoxia exists, such that the radiation responses of these tissues are less sensitive than with a full supply of oxygen (12). Thus even a modest consumption of available oxygen by reaction with thiol-containing radioprotective drugs could lead to a decrease in radiation sensitivity.

Radical scavenging

As described above, there are two main theories currently held to explain the action of thiol-based radioprotectors and both are related to oxidation and reduction reactions which modify the nature of radicals after they have been produced on the critical target of the cell. Another mechanism of radioprotection should be mentioned and this is radical scavenging. Agents which are believed to protect by this mechanism include various alcohols and glycols and dimethyl sulphoxide. As with thiol-based compounds, their radioprotective action is generally greater in oxically than in hypoxically irradiated cells. It is believed that they act by scavenging various primary radicals, especially those derived from water ($H^\bullet$, $OH^\bullet$ and e^-_{aq}) before they attack the critical target molecules to produce secondary radical damage sites on them. Radioprotectors of this type have to be present in high concentration (ca. 1 mol dm^{-3}) to show an appreciable effect, whereas thiol-based compounds are effective at much lower concentration (ca. 1 mmol dm^{-3}). This is because the lifetimes of the primary radicals are much shorter (13) than those of the secondary radicals that they produce in the target (6-9).

DEVELOPMENT OF RADIOPROTECTIVE DRUGS

Background

The pioneering work of Patt and colleagues (14) demonstrated that an injection of cysteine prior to irradiation protected rats against radiation-induced haematopoietic death. This result stimulated the search for other radioprotective compounds and lead to the finding that a number of thiols were effective protectors *in vivo* and *in vitro*. It was recognized that aminothiols were particularly effective provided there were not more than 3 carbon atoms separating the thiol and amino groups (15). The requirement for a thiol group is explainable, as described above, in terms of its known free-radical reactivity and ability to participate in chemical repair reactions (2-4,7). However, the function of the amino group, or groups, is less clear. It has been proposed that they enable the protector molecules to bind to and stabilize the DNA and facilitate enzymatic repair (16). A more recent and attractive proposal is that amino groups enable the protector to bind to DNA in a manner similar to that of polyamines, making the radioprotective SH function more effectively available to the DNA (17, 18). An alternative explanation is that the presence of one or more amino groups influences the hydrophilicity of the protector in such a way that it can readily cross cell membranes due to the formation of neutrally charged zwitterionic forms at or near to the isoelectric pH (19).

Synthesis and evaluation

The main effort in the development of radioprotective drugs has been to identify compounds that could be used to protect military personnel against the lethal and functional effects of exposure to radiation from nuclear weapons. From 1959 to 1973 the U.S. Army Medical Research and Development Command sponsored an Anti-radiation Drug Development Programme and a summary of the results has been published (20). The technical part of the programme was carried out by the Walter Reed Army Institute of Research and consequently the identification numbers of the compounds tested are preceded by the letters WR. Approximately 4400 compounds were synthesized and tested in mice. The very best compounds found were ultimately tested in dogs and/or monkeys. New drug applications were made on five compounds and limited human tolerance studies were conducted on three of these.

The main thrust of the programme was on aminothiols and their derivatives. Over 1500 other compounds were tested, but no new classes of protectors were found. Thus the aminothiols remained the best compounds for effect, although the function of the amino group, or groups, is not as certain as that of the thiol group, as described above. The programme was originally designed to explore, in a straightforward manner, the structure-activity relationships for

a range of compounds in respect of their radioprotective actions and toxicities in higher species. This simple medicinal chemistry approach proved unsuccessful because the properties of the compounds were found not to vary in a logical way with structure but to be highly specific and unpredictable.

It was considered in the programme that the aminothiols and their derivatives were the only compounds offering "true" radioprotection against free-radical damage and that, even so, their mechanism of action could involve the production of radiobiological hypoxia, as described above. It was recognized that the maximum protection factors likely to be achieved were about 2-3.

In the development programme a wide range of structures and substituents was tested. An important example of this was the finding that activity and toxicity could be controlled by blocking the thiol group with a covering function, The covering functions were designed to be cleaved in vivo to release a free thiol group. Of over 50 different covering functions tested, the most interesting proved to be thiosulphate, phosphorothioate, disulphide and thiazolidine. The relative effects of the thiol-covering functions were found not to be systematic and varied according to the common structure of the series of compounds in which they were tested. Examples of the effects of these covering functions in one series of compounds are shown in Table 1 (20).

TABLE 1

THIOL-COVERING GROUPS

2(3-Aminopropylamino)ethanethiol and Derivatives. Effect of Varying the Thiol-covering Group (ref. 20).

WR No.	Drug Structure	Approximate LD_{50} m mol/kg	Drug Dose m mol/kg	Radiation Survival (%)
1065	$H_2N(CH_2)_3NHCH_2SH$	1.68	1.12 0.56	85 0
33278	$[H_2N(CH_2)_3NHCH_2CH_2S-]_2$	0.52	0.24	0
9720	$H_2N(CH_2)_3NHCH_2CH_2SSO_3H$	3.50	2.33 1.17	0 0
2721	$H_2N(CH_2)_3NHCH_2CH_2SPO_3H_2$	4.76	2.80 1.40 0.70 0.35	100 100 40 20

The data in Table 1 show that the phosphorothioate group as a thiol-covering function gave the lowest toxicity (LD_{50} = 4.76 m mol/kg) and the highest radioprotection (100% 30-day radiation survival of the mice at 1.4 m mol/kg and 40% at 0.7 m mol/kg). It is interesting to note that WR 2721 is the best radioprotector that was found in the U.S. Army programme. Its dephosphorylated and active thiol form, WR 1065, is also shown in Table 1. Studies have been made of the dephosphorylation processes (21, 22).

RADIOPROTECTIVE DRUGS AND RADIOTHERAPY

WR 2721 continues to be the most widely studied and effective radioprotective drug although efforts to find compounds with better activity and/or lower toxicity have been continued (23). The interest here is in the possibility that there may be a therapeutic benefit to be gained from the use of radioprotectors in conjunction with radiotherapy. This stemmed from work showing that in mice there was a greater radioprotection of certain normal tissues by WR 2721 than of an endpoint of tumour transplantability (24). This work suggested a favourable differential protection of normal tissues rather than tumours which might be exploited to improve the effectiveness of radiotherapy. To achieve a benefit in terms of improved tumour control would require an increase in the radiation treatment dose - and this benefit would be offset by any radioprotection by the drug of the tumour. Any increase in the radiation treatment dose would carry the grave risk of an unacceptable increase in normal tissue damage and this risk would only be overcome if it were certain that the drug would act at or above the minimum level of protection expected in all the normal tissues necessarily exposed in the radiation treatment field. In other words, the use of radioprotector drugs with radiotherapy is not "fail safe" for normal tissue damage. Radiotherapy is normally fractionated into daily treatments given over a number of weeks and the radioprotector should ideally be administered at each fraction. Unfortunately, the dose of drug that can be tolerated in these multiple treatments is appreciably less than for a single administration (26). Nevertheless, in the first clinical tests of WR 2721 on patients with head and neck tumours, using daily treatments, significant radioprotection of oral mucosa appears to have been obtained (27).

THE CRITICAL ROLE OF OXYGEN TENSION

As indicated earlier, chemical radioprotection involves a shift in the balance between oxidizing and reducing equivalents in the cell (2,3). It follows that a given amount of protector would be expected to have less effect if the cell were fully oxic or hypoxic than if it were in an intermediate state of oxygenation and therefore more sensitive to a shift in the balance. This

has been demonstrated in mouse skin using a clone assay to measure survival (and therefore radiation sensitivity) of irradiated basal cells (28). Mice were given 400 mg/kg of WR 2721 and were made to breathe various concentrations of oxygen during irradiation. Results from two series of experiments at different radiation intensities but at similar dose levels are shown in Figure 1.

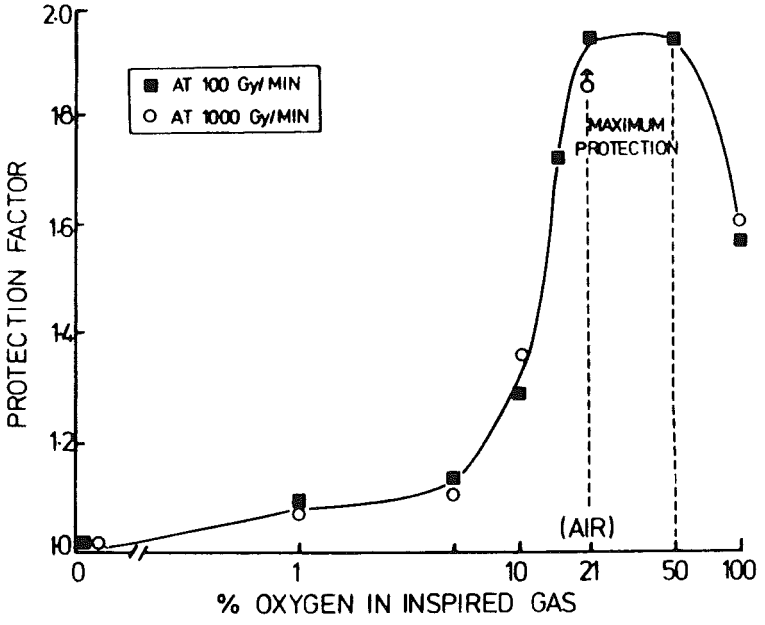

Figure 1 Radioprotection of mouse skin by 400 mg/kg of WR 2721 as a function of concentration of oxygen breathed. Note maximum protection for 21-50% oxygen, no protection for 0% (hypoxia). (ref. 28).

When the mice were supplied with oxygen at 21 and 50 percent concentration the radioprotective effect of WR 2721 was at a maximum but fell at oxygen concentrations above and below this range. This confirms the idea of the protector operating best close to the balance point (or radiobiological "K-value") between oxic and hypoxic conditions. It is also consistent with the finding that in rodents breathing air most normal tissues are in a state of mild hypoxia (12). Clearly, tissue oxygen tension is a major determinant of radioprotector effectiveness.

SUMMARY

Thiol-based compounds remain the most effective radioprotectors known to work in animals and man. The maximum protection factor likely to be achieved with these compounds is 2-3 and indeed such protection has been demonstrated in animals. The maximum protection factors that have been observed in man are much less than this, even using the best drug currently available, WR 2721 (aminopropylaminoethylphosphorothioic acid). Drug toxicity limits the dosage and, therefore, the protection factor that can be achieved. Blocking the SH group generally reduces toxicity and phosphorothioate is one of the best covering functions.

Thiol-based compounds probably operate by two mechanisms: chemical repair by the SH group of free-radical damage and induction of tissue hypoxia. Tissue oxygenation is a major determinant of radioprotector drug action. Aminothiols are the most effective radioprotectors, but the exact function of the amino group, or groups, is unclear. Radioprotective action is lost if there are more than three carbon atoms between the latent thiol group and its associated amino group.

ACKNOWLEDGMENTS

The author would like to acknowledge the support of the Cancer Research Campaign and to thank Dorothy Woodman for preparation of the manuscript.

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Discussion - Radioprotective drugs

F. Planas-Bohne

I have two questions. When do you have to administer the protector, because radicals will react with other substances within seconds, and do the surviving animals develop late effects such as leukemia?

B.D. Michael

The protector has to be present at the time of irradiation, and as far as late effects are concerned, one would expect them to appear in animals surviving, whether or not they had received a radioprotective drug. However, I believe that some radioprotection against carcinogenesis can be achieved.

B.P. Schmid

Have you done any mutagenicity studies to see whether there is some protective effect against mutational events?

B.D. Michael

I have not done them, but I can mention that some protection is achieved. However, the degree of protection is usually rather less than that achieved on mortality.

L.F. Prescott

I wonder if there is any correlation between the activity of these radioprotective agents and other thiols that offer protection against chemical alkylating tissue injury?

B.D. Michael

There is probably some. WR 2721 shows a differential uptake into normal tissues rather than tumors and as a consequence it has been tried in association with cyclophosphamide with some interesting results, but I do not have more extensive information.

F.J.C. Roe

Have any of these compounds been tried in people such as uranium miners or other subjects exposed to natural radiation?

B.D. Michael

There has been quite a lot of interest in whether protectors could be used in these environmental exposures, but the general feeling is that the toxicity of the products now available precludes their continued use at the high doses that are necessary.

G. Zbinden

All of the studies that you mentioned were done with X rays. Are they representative of other types of ionizing radiation?

B.D. Michael

No, they are not. In general, radioprotection is more difficult to achieve with the more densely ionizing radiations such as neutrons and alpha particles. Another factor is that the dependence on available oxygen varies with the type of radiation.

J.V. Castell

What is the toxicity of the radioprotective agents that have undergone clinical trials?

B.D. Michael

In the case of WR 2721 it is mostly vomiting, but hypotension may also occur. Altogether the limitation in dosage due to side effects makes that only protection factors of the order of 1.5 can now be achieved in man.

P. Moldeus

What is the general mechanism of oxygen potentiation of radiation?

B.D. Michael

There is currently a great deal of interest in active oxygen species in various fields, but the evidence that active oxygen species are involved in oxygen potentiation of radiation effects is rather slim. Broadly, it looks like oxygen potentiates radiation by competing with and blocking reductive radical repair or restitution processes within the cell. O_2^- may contribute, but it seems that it produces only a very small fraction of the whole

potentiating effect.

J. Brodeur

Turning around the matter of your presentation and no longer talking about radioprotection but about radiosensitivity, is it right to assume that reduction in endogenous thiols will eventually lead to increased radiosensitivity? Has this approach been tried in the treatment of tumors?

B.D. Michael

Well yes, there is a lot of interest in the possible use of agents, such as buthionine sulphoximine, which reduce the levels of endogenous thiols in the treatment of cancer by radiation. Broadly, an aspect which has to be looked at is the oxygen status of the tissues involved. Tumors are generally regarded as being more poorly oxygenated than normal tissues. Decreasing the level of endogenous thiols by buthionine sulphoximine increases the radiosensitivity of tissues to a modest extent. The sensitization factor that could be achieved in a clinical setting could be of the order of 1.1 or 1.2. However, in the better oxygenated tissues the sensitization factors could be somewhat larger than in poorly oxygenated tumors.

P. du Souich

You have shown that moderate hypoxemia offers some radioprotection, and it is known that moderate hypoxemia increases reduction reactions. Can this be involved in the radioprotection?

B.D. Michael

Yes, it seems likely, particularly from what we know about the increase in radiosensitivity produced by oxidizing agents. They work by competing with and blocking the reduction reactions of gluthatione and similar substances.