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RADIOPROTECTIVE AGENTS

There has been substantial progress in the area of radioprotective agents since the early 1980s, especially in terms of the biological and mechanistic evaluation of the large number of compounds synthesized prior to that time. This information has also begun to direct the development of a new generation of derivative compounds. Advances have been made in understanding the fundamental mechanisms of chemical radioprotector action, an area long dominated by thiol compounds (see Thiols). Nonthiol protectors, including protease inhibitors, vitamins (qv), metalloelements, and calcium antagonists, are playing a larger role as of the mid-1990s. There has been an explosion of interest in biological, as opposed to chemical, modifiers of radiation injury. Some of these biologics act best when given prior to irradiation; many can modulate radiation injury when given after irradiation, presumably by affecting the recovery and repopulation of critical tissue elements. Biologics thus afford an opportunity for therapeutic intervention following accidental radiation exposure (1) as well as in radiation therapy (XRT). Demonstrations of a potential therapeutic advantage from combining chemical radioprotectors, which decrease the extent of initial damage, and nonthiol biologics, which accelerate tissue recovery, provides an attractive approach to radioprotection (2).

1. Thiols

Some important radioprotective thiols are listed in Table 1. The most effective compounds have a sulfhydryl, –SH, group at one terminus and a strong basic function, usually an amino group, at the other. The general structure of these aminothiols is $H_2N(CH_2)_xNH(CH_2)_ySH$, where x is optimally 3 and y is optimally 2 or 3. Because of lower toxicity, clinical interest has focused on pro-drugs in which the –SH group has been derivatized. Phosphorothioates such as WR-2721, WR-3689, and WR-151327 are the most practicable (3). These pro-drugs are dephosphorylated by enzymes such as alkaline phosphatase, generating the corresponding active free thiols WR-1065, WR-255591, and WR-151326. Initially developed for military applications, the emphasis for the thiols (qv) has shifted to their potential use in XRT to protect against damage to normal tissues, based on reports that concentrations of WR-2721 that are relatively well tolerated by mice can protect normal tissues more than tumors (4).

1.1. General Mechanisms of Radioprotection by Thiols

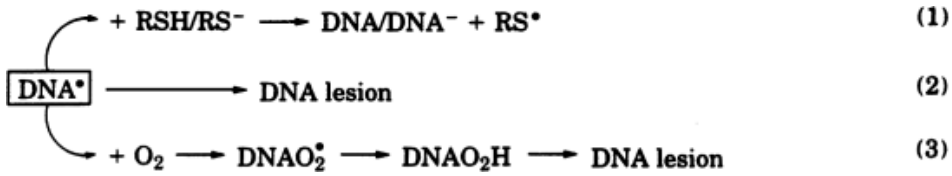
Thiols protect mammalian cells from radiation effects primarily by reducing the severity of the initial damage inflicted to genomic deoxyribonucleic acid (DNA). The hydroxyl radical, $OH^{\bullet}$, is a primary cause of damage to cellular DNA. Thiols, RSH; thiolate anions, RS^-; and disulfides, RSSR; react rapidly with $OH^{\bullet}$ and may thus protect cells by scavenging $OH^{\bullet}$ before this radical can react with DNA. Scavenging of secondary radicals may also contribute to protection. Thiols may also enhance cell survival by chemically repairing DNA radicals caused both by $OH^{\bullet}$ and by the direct ionization of DNA. Either an H atom, $H^{\bullet}$, or an electron can be transferred from the thiol to a DNA radical (see eq. **1**). These reactions are embodied in the fixation-repair model, the cornerstone

Table 1. Radioprotective Thiols and Phosphorothioates

Compound[a]	CAS Registry Number	Structure
Thiols		
dithiothreitol (DTT)	[27565-41-9]	$HSCH_2CH(OH)CH(OH)CH_2SH$
2-mercaptoethanol (WR-15504)	[60-24-2]	$HOCH_2CH_2SH$
cysteamine (MEA, WR-347)	[156-57-0]	$H_2NCH_2CH_2SH$
2-((aminopropyl)amino)ethanethiol (WR-1065)	[31098-42-7]	$H_2N(CH_2)_3NHCH_2CH_2SH$
WR-255591	[117062-90-5]	$CH_3NH(CH_2)_3NHCH_2CH_2SH$
WR-151326	[120119-18-8]	$CH_3NH(CH_2)_3NH(CH_2)_3SH$
Phosphorothioates		
WR-638	[3724-89-8]	$H_2NCH_2CH_2SPO_3H_2$
WR-2721	[20537-88-6]	$H_2N(CH_2)_3NHCH_2CH_2SPO_3H_2$
WR-3689	[20751-90-0]	$CH_3NH(CH_2)_3NHCH_2CH_2SPO_3H_2$
WR-151327	[82147-31-7]	$CH_3NH(CH_2)_3NH(CH_2)_3SPO_3H_2$

[a] WR = Walter Reed Army Institute of Research (4).

of which is the hypothesis that, in the absence of oxygen, DNA radicals, which are presumed to be responsible for radiation lethality, are either inherently irreparable (eq. **2**); can be chemically repaired, usually by reaction with a thiol (eq. **1**); or can be fixed by a competing reaction with oxygen (eq. **3**). Direct evidence for such a competition between oxygen and thiols for DNA radicals in mammalian cells has come from fast-kinetic studies (5).



A variety of additional factors are considered in derivatives of this model, such as fixation by species other than oxygen, repair by species other than thiols, and subsets of DNA radicals that do not react with oxygen or thiols (6). In addition to increasing the cells' capacity for $OH^{\bullet}$ scavenging and chemical repair, thiols can undergo autooxidation, resulting in the depletion of oxygen and a decrease in O_2-fixation reactions. Although oxygen depletion is unlikely to represent a general mechanism for radioprotection *in vitro,* it may be a significant factor in tissues and tumors *in vivo* where oxygen levels are much lower.

The outcome of rapid radiation chemical processes in mammalian cells is to cause a variety of longer-lived physical alterations in the DNA. Of these, double-strand breaks (DSBs) appear to be most frequently involved in cell killing if not correctly repaired. In general, thiols protect against DSB induction in proportion to their effect on cell killing (7), although there are exceptions (8).

1.2. Modulation of the Killing of Mammalian Cells by Thiols

Important aspects of the effects of exogenous thiols on clonogenic cell survival following exposure to low linear energy transfer (LET) radiations include the following.

(1) Thiols must be added before or within a very short time after irradiation to protect against cell killing. This is apparent from conventional cell survival data (9) but is even better illustrated by kinetic studies showing that 2-mercaptoethanol (see Table 1) protects oxic V79 cells when added just before but not 7 milliseconds after irradiation (5).

(2) Subcellar distribution may be an important aspect of the biological activity of thiols. Cationic thiols such as MEA and WR-1065 protect cultured cells from radiation injury at much lower concentrations than the uncharged thiols such as 2-mercaptoethanol and DTT. This behavior cannot be explained by differences in the efficiency of $OH^{\bullet}$ scavenging or chemical repair (10). The variable term may be the thiol concentration close to the DNA, where cationic thiols could be sufficiently concentrated to scavenge $OH^{\bullet}$ effectively and/or to participate in chemical repair. Uncharged thiols should be neither condensed nor excluded, whereas anionic thiols such as glutathione [70-18-8] (GSH) should be excluded from the vicinity of the DNA. A variety of experimental data are consistent with this counterion condensation model (10, 11), although other studies suggest that GSH is inherently limited compared with MEA in its ability to protect against DSB induction (12).

(3) Protection by thiols depends on the concentration of oxygen and of the thiol. Thiols generally protect cells at intermediate oxygen concentrations, $[O_2]$, to a greater extent than fully oxic or anoxic cells (6, 13). A bell-shaped relationship has also been observed between thiol concentration and oxygen enhancement ratio (OER) (6, 14). Discrepancies among earlier studies with respect to the effect of thiols on the OER may thus be a consequence of the different thiol concentrations used.

(4) Alteration in the levels of endogenous thiols is not required for protection by exogenous thiols. A number of thiols elevate intracellular GSH (15, 16), although this is not always the case (17). Elevation of endogenous thiol levels is generally attributed to the exogenous thiol reducing cystine in the growth medium to cysteine, followed by transport of the cysteine into the cell and stimulation of *de novo* GSH synthesis. In most of these studies the addition of buthionine sulfoximine, an inhibitor of γ-glutamyl cysteine synthase and thus of GSH synthesis, abolished the elevation of GSH but had little effect on protection by the exogenous thiol (15, 16). GSH elevation is thus unlikely to be a mechanism of radioprotection.

(5) Different types of cells can be protected by thiols to different degrees. WR-1065 protects normal human fibroblasts, but not fibrosarcoma tumor cells, against the DNA-damaging and lethal effects of x-irradiation *in vitro* (18). Cell-type differences in chromatin organization and DNA-drug associations may be responsible. WR-1065 also protects the DSB-repair-deficient xrs5 Chinese hamster ovary (CHO) mutant much less efficiently than the parent line against killing by x-rays and neutrons (19). In contrast, WR-1065 has an equally protective effect on two human glioblastoma lines (20), one of which is DSB-repair-deficient because of a defective DNA-dependent protein kinase activity. Two human squamous cell carcinoma lines, which have different DSB-repair capability, are also protected equally by MEA (8).

(6) Thiols can protect against the lethal effects of high LET radiations. Pretreating cells with thiols such as WR-255591 protects against injury by high LET radiations such as neutrons (21), but invariably the magnitude of protection is lower than with x- or γ-rays. It is generally presumed that the greater severity of the DNA lesions induced by high LET radiations makes these less amenable to chemical modification.

(7) Thiols appear to be effective modulators of apoptosis and oxidative injury to the cell membrane. Apoptotic cell death may be important in the radiation responsiveness of some tumors and normal tissues. The identification of agents that can decrease apoptosis in normal cells while not affecting or enhancing the response of tumor cells has clear therapeutic benefits (22). Alterations in thiol redox status regulate various aspects of cell function, including signal transduction, transcriptional activity, and the apoptotic pathway (23). WR-1065 protects against internucleosomal DNA fragmentation and loss of viability when added to thymocytes that had been exposed to γ-radiation. Pretreatment with WR-1065 does not protect against DNA fragmentation. Clearly the post-irradiation effect is unrelated to the ability of the thiol to protect against DNA damage. Rather, WR-1065 may either inactivate the endonuclease responsible for DNA degradation,

alter chromatin structure so that the internucleosomal region is not accessible to the endonuclease, or regulate cation mobilization (24). Pretreatment with WR-1065 exerted an equal protective effect on the M059J and M059K human glioblastoma cell lines. The former cells undergo significant apoptosis following irradiation, which is a minor mode of death in the latter. Thus in this case preirradiation WR-1065 appears to modulate apoptotic death (20).

(8) Although DNA damage may trigger apoptosis (25), non-DNA targets such as the cell membrane may also be involved (26). Some studies have demonstrated effects of thiols on non-DNA targets. WR-1065 and GSH (both at 5 m*M*) have efficiently inhibited lipid peroxidation in mitochondrial membranes (27). WR-1065, *N*-acetylcysteine [616-91-1] (NAC), and DTT have protected against ^{90}Sr β-ray-induced perturbation in synchronously contracting chick embryo cardiac myocytes, which is suggested to reflect protection at the cell membrane/cytosol level (28).

1.3. Modulation of Cellular Recovery and Stress Responses

Thiols have frequently been suggested to alter cellular radiosensitivity on a longer time scale than can be explained by free-radical events. This may be because thiols can also produce profound changes in cell metabolism, including effects on cell progression, DNA synthesis, and protein synthesis (29, 30). These combined effects are often referred to as biochemical shock and, in some cases, may contribute to protection by allowing additional time for the repair of DNA damage. However, the lack of protection against cell killing by thiols added immediately after irradiation (5) is inconsistent with such a scenario. Biochemical shock may therefore be more important for antimutagenic or anticarcinogenic activity which, in contrast to effects on survival, are manifested when the thiol is added after irradiation (9, 31). The post-irradiation modulation of transformation and mutation, but not of cell killing, may relate to the hypothesis that the initial response of cells is to initiate constitutive DNA repair processes required for survival, regardless of the mutagenic/carcinogenic consequences (32). The subsequent slow phase of repair, which requires protein induction, may correct potentially carcinogenic lesions that are missed or misrepaired during the initial error-prone phase. Biochemical shock may thus allow additional time for such processes to function. Cell division is an important step in the fixation of mutational and transformational events.

It is becoming apparent that alterations in redox status may provide a more general control of cell metabolism, analogous to that mediated by phosphorylation. Thiols may thus affect cellular radiosensitivity by modulating the activity of a variety of proteins involved in the signaling pathways that regulate cell progression, DNA repair, apoptosis, etc (23). Radiation itself activates a cascade of signaling events that alter the expression and/or activity of specific genes and proteins involved in cell cycle progression and apoptosis, and of growth factors that stimulate the proliferation of surviving cells. This can lead to enhanced survival or cell death, depending on which pathway a cell follows, which in turn depends on the cell type and its environment, as well as on the extent of DNA damage (25). Protein kinase activation and subsequent phosphorylation reactions appear to play an important role in these responses. Thiols can attenuate the radiation-induced activation of these pathways by a number of mechanisms. By scavenging $OH^{\bullet}$, thiols prevent the oxidation of membrane phospholipids and associated generations of arachidonate and diacylglycerol, which activate protein kinase C (PKC), and inositol triphosphate, which causes Ca^{2+} mobilization and activation of PKC. The activation of c-jun, a cellular proto-oncogene transcription factor, by x-rays has been suggested to be mediated by reactive oxygen intermediates based on the inhibition of this effect by NAC (33). Similarly, WR-1065 blocks the activation of PKC in CHO cells exposed to 20 Gy (1 Gy = 100 rad = cGy) of x-rays (34). Signal transduction pathways can also be activated by DNA damage, and are thus sensitive to the effects of thiols on DNA damage.

1.4. Modulation of Mutation

Because mutations in cells that survive radiation exposure may be expressed as undesirable late effects in XRT, the potential use of thiols to protect against mutation and transformation has generated considerable interest. The phosphorothioates WR-2721 and WR-151327 inhibit the induction of tumors in mice exposed to both low and high LET radiations (35, 36). WR-2721 given to mice 30 min before irradiation also protects against hypoxanthine phosphoribosyltransferase (HPRT) mutations induced by both γ-rays ($\sim$2.4−fold) and neutrons ($\sim$1.4−fold) (37). The free thiols WR-1065 and WR-151326 also display antimutagenic (9) and antitransforming (38) activity *in vitro*. Studies have shown several important aspects. (*1*) Timing. Adding aminothiols to V79 cells up to 3 h after irradiation has no effect on survival following either γ-rays or neutrons, but markedly reduces the induction of HPRT mutations for both radiation types (9, 31). WR-2721 also protects against neutron-induced HPRT mutations *in vivo* when it is given 3 h after irradiation (37). These data suggest a role in the treatment of individuals who have already been exposed to radiation. (*2*) Concentration. WR-1065 protects against transformation and HPRT mutations at much lower concentrations than are necessary to protect against cell killing (16). (*3*) LET dependency. Thiols are equally effective against neutron and γ-ray-induced HPRT mutations *in vitro* (31). Protection by WR-151326 and WR-1065 against neutron-induced transformation *in vitro* has also been reported (38). (*4*) Form of the drug responsible. Although WR-1065 per se, rather than its disulfide WR-33278 (*N,N′*-(dithiodi-2,1-ethanediyl)bis-1,3-propanediamine), appears to be responsible for protecting V79 cells against killing by γ-rays, this distinction is not so clear-cut for CHO cells (16). The polyamine spermine and WR-33278 are structurally similar agents capable of binding to DNA. When introduced into CHO cells by electroporation at 0.01–0.001 m*M* either 30 min prior to or 3 h following exposure to 7.5 Gy (750 rad), both agents protect against HPRT mutations (39). (*5*) Alterations in the distribution of mutations by thiols. A different distribution of HPRT mutations was observed in cells irradiated in the presence or absence of MEA. Thiols preferentially protect against those events that generate large-scale molecular changes as opposed to point mutations (40).

1.5. Preclinical and Clinical Studies using Phosphorothioates

Because radiation does not discriminate between normal and tumor cells, XRT may cause adverse normal tissue reactions that can limit the intensity of treatment and also be life-threatening. An improved therapeutic index would be possible if the differential response between the tumor and irradiated normal tissues could be increased. Promising agents in this context include the phosphorothioates WR-2721, WR-151327, and WR-3689, which protect a variety of dose-limiting normal tissues, both early and late responding (3, 4, 13, 41–43). An enormous literature has accumulated relating to the effects of phosphorothioates on animals and humans. On the basis of these studies, several generalizations can be made.

(1) Phosphorothioates generally protect normal tissues more than tumors. Tumor protection reported in some animal studies can partly be explained by physiological effects of the particular drugs, which are specific to rodents (4). WR-2721 does not appear to protect human and most animal tumors, apparently because of the low availability of the drug to tumor cells (4). Many tumors appear to have a reduced capillary density (44), which may mean that these tumors have altered levels of alkaline phosphatase, the enzyme that converts WR-2721 to WR-1065. A reduced ability of thiols to protect the hypoxic cells characteristic of many tumors may also contribute to their selectivity for normal tissues. The observation that WR-1065 protects cultured normal human fibroblasts, but not fibrosarcoma tumor cells, suggests that additional factors may contribute to the selectivity of radioprotection by WR-2721 *in vivo* (18).

(2) Thiols protect different normal tissues to different degrees. Differences in the extent of protection of normal tissues by WR-2721 have been attributed to differential drug accumulation. A potential mediator of this selectivity is alkaline phosphatase. However, there is no simple relationship between protection and tissue

drug concentration or alkaline phosphatase levels (42). Another potential factor is tissue oxygenation, or more specifically the oxygen concentration, pO_2, of the critical target cells for radiation injury. Protection by WR-2721 appears to be maximal when the pO_2 of target cells is at or just above the transition region from hypoxic to oxic sensitivity, ie, the K-value, which may be different in different tissues (13, 42, 43). The likelihood that many normal tissues exist at intermediate oxygenation may in fact contribute to the therapeutic ratio. The importance of considering the target cells in this regard is illustrated by the murine bone marrow (BM), which is regarded as well oxygenated but is efficiently protected by WR-2721. However, the BM stem cells may exist in a more hypoxic environment than the committed lineage-specific progenitor cells and the proliferating cells (45).

(3) Fractionated and low dose-rate XRT schedules may be less amenable to modification. There has long been a concern that radioprotective agents are less effective when fractionated irradiation is employed. The loss of protection of normal tissues by WR-2721 found in some studies is probably a result of the reduced drug dosage used with each fraction to avoid cumulative drug toxicity (43). The same appears to be true in clinical fractionated XRT, where the daily dose of WR-2721 would have to be reduced by ∼50% to minimize toxicity (4, 44). WR-2721 efficiently protects the BM after acute doses and could thus be effective in radioimmunotherapy, where myelosuppression is the dose-limiting toxicity, for protecting against the early phase of injury associated with the initial systemic distribution of the radioisotope. Although initial results showed promise (42), subsequent studies, in which WR-2721 was given to mice prior to an $LD_{90/30}$ dose of radiolabeled antibody, followed by further injections of the drug every 4 h up to 72 h, failed to show significant benefit (46). A use for WR-2721 has also been suggested in single high dose intraoperative XRT, in which dogs given WR-2721 in their duodenal lumen prior to irradiation showed significantly reduced normal tissue injury (47).

(4) Clinical studies of WR-2721 using XRT for certain types of cancer show promise, but toxicity remains a concern. Although systemic WR-2721 is generally well tolerated as a single iv infusion, the achievable concentration of WR-2721 is still limited by toxic side effects. These include nausea, vomiting, sneezing, a warm or flushed feeling, mild somnolence, a metallic taste in the mouth, transient hypocalcemia, and allergic reactions (4). Emesis may be moderately severe, but can be reduced by antiemetics such as ondansetron and dexamethasone. The potentially dose-limiting toxicity is hypotension, which is rapidly reversed by discontinuing the drug. WR-2721 shows promise for patients with inoperable, unresectable, or recurrent rectal adenocarcinoma who receive pelvic irradiation (48). In these studies there is no evidence of tumor protection. Some acute reactions are less frequent in the WR-2721-treated group. There are no moderate or severe late effects in the WR-2721-treated group, compared to 14% in the XRT-only group. Patients receiving WR-2721 15 min before each fraction show no significant hypotension or hematologic toxicity up to 18 months. Mild to moderate emesis occurred in 80% of the courses. These encouraging results are being extended worldwide, and trials are planned for patients having head and neck or lung cancer, with and without concomitant antiemetics (5, 48). WR-2721 also shows promise for protecting the BM of patients receiving total body irradiation (TBI) for lymphoma or chronic lymphocytic leukemia (CLL) (49, 50).

(5) Although WR-2721 is generally superior to other phosphorothioates in protecting normal tissue in mice, other drugs also show some potential. No phosphorothioate is markedly superior to WR-2721. However, WR-3689 is equivalent in most respects and is less toxic (41). Whereas both WR-2721 and WR-3689 are behaviorally toxic at radioprotective doses in rats, the combining of these with caffeine appears promising for ameliorating these effects (2, 51). Other phosphorothioates such as WR-44923 (41), WR-638, and WR-77913 (52) provide radioprotection comparable to WR-2721 in the small intestine of mice. Similarly, WR-151327 effectively protects the intestine and BM and modulates late normal tissue reactions in mice (53, 54). WR-151327 is active orally and is undergoing preclinical testing (4, 55). Clinical trials are anticipated.

(6) Alternative methods of delivering radioprotective agents may provide an improved therapeutic index. Topical or regional application can circumvent problems related to systemic toxicity and tumor protection,

eg, during irradiation of the chest wall after breast tumor surgery. Unfortunately, WR-2721 is ineffective topically for skin protection, presumably because of inadequate penetration. Potential enhancers of transdermal drug delivery (qv) have been screened in rodents, although even the best WR-2721 levels attained may be insufficient for protection (56). WR-2721 applied topically before irradiation protects the mucosa of the large bowel of rats by ∼1.8−fold (57). A subsequent phase-I/II evaluation of WR-2721 as a protector of the rectosigmoid mucosa, when given topically in enema form daily before fractionated XRT to the pelvis, showed no unfavorable side effects or toxicity in patients given WR-2721 daily during a five-week course of external beam XRT. However, WR-2721 did not protect the rectal mucosa from radiation damage, possibly because of the low drug doses used (58). Another approach to targeting phosphorothioates can be found in the demonstration that intraventricular injection of WR-2721, WR-3689, or WR-77913 into the brains of rats protects against hind-limb paralysis caused by irradiation of the cervical spinal cord (59). This strategy could be useful during XRT for noncentral nervous system (non-CNS) tumors where the spinal cord is in the radiation field. Thiols can also be targeted at the subcellular level. A series of compounds in which an aminothiol is bound to the DNA-binding chromophores quinoline and acridine have been synthesized and shown to protect against guanine oxidation more efficiently than the parent aminothiol (60).

(7) Phosphorothioates attenuate the effects of neutrons *in vivo,* but are generally less protective than for x-rays. WR-2721 and WR-151327 protect against neutron lethality in mice and against damage to various normal tissues and tumors (53, 54, 61). Whereas WR-2721 is generally superior for protecting against low LET radiations, other drugs may be equal or better protectors for high LET radiations. WR-151327 is more effective than WR-2721 in protecting against gastrointestinal (GI) injury caused by neutrons (53), whereas the phosphorothioate WR-2529 exhibited the optimal protection in the $LD_{50/30}$ assay and, in view of its lower toxicity, is a significantly better protector than WR-2721 (61).

1.6. Other Sulfur-Containing Compounds

1.6.1. Glutathione and Cysteine Analogues

Endogenous thiols such as GSH and cysteine [7048-04-6] are present in all mammalian cells and perform a variety of metabolic functions, including the protection of cellular components from the damaging effects of radiation and other oxidative stresses (62). Radioprotection *in vitro* cannot generally be achieved by adding cysteine or GSH per se, as these agents can be quite cytotoxic and often have irregular uptake into cells. However, GSH given to rats 15 min prior to irradiation does protect the parotid glands from radiation injury (63). Similarly, a randomized pilot trial indicates that administration of GSH (1200 mg iv) before XRT reduces the occurrence of diarrhea resulting from damage to the GI mucosa of patients undergoing surgery for endometrial cancer and receiving adjuvant XRT to the pelvis (64).

An alternative method for elevating intracellular GSH levels is to use agents that promote GSH synthesis, such as 2-oxothiazolidine-4-carboxylate (OTZ), a cysteine delivery system, or glutathione ethyl ester [118421-50-4] or methyl ester, which is readily taken up by a variety of cell types and converted to GSH (62). When OTZ has been used to elevate intracellular GSH by two- to three-fold, conflicting results have been obtained for oxic cells where either modest or no protection has been found (65). However, GSH should be more effective at lower O_2 tensions. Some protection of V79 cells by OTZ at low or intermediate oxygenation, but none at high $[O_2]$, was observed. The conversion of OTZ to cysteine requires the activity of 5-oxoprolinase. Whereas abundant in normal tissues, this enzyme is deficient in many experimental tumors, which may give rise to a therapeutic advantage (62).

Another alternative is NAC, which enters cells, is rapidly hydrolyzed to cysteine, and can then enter the GSH-synthetic pathway. Radioprotective effects of NAC have been reported in a variety of *in vitro* systems (28, 66) and in mice (67). Treating cultures of nonadherent low density human BM cells with NAC (2 mg/mL) before and after irradiation protects granulocyte/macrophage colony-forming units (CFU-GM) by ∼1.56−fold (68). A

radioprotective effect has also been obtained using NAC applied topically on normal human skin (69). In contrast, NAC given to patients with inoperable nonsmall-cell lung cancer who were receiving hyperfractionated XRT (60 Gy in 48 fractions over 32 days) had little effect on either normal tissue reactions or tumor responses (70). Ribose-cysteine (RibCys) (RibCys), a cysteine pro-drug that stimulates GSH biosynthesis, shows some ability to protect against radiation-related deaths and anastomotic leaks in a swine model of surgical injury to the rectosigmoid (71).

1.6.2. 2-Mercaptopropionylglycine

Preirradiation administration of 2-mercaptopropionylglycine [1953-02-2] (MPG) protects against alterations in the esophageal epithelium of mice receiving a single dose of 7.5 Gy of γ-rays TBI (72) and ameliorates the depletion of various types of ovarian follicles (73). Combining nontoxic doses of MPG and WR-2721 can lead to improved protection of tissues such as BM and GI tract (74).

1.6.3. 2-Aminoethyl Isothiuronium Bromide Hydrobromide and 5-Hydroxy-L-Tryptophan

Synergistic protection by the thiol compound aminoethylisothiuronium bromide hydrobromide (AET) and 5-hydroxy-L-tryptophan (5-HTP) at doses of each agent that were individually ineffective and nontoxic has been described (75, 76). Treating mice with AET (20 mg/kg) plus 5-HTP (100 mg/kg ip) 30 min before TBI modified the decline in sperm counts, significantly decreased the level of sperm abnormalities, and protected against sterility associated with oligospermia. This same combination of agents markedly protected normal hemopoietic mouse tissues, showing a $\sim$1.76−fold increase in $LD_{50/30}$ as well as enhanced recovery of 10-day spleen colony-forming units (CFU-S), but did not protect a transplanted mammary carcinoma or a significantly modified injury to rat splenic tissue following 8 Gy TBI. This combination notably protected against micronucleus induction in the femoral BM cells of mice exposed to 8 or 12 Gy of γ-rays TBI. Both the magnitude and duration of radioprotection in the 30-day survival assay were further improved by the administration of hydroxylamine, a decarboxylase inhibitor, prior to the combination of AET/5-HTP (76).

1.6.4. Other Thiols

A variety of sulfur-containing organic compounds have been synthesized and screened for radioprotective activity. Compounds having a high ability to protect mice against lethal doses of TBI γ-radiation have been identified among a series of quinolinium and pyridinium bis(methylthio) and methylthio amino derivatives (77). Several 1,2-dithiol-3-thione and dithioester compounds are also highly effective protectors of EMT6 cells *in vitro,* and in some cases are differentially cytotoxic toward hypoxic cells (78). Lipoic acid [1077-28-7] (6,8-dimercaptooctanoic acid), a lipophilic endogenous disulfide that can be reduced to the dithiol dihydrolipoic acid [7516-48-5], protects against free-radical-mediated injury both *in vivo* and *in vitro.* Lipoic acid given before, but not after, irradiation markedly protects murine hemopoietic tissues in the $LD_{50/30}$ ($\sim$1.26−fold), endogenous CFU-S ($\sim$1.5−fold), and exogenous CFU-S ($\sim$1.34−fold) assays (79). Dihydrolipoic acid, but not lipoic acid, increases the survival of x-irradiated V79 cells, thus suggesting that dihydrolipoic acid is the active cellular radioprotective agent.

2. Other Classes of Protectors

2.1. Tempol

Nitroxides are low molecular weight, stable free radicals that protect against various biological manifestations of oxidative stress, including mutagenic effects. Tempol [2226-96-2], a water-soluble piperidine nitroxide derivative having nonspecific radical-scavenging and superoxide dismutase (SOD) activity, protects cultured aerobic, but not hypoxic, cells against radiation-induced killing (80). Protection does not depend on intracellular thiols

and does not involve O_2-depletion. Tempol reacts with peroxyl radicals and can also oxidize DNA-bound metal ions, thereby interfering with $OH^{\bullet}$ generation. Analogues such as 3-aminomethyl-Proxyl and Tempamine show superior protective activity to Tempol in V79 cells. These analogues have amino groups that may confer an affinity for DNA comparable to that of the aminothiols, a suggestion supported by equilibrium dialysis studies and by the ineffectiveness of the negatively charged derivative 3-carboxy-Proxyl (80).

Tempol protects against alopecia in guinea pigs, possibly by directly protecting the hair follicle stem cells. Tempol administered 5–10 min before TBI protected mice by $\sim$1.3−fold based on $LD_{50/30}$ values, but did not compromise the ability of radiation to control the RIF fibrosarcoma, presumably because of enhanced reduction of the drug in tumor cells (80). A potential application for nitroxides as selective normal tissue radioprotectors, especially in view of the modest radiosensitizing effect in hypoxic cells, has been suggested. Nonclassical radioprotectors such as Tempol, Zn aspartate, and Ca^{2+} antagonists, which are effective protectors against TBI at low concentrations, may act by inhibiting apoptosis (81).

The potential for improved radioprotection by combining Tempol with growth factors such as stem cell factor (SCF), which protects by quite different mechanisms, has been examined in mice (82). Both SCF alone, given 20 and 4 h before and 4 h after TBI, and Tempol alone, given 10 min before TBI, increased 30-day survival, but protection was greater than additive when the two agents were combined.

2.2. Captopril

The antihypertensive agent captopril [62571-86-2] ((2*S*)-1-(3-mercapto-2-methylpropionyl)-L-proline) is an inhibitor of angiotensin-converting enzyme (ACE) (see Cardiovascular agents; Enzyme inhibitors). ACE converts angiotensin I to angiotensin II, a potent vasoconstrictor. Captopril is well tolerated and approved by the U.S. FDA for chronic use in humans. ACE inhibitors protect against radiation-induced injury to lung and skin as well as early hemodynamic changes after local renal irradiation (83–85). Post-irradiation captopril also ameliorates radiation nephropathy in rats receiving 15–27 Gy (1500–2700 rad) in 12 fractions, especially in animals having less extensive renal injury. Captopril and other ACE inhibitors may also be useful for the treatment and prophylaxis of nephropathy, which is a significant cause of late morbidity in BM transplantation patients receiving total body irradiation (TBI) (83).

Captopril protects against pulmonary endothelial dysfunction and fibrosis and reduces the severity of the histopathological reaction in the irradiated rat lung. When added to the feed after the final irradiation, it protected against various manifestations of early lung reaction in rats receiving fractionated hemithorax irradiation (60 or 80 Gy (6000 or 8000 rad) in 10 daily fractions) (84). Captopril prevented or attenuated hypertensive reactions possibly in part by limiting edema in the irradiated lung. However, this drug is not able to modify all aspects of acute radiation lung damage, as it had no effect on the early increase in permeability surface area and delayed the onset of hypoperfusion only slightly.

Whereas daily doses of captopril following irradiation ameliorates several indexes of cardiac damage measured three and six months after 20 Gy (2000 rad), it fails to prevent the progressive functional deterioration of the heart, thus indicating that captopril is ineffective for the treatment of late cardiac complications (85). Captopril administered continuously in the drinking water from day seven before irradiation protects against acute (3- and 5-day) damage to the jejunal mucosa of mice exposed to 9 or 15 Gy (900 or 1500 rad) TBI (86).

Assigning a specific mechanism to the various therapeutic effects of captopril is difficult because the drug has diverse biological effects. Both the antihypertensive activity via ACE inhibition and the nonspecific thiol function may contribute. The thiol group is unlikely to contribute in cases where captopril is administered only after irradiation. Inhibition of ACE may be important mechanistically because enalapril [75847-73-3], a nonthiol ACE inhibitor, is an effective radioprotector in some situations. The ability of captopril to control hypertension may be a factor in kidney response and may play a minor role in protection of lung, but is unlikely to contribute to protection of skin. Comparing antihypertensives that are or are not ACE inhibitors indicates

that, whereas hydrochlorothiazide [58-93-5], a diuretic, and enalapril, an ACE inhibitor, are equally effective in preventing hypertension, only the latter prevents proteinuria.

Captopril does appear to be capable of acting as a traditional thiol under some conditions. Pretreatment using captopril inhibited the induction of single-strand break (SSB)s and DSBs in plasmid pBR322 DNA and protected against the loss of clonogenicity of cultured human SCL-1 keratinocytes irradiated with neutrons, apparently by an $OH^{\bullet}$ scavenging mechanism (66).

2.3. Metallothioneins

The metallothioneins, a group of low ($<10,000$) molecular weight proteins containing $\sim$30% cysteine residues, are efficient $OH^{\bullet}/O_2^{\bullet}$ scavengers. Transcription of metallothionein genes can be induced by a variety of agents, including heavy metals such as cadmium, Cd; glucocorticoids (GC); interferon (IFN); Ha-*ras;* and mediators of the inflammatory stress response such as tumor necrosis factor (TNF) and interleukin 1 (IL-1). In some but not all studies induction of metallothioneins prior to irradiation *in vitro* is associated with a radioresistant phenotype (87). Cadmium-treated mice also show a significantly increased $LD_{50/30}$ for x-rays (88). Bismuth nitrate modifies both acute and late effects of x-rays (30-day lethality, number of endogenous CFU-S, and induction of thymic lymphomas) in mice by inducing metallothionein in the target tissues, but does not protect against GI lethality (89). In contrast to these reports of radioresistance following gene induction, no change in radiosensitivity has been observed in several studies where mammalian cells are transfected with metallothionein genes (90).

2.4. Protease Inhibitors

Protease inhibitors such as antipain [149116-08-5], a tripeptide analogue derived from actinomycetes, and the soybean-derived Bowman-Birk inhibitor [37330-34-0] family exhibit antitransforming and anticarcinogenic activity *in vivo* and *in vitro* at nontoxic doses but generally do not modify cell killing (91). The Bowman-Birk inhibitor suppressed x-ray-induced transformation *in vitro* at nanomolar concentrations. Many protease inhibitors suppress radiation-induced transformation in a variety of *in vitro* systems. The most effective of these is chymostatin, a quite specific and potent inhibitor of chymotrypsin. Protease inhibitors are also antiteratogenic, decreasing the induction of exencephaly in irradiated mice (92).

Although the mechanism by which protease inhibitors block carcinogenesis is unknown, these inhibitors appear to be able to reverse the initiating event, presumably by stopping an ongoing process begun by the carcinogen exposure. At the subcellular level, protease inhibitors suppress c-myc transcripts in irradiated $C3H10T\frac{1}{2}$ cells and alter radiation-induced gene amplification in a manner that correlates with their ability to suppress transformation (91). Protease inhibitors can suppress transformation even when applied after carcinogen exposure both *in vitro* and *in vivo,* provided that the cells are able to proliferate at the time of exposure to the inhibitor (91).

2.5. Antioxidant Vitamins

The natural antioxidant vitamins A, C, and E (retinoic acid, ascorbic acid, and α-tocopherol, respectively), as well as the vitamin A dietary precursor β-carotene, exhibit a range of radioprotective effects (see Vitamins). These protect against lethality, mutation, and transformation in a variety of cultured cell types and laboratory animals. Effects have been reported for both pre- and post-irradiation treatments (3, 93). Although vitamins protect much less efficiently than WR-2721, the vitamins have low toxicity and may thus be useful in some situations. Protection against 30-day lethality has been observed in mice given vitamin A or β-carotene within two days after TBI (93). Post-irradiation, but not preirradiation, treatment with vitamin E also enhances the 30-day survival of mice exposed to 8 Gy (800 rad) of γ-rays, which suggests an enhancement of recovery of

BM function. Although such preirradiation activity is likely to involve radical scavenging, these mechanisms are unlikely to explain the ability of these agents to protect when administered post-irradiation. Vitamin C, however, does suppress radical formation in irradiated hamster cells (94).

Supplemental vitamin A, begun prior to or directly after local irradiation (30 Gy (3000 rad) to the hind limb), decreases radiation-induced toxicity. Supplemental vitamin A or β-carotene also diminishes systemic toxicity owing to local x-radiation in tumor-bearing mice without diminishing the antitumor effect of the radiation. In addition to the effects on lethality ($LD_{50/30}$) and survival time, these vitamins also protect rodents against gastric and intestinal bleeding, adrenal gland hypertrophy, thymic involution, lymphocytopenia, weight loss, and carcinogenesis. Vitamin A and β-carotene can stimulate immune reactions, including those directed against tumors. In some instances this results in an enhanced antitumor effect of local irradiation (93).

A number of studies have shown that vitamins moderate the induction of chromosomal aberrations by radiation. Vitamins C and E given orally to mice either 2 h before, immediately after, or 2 h after 1 Gy (100 rad) of γ-ray TBI significantly reduce the frequencies of micronuclei and chromosomal aberrations in BM cells. Vitamin E is the more effective (95). Administration of vitamins C and E within 5 min of irradiation is as effective as pretreatment. Protection by vitamin C has also been shown in humans. Whereas chronic treatment of rats using vitamin C (100 or 300 mg/(kg/d)) for six months prior to TBI protects against chromosomal aberrations, vitamin E is not radioprotective in this setting (96).

There are numerous reports of the effects of antioxidant vitamins on transformation. Vitamin C suppresses x-ray-induced transformation when $C3H10T\frac{1}{2}$ cells are treated daily for one week following irradiation (97), suppresses transformation by γ-rays or neutrons, and prevents the promotion of radiation-induced transformation by 12-*O*-tetradecanoylphorbol 13-acetate (TPA), but has no effect on cell survival (98). In these studies, the continuous presence of vitamin C for a critical period appears to be necessary for suppression of transformation. Vitamin C may act on the promotion stage of transformation which, for TPA, may involve activation of PKC or production of $O_2^{\bullet}$ (98).

Some radioprotective agents can ameliorate the effects of radionuclides, which irradiate tissues chronically. Many radionuclides emit low energy Auger electrons that may have severe biological effects. Vitamins A and C, as well as traditional thiol radioprotectors such as MEA, offer significant protection against the effects of tissue-incorporated radionuclides on murine spermatogenesis (99). The radioprotective ability of vitamin C appears to depend on the dose rate and the radiation type.

One vitamin E analogue, TROLOX, inhibits radiation-induced apoptosis in murine thymocytes (26). Chicks given vitamin E prior to exposure to a sublethal dose (2.25 Gy (225 rad)) of γ-radiation demonstrate a more rapid recovery from damage to the thymus (100).

The potential for combining vitamins and aminothiols shows some promise. Vitamin E (100 IU/kg) injected subcutaneously (sub-q) either 1 h before or within 15 min after irradiation significantly increases the 30-day survival of mice. Improved protection has been obtained when WR-3689 (150–225 mg/kg) is also given ip 30 min before irradiation (101). Preirradiation treatment using vitamin E and cysteine in combination also offers better radioprotection than the individual agents against alterations in various hematological parameters (102). An additional potential benefit is that vitamin A and β-carotene may actually inhibit some of the side effects, such as ulceration and bleeding, associated with the use of aminothiols (93).

2.6. Metalloelements

Radioprotection by the essential metals Cu, Fe, Mn, and Zn and their intracellular complexes has been reviewed (103). Metalloelement-dependent enzymes include SOD, catalase, metallothioneins, lipoxygenases and cyclo-oxygenases involved in arachadonic acid metabolism, alkaline phosphatase, DNA polymerase and gyrase involved in DNA synthesis, and Zn-finger proteins involved in the regulation of transcription. These may modulate recovery from radiation-induced injury, and the depletion of their activity may partially account for the biological effects of ionizing radiation, as well as explaining the radioprotective activity of exogenous metal

compounds. Cytokine-mediated redistribution of metalloelements may also be an important factor in radiation response. The SOD-mimetic tetra(3,5-diisopropylsalicylato)dicopper(II), $Cu(II)_2(DIPS)_4$, protects mice by $\sim$1.2−fold at nontoxic doses and protects against radiation carcinogenesis. Protection by $Cu(II)_2(DIPS)_4$ may be related to the induction of hypoxia, stimulation of lympho/hemopoiesis, and/or Cu-dependent effects on the transcription and translation of stress response genes, rather than to its SOD activity. Various other essential metal compounds such as $ZnCl_2$ and $Zn(II)(DIPS)_2$ exhibit some degree of radioprotective activity in the mouse $LD_{50/30}$ assay (103, 104). The involvement of zinc compounds in transcription and translation may be important for cellular/tissue recovery processes.

Metalloelement complexes may be useful for the post-irradiation treatment of radiation injury, based on the observation that several of these compounds accelerate recovery of, among other things, lympho/hemopoiesis. Preirradiation $Mn_3(O)(DIPS)_6$ increases the survival of γ-irradiated mice (103). Treatment of mice that have been exposed to an $LD_{50/30}$ dose of γ-rays plus $Mn_3(O)(DIPS)_6$ either 1 or 3 h after irradiation also increases survival, which supports the hypothesis that this compound is an effective radiorecovery agent (105). Again, this increase in survival may result from the resynthesis of radiation-depleted Mn-dependent enzymes that facilitate the recovery of immunocompetence and tissue repair, as reported for $Cu(II)_2(DIPS)_4$.

Significant data support the approach of combining metalloelement complexes such as $MnCl_2$, zinc aspartate, and $ZnCl_2$, with aminothiols such as MEA and WR-2721 to minimize acute toxicity and improve radioprotective efficacy by minimizing tumor protection (2, 103). Nontoxic doses of metalloelements appear to decrease the toxicity of aminothiols (103). Combining WR-2721 and $ZnCl_2$ results in enhanced normal-tissue radioprotection (2). Intraperitoneal injection of mice with $ZnCl_2$ and WR-2721 also decreases the extent of tumor protection observed using WR-2721 alone, which suggests an additional favorable therapeutic gain factor for this combination (103). Although normal tissue specificity has been shown for zinc aspartate with and without WR-2721 (106), the level of zinc aspartate that humans can tolerate is not known.

A variety of other metals and their complexes have been studied for radioprotective activity. Among these are copper glycinate, strontium chloride, $ZnNa_3$-diethylenetriaminepentaacetate (ZnDTPA), and selenium, which has been studied because of its relationship to endogenous antioxidant mechanisms, especially GSH peroxidase and vitamin E.

2.7. Calcium Antagonists

The potential use of Ca^{2+} antagonists as radioprotective agents has been suggested based on the importance of maintaining Ca^{2+} homeostasis for cell viability following a variety of cytotoxic insults (107). Alterations of cytosolic Ca^{2+} levels can result in changes in the activity of Ca^{2+}-dependent degradative enzymes, which may contribute to cell death, and of the PKC-mediated signal transduction pathway. Although the role of perturbations in Ca^{2+} homeostasis in cellular radiation response remains poorly defined, the realization that modulation of intracellular Ca^{2+} can prevent apoptosis in some cell types has stimulated even greater interest in Ca^{2+} antagonists (22, 25).

A variety of drugs that inhibit cellular Ca^{2+} uptake exhibit radioprotective activity. The Ca^{2+} channel blocker diltiazem [33286-22-5] protects mice against radiation lethality when administered sub-q or ip, and even orally, 10 or 30 min preirradiation (108). The Ca^{2+} channel blockers nifedipine [21829-25-4] and nimodipine [66085-59-4] are also effective. Protection by diltiazem may result from inhibition of the cellular influx of Ca^{2+} subsequent to membrane injury or to free-radical scavenging. Diltiazem is both a thiol and a Ca^{2+} antagonist. Diltiazem, however, has some effectiveness when administered after irradiation, a feature that distinguishes it from other thiols. Flunarizine [52468-60-7], a Ca^{2+} antagonist that also enhances local blood flow, is not radioprotective in these studies, which indicates that radioprotection is not a general property of Ca^{2+} antagonists, and that some specificity exists (108).

As for all radioprotective agents, the clinical usefulness of these drugs depends on an ability to protect normal tissue better than tumor cells at nontoxic levels. Diltiazem, nifedipine, nimodipine, and nitrendipine

do not appear to alter the radioresponsiveness of human tumors grown in immunosuppressed mice (108). Although providing only modest normal tissue potection, Ca^{2+} antagonists have mild side effects and may thus be useful in combination with other agents. Synergistic effects have been observed when diltiazem is combined with zinc aspartate, dimethyl sulfoxide (DMSO), and nifedipine. In fact, zinc aspartate combined with diltiazem protects to a similar extent to combinations of zinc aspartate with WR-2721 (108).

2.8. Adenosine Analogues

Exogenous adenine nucleotides are moderately radioprotective when given to animals shortly before irradiation. Protection appears to be mediated by extracellular adenosine receptors that are coupled to the inhibition/activation of adenylate cyclase, which in turn regulates intracellular cyclic adenosine monophosphate (AMP), which is itself radioprotective *in vitro* (109). The combination of AMP, a soluble adenosine pro-drug, and dipyridamole, an inhibitor of adenosine uptake, leads to an elevation of extracellular adenosine and activation of cell surface adenosine receptors. The combination enhances the proliferation of hemopoietic cells, including CFU-GM, in nonirradiated mice. In mice evaluated 24 h after 3 Gy (300 rad), a greater number of DNA-synthesizing hemopoietic cells have been observed when dipyridamole-AMP is given 60–90 min prior to irradiation. This drug combination also protects against the increase in free polynucleotide levels in the thymus and spleen of mice receiving 1 Gy (100 rad) TBI (110). Dipyridamole-AMP given either 15 or 60 min preirradiation is radioprotective in mice based on $LD_{50/30}$ values ($\sim$1.11−fold), endogenous CFU-S survival, and post-irradiation BM CFU-GM recovery (109). Protection is also apparent for fractionated irradiation upon repeated drug administration. In addition to the stimulation of hemopoietic recovery, protection appears to involve induction of hypoxia resulting from effects on the cardiovascular system. However, noradrenaline given along with dipyridamole-AMP eliminates hypoxia induction but preserves the radioprotective action of dipyridamole-AMP in terms of hemopoietic recovery and partially with respect to survival enhancement (111).

Administration of dipyridamole-AMP to mice 5–25 min after 1 Gy (100 rad) of TBI γ-irradiation is also protective, as indicated by plasma thymidine levels and the amount of saline soluble polynucleotides in the thymus (112). Adding dipyridamole-AMP to *in vitro* irradiated suspensions of thymocytes enhances the rejoining of DNA strand breaks (112). These post-irradiation effects are presumably mediated by the activation of extracellular adenosine receptors.

Exogenous adenosine triphosphate (ATP) protects various tissues of mice subsequently exposed to a lethal TBI dose of neutrons (113). ATP (700 mg/kg ip) increases the 30-day survival from 40 to 85%, protects against damage to the testes, and increases the seven-day survival from 26 to 86%. The increase in activity of acid phosphatase after neutron irradiation, an indicator of lytic processes, is ameliorated by ATP in both the testes and small intestine. ATP also differentially ameliorates the increase in cholinesterase activity observed in rhabdomyosarcoma tumors and the small intestine of neutron-irradiated mice. ATP-$MgCl_2$ (60 μmol/kg iv) given to pigs receiving preoperative fractionated external-beam pelvic XRT also protects against various manifestations of colorectal injury (114).

2.9. Priming with Other Antitumor Agents

Priming animals with low doses of some chemotherapeutic agents enhances resistance to subsequent exposures to radiation (see Chemotherapeutic agents, anticancer). Priming mice with vincristine [57-22-7] increases the radioresistance of the GI epithelium and of the BM, primarily by accelerating post-irradiation recovery of the hemopoietic stem- and progenitor cells, rather than altering their intrinsic radiosensitivity (115). Giving vincristine 24 h prior to irradiation provides optimal radioprotection of 12-day CFU-S, possibly by initiating a recruitment of stem cells into the cycle prior to irradiation. At the time of irradiation, cells may be in a more radioresistant phase (S) of the cell cycle, or have a decreased tendency to apoptose or increased radical-scavenging capacity. Many biologics that prime against radiation are also potent immunostimulants that

activate macrophages and cells of the immune system to release cytokines, such as IL-1, that can confer radioprotection and enhance hemopoietic stem cell recovery. The similarity between priming with vincristine and these immunostimulants suggests that radioprotection by vincristine may be cytokine-mediated. A number of antitumor agents increase cytokine production by macrophages (116). Because cytokines such as IL-1 and TNF can induce MnSOD (115), it is possible that this could mediate priming by vincristine. Although treatment of nontumor-bearing mice with vincristine increases their MnSOD activity on a time scale similar to that for radioprotection, no such increase has been found in tumor-bearing mice, which suggests that increased antioxidant enzyme activity is not responsible for this vincristine effect (115).

An additive acceleration of post-irradiation regeneration of BM function (white blood cells, 12-day CFU-S, burst-forming unit-erythroid (BFU-E), colonyforming unit-fibroblast (CFU-F), colony-forming unit-granulocyte/erythroid/macrophage/megakaryocyte (CFU-GEMM), and CFU-GM), has been observed in the BM of mice treated with a combination of vincristine and lithium prior to 4.5 Gy (4500 rad) TBI (117). Whereas vincristine does not appear to increase stromal production of hemopoietic growth factors, lithium does enhance the production of some of these factors, which may contribute to its activity.

2.10. Methylxanthines

Pentoxifylline [6493-05-6] (Trental) a synthetic dimethylxanthine derivative, is a hemorrheologic agent that can prevent or ameliorate late radiation injury to soft tissues and lung in animals and humans, and may be useful in the management of fibrotic sequelae, particularly if administered prophylactically or in the inflammatory early stages of fibrosis (118–120). Pentoxifylline administered concurrently with and following irradiation has reduced high grade late soft tissue injury in a murine experimental model (118). Pentoxifylline given just prior to irradiation and continued for 40 weeks has also decreased late lung injury in rats (120). In humans, pentoxifylline is used as an interventional therapy for persistent soft tissue ulceration or necrosis. The drug treatment is initiated $\sim$30 weeks after irradiation (119). Pentoxifylline may influence late radiation injury via several mechanisms, including protection of endothelial cells soon after radiation exposure by enhancing blood flow in injured microvasculature and down-regulating TNF (121). In contrast to its effects on late radiation injury, pentoxifylline had little or no effect on acute skin or lung reactions in animals (118, 120, 122). Pentoxifylline, given at various times after a single dose of 20–24 Gy (2000–2400 rad) x-rays, also fails to moderate the development of late rectal ulcers in rats (123). Pentoxifylline generally increases tumor radiosensitivity (124).

Pentoxifylline is structurally related to other methylxanthine derivatives such as caffeine [58-02-2] (1,3,7-trimethylxanthine), theobromine [83-67-0] (3,7-dimethylxanthine), and theophylline [58-55-9] (3,7-dihydro-1,3-dimethyl-1*H*-purine-2,6-dione or 1,3-dimethylxanthine), which also show radioprotective activity in some instances, suggesting that methylxanthines as a drug class may radioprotect through a common mechanism (see Alkaloids). In a retrospective analysis of cervical and endometrial cancer patients receiving primary or adjuvant XRT, no association between caffeine consumption and incidence of acute radiation effects has been found. However, there was a decreased incidence of severe late radiation injury in cervical cancer patients who consumed higher levels of caffeine at the time of their XRT (121). The observed lack of correlation between caffeine consumption and acute radiation effects is consistent with laboratory investigations using pentoxifylline.

2.11. Superoxide Dismutase

Superoxide dismutase (SOD) exhibits radioprotective activity in a variety of systems, including protection against 30-day lethality in mice and some late radiation effects in humans when given after irradiation (125). The mechanistic basis for these effects is controversial. The preirradiation activity of SOD, and its activity in cultured cells, has generally been attributed to radical-scavenging effects, whereas its activity when given to

animals or patients after irradiation is probably related to its antiinflammatory and/or immunostimulatory properties.

Several studies have reported radioprotective effects following transfection of SOD genes into mammalian cells (126). MnSOD protects against events occurring as early as 24 h after irradiation, which suggests that it may prevent apoptosis. Whereas the presence of SOD during irradiation increases the resistance of murine leukemia BCL1 cells *in vitro,* injecting BCL1-bearing mice with SOD (100 mg/kg iv) 30 min prior to irradiation does not protect against the development of leukemia. Thus, SOD may be useful in normal tissue protection in patients receiving TBI as part of the conditioning prior to allogeneic BM transplantation (127).

Liposomally encapsulated SOD has better pharmacological properties than the free enzyme and has been tested as an antiinflammatory agent. A suggested clinical benefit of liposomal SOD in two patients with severe fibrosis and necrosis caused by exposure to high dose XRT has been confirmed in a clinical trial of systemic CuZnSOD for the treatment of patients with preexisting severe symptomatic radiation-induced fibrosis of the skin and underlying tissue (128). A three-week course of liposomal SOD caused some regression of fibrosis in all subjects. Although several drugs, including antiinflammatory agents or vascular modifiers such as captopril, may be useful for the management of fibrotic sequelae, particularly if administered prophylactically or in the inflammatory early stages of fibrosis, SOD is the first agent having a demonstrated ability to reduce preexisting fibrosis. A preliminary account of a second clinical study using topical liposomal SOD also suggested an improved control of fibrosis (129). Little is known of the effects of liposomal SOD on tumors.

2.12. Chinese Herbal Medicines

Many traditional Chinese medicines have been screened for radioprotective activity in experimental animals. In one study of more than a thousand Chinese herbs, a number of agents increased the survival rate of dogs exposed to a lethal dose of γ-rays by 30–40%, and some symptoms of radiation injury were ameliorated. These effects are potentially related to stimulation of the hemopoietic and immune systems (130). Extracts of five Chinese drug plants, as well as aspirin, effectively protected mice exposed to 7.5–8.0 Gy (750–800 rad) of γ-radiation, and increased survival rates by 8–50% (131). Several Chinese traditional medicines, administered ip before or after irradiation, protected against lipid peroxidation in a variety of mouse tissues, including BM, liver, and spleen, as well as in mouse liver microsomal suspensions irradiated *in vitro* (132). Jen-Sheng-Yang-Yung-Tang, when administered ip at 1 mg/g after γ-irradiation, appeared to facilitate the recovery of cellular immunocompetence in mice. When injected ip at 1 mg/(g·d) for seven days after irradiation, this herb accelerated the recovery of hemocyte counts and 10-day exogenous CFU-S in 4 Gy (400 rad) irradiated mice (133). Both the aqueous (2 mg/mL in the drinking water) and alkaloid fractions (5.4 mg/d po) of Panax ginseng extract protected the jejunal crypts of γ-irradiated mice (both as pre- and post-irradiation treatments). The incidence of radiation-induced micronuclei in splenic lymphocytes was also reduced by pretreatment with both fractions (134). Thus, Panax ginseng may act as a scavenger and may also promote the repair or regeneration of damaged cells following γ-rays.

2.13. Antibiotics

Although not strictly speaking radioprotective agents, antibiotics (qv) are an important component of the treatment of radiation injuries. By preventing or delaying the onset of systemic infection owing to endogenous and exogenous organisms, these agents can allow greater recovery of tissues such as BM and intestine (135). Indeed, mortality following exposure to radiation in the Chernobyl nuclear accident was mainly attributed to bacteriemia under immunocompromised conditions (136). Antibiotics such as penicillin and synthetic antibacterials such as quinolone (see Antibacterial agents, synthetic), alone and in combination, can reduce bacterial translocation from the intestine, treat the subsequent sepsis, and reduce mortality (137, 138). Combined

glycopeptide, ie, vancomycin or teicoplanin, and antimicrobial, ie, the quinolone L-ofloxacin, therapy has also been evaluated in mice exposed to mixed-field neutrons plus γ-rays (138).

2.14. Other Radioprotective Chemicals

The bis-methylthio- and methylthioamino-derivatives of 1-methylquinolinium iodide and 1-methylpyridinium-2-dithioacetic acid provide reasonable protection to mice at much lower doses than the aminothiols, which suggests a different mechanism of action (139). One of these compounds, the 2-(methylthio)-2-piperidino derivative of the 1-methyl-2-vinyl quinolinium iodide (VQ), interacts with supercoiled plasmic DNA primarily by intercalation. Minor substitutions on the aromatic quinolinium ring system markedly influence this interaction. Like WR-1065, VQ is positively charged at physiological pH, and the DNA-binding affinities of VQ and WR-1065 appear to be similar.

Cimetidine [51481-61-9], a histamine H2 receptor antagonist, given at 15 mg/kg ip 2 h prior to irradiation of mice using 0.25–1 Gy (25–100 rad) of γ-rays, protects $\sim$1.5−fold against the induction of micronuclei in erythrocytes (140). Cimetidine also protects $\sim$1.5−fold against lymphoid tissue injuries in mice receiving 1–8 Gy TBI. Protection may involve radical scavenging, although cimetidine may also augment the proliferative and cytotoxic response of lymphocytes and can prevent the interaction of histamine with leukocytes and endothelial cells and block inflammatory reactions (see Histamines and histamine antagonists).

Diethyldithiocarbamate [20624-25-3] (DDC) is both an inhibitor of SOD and a thiol, and exerts both radiosensitizing and radioprotective properties in mice, depending on factors such as the time of its administration relative to irradiation. For neutrons, DDC shows only protective effects (141). DDC (1 mg/g ip) given 30 min before 15 Gy (1500 rad) also protects mouse jejunal crypt cells and reduces the frequency of micronuclei in splenic lymphocytes (134).

A 24-h pretreatment of V79 cells using the DNA methylation-disrupting agent 5-azacytidine decreases the cells' 5-methylcytosine content by 50% and protects them by $\sim$1.8−fold from killing by γ-radiation, possibly by activating repair enzymes (142). Deproteinized calf blood serum (ActoHorm), given to rats at various times after 20–24 Gy (2000–2400 rad) of x-rays, stimulates regeneration of the mucosal epithelium and may be effective in promoting the healing of the GI mucosa (123). A slight radioprotective effect of inosine given ip to mice shortly before γ-irradiation can be enhanced by magnesium aspartate, apparently owing to the additive vasodilatory activity of the two agents (143).

Glutamine has been widely examined as a potential agent for enhancing intestinal repair following radiation injury, although its value in this regard remains to be clearly established (144). Glutamine does exert radioprotective effects in cultured CHO cells.

Radioprotective effects of the bibenzimidazoles Hoechst 33342 [23491-52-3] and 33258 [23491-45-4] in cultured cells have been reported. These drugs bind in the minor groove of DNA and protect against SSBs in pBR 322 plasmid DNA, providing a possible basis for the observed protection (145). The pattern of protection suggests both a general reduction in SSBs throughout the entire plasmid, probably owing in part to $OH^{\bullet}$ scavenging by unbound ligand, as well as a binding site-specific component that may involve $H^{\bullet}$ donation from the ligand to 4′-sugar radicals (145). DNA binding represents a focal feature for the design of more efficient radioprotectors, and substitution of these bibenzimidazoles, eg, halogenation, markedly alters their protective activity.

Several antiulcer drugs have been evaluated for their ability to protect against acute skin reactions, such as erythema and moist desquamation, which are significant problems during XRT of superficially located tumors. Atropine [51-55-8] (*dl*-hyoscamine), a naturally occurring alkaloid, is the prototypal antimuscarinic agent. It can decrease gut motility and, when given with irradiation, reduce the extent of deformation of villous shape and protect against crypt cell depletion (146). Sucralfate, an antiinflammatory agent that activates cell proliferation, when applied as a cream to breast cancer patients receiving post-operative electron beam XRT to their chest wall, significantly decreases acute skin reaction and enhances skin recovery (147). Intraperitoneal

injection of irsogladine maleate, an antiulcer drug, immediately after irradiation and then daily for three days protects intestinal stem cells by ~1.16−fold, which suggests that the drug may be useful for alleviating GI injuries in radiation accident victims (148).

Many agents that alter blood flow to tissues have been examined as possible radioprotective agents. BW12C, ie, 5-(2-formyl-3-hydroxyphenoxyl) pentanoic acid, a substituted benzaldehyde that stabilizes oxyhemoglobin and reduces oxygen delivery to tissues, is of interest as a possible potentiator of bioreductive agents and/or hyperthermia. Owing to these changes in oxygen availability, BW12C can act as a protector against radiation-induced injury to normal tissues. However, variable results have been reported with respect to BM12C's ability to protect normal tissues and tumors (149).

3. Cytokines and Related Factors as Radioprotective Agents

Although thiols dominated the field of radioprotection from the 1950s through the 1980s, radioprotective, or rather radiomodulating, activity of biologics, and especially cytokines, has received considerable attention in the 1990s (3, 150). The term cytokines herein means proteins that modify cellular responses through ligand–receptor interactions, including growth factors and interleukins, and discussion is extended to immunomodulating agents, which function mainly through the generation of cytokines. Eicosanoids are included because these can be generated during cytokine responses and mediate such diverse processes as vasoregulation and inflammation. These are potent protectors of jejunal and hemopoietic stem cells when given prior to irradiation, and are therefore implicated as potential mediators of radioprotection by cytokines, although this does not seem to be the case for IL-1 (151). Some cytokines are most effective as radioprotectors when given between one and several days prior to irradiation; others work best when administered after irradiation. Biologics generally protect tissues by 1.3-fold or less, whereas WR-2721 can protect by as much as 2.7-fold. However, clinically, protection as low as 1.1-fold could be beneficial. The ability to increase the tumor dose by 10% while maintaining the same level of complications consequently translates into a significant increase in tumor control rates (135). Certain cytokines, such as G-CSF, SCF, and GM-CSF, are well tolerated at effective doses (44). Others, such as TNF-α and IL-1, are more toxic, especially if administered systemically, as is consistent with their roles in inflammation and septic shock.

Elucidation of the mechanisms of cytokine action can be complicated. Cytokine action may be indirect and mediated by the products of responding cells, such as other cytokines, eicosanoids, and other biologically active molecules. The specific cellular response therefore feeds into a network, involving soluble factors, cell adhesion molecules, and extracellular matrix components, that determines cell maturation, proliferation, and even death, within tissues. Disturbing this delicately balanced network can therefore cause wide ranging effects (152). Tissue response to a cytokine is determined by the receptor profiles of the cells, the microenvironmental signals that are present and induced, and the programmed agendas of the cells in the tissue. Many cytokines cause a cellular influx which forms part of the response, and radiation itself induces imbalances in the system by directly generating positive cellular responses, including cytokine expression, and causing cell death. It is axiomatic that cytokine responses are generally highly predictable and reproducible within any one tissue, and can readily be investigated. Anticytokine antibodies can be used to determine the role of individual cytokines in radioprotection. Cytokine and cytokine receptor knockout mice have been invaluable in defining those situations in which individual cytokines are critically involved and in determining the extent of redundancy.

Any one cytokine can have more than one function (pleiotropy). A few examples from the lympho/hemopoietic system clearly demonstrate the selective and specific action of these factors in context. For example, stem cell factor (SCF) or c-kit ligand, IL-1, and IL-6 are involved in the proliferation, differentiation, and functional activation of the earliest pluripotent stem cells. IL-3 (multi-CSF) stimulates colony formation by more committed myeloid progenitors. IL-7 is needed for the clonal development of T and B lymphocyte precursors; erythropoietin (EPO) for red cells; granulocyte–macrophage colony-stimulating factor (GM-CSF)

for macrophages, granulocytes, and dendritic cells; and granulocyte colony-stimulating factor (G-CSF) for granulocytes. The CSFs therefore differ in their lineage specificity and the maturity of their primary targets. This, in turn, reflects the cellular receptor profile and the other accessory factors that are needed for development of each cell type (151).

3.1. G-CSF and GM-CSF

Because of availability in recombinant form and enormous clinical potential in both XRT and chemotherapy, hemopoietic growth factors have moved rapidly from the laboratory to the clinic, in spite of their expense (44). Clinical use of cytokines is largely limited to accelerating lympho/hemopoietic regeneration using exogenous CSFs, in particular G-CSF and GM-CSF (135). Such agents have the potential to protect selectively normal hemopoietic and other tissues, allowing higher doses of radiation or chemotherapy to be delivered to the tumor. They also have roles in the management of disease.

Injection of these factors rescues mice (153), dogs (154), and monkeys (155) from radiation injury. Whether GM-CSF and G-CSF can protect mice against supralethal doses of irradiation is controversial and appears to vary with the strain, although there is little evidence that these cytokines, on their own, increase stem cell survival in addition to accelerating recovery of progenitor cells. GM-CSF is optimally protective when given before irradiation (150), and G-CSF is even more potent than GM-CSF when given to mice prior to an $LD_{100/30}$ TBI dose (156). The most dramatic increases in survival of mice and neutrophil recovery after irradiation are found when multiple doses of G-CSF are administered (157). In animals given 4 Gy (400 rad) TBI, continuous treatment using G-CSF over 21 days initiated shortly after TBI causes complete and sustained recovery (154). Several other studies have reported that G-CSF enhances recovery from neutropenia in mice caused by single high dose TBI (158) and fractionated TBI (159). Daily treatment of rats using sub-q G-CSF for 21 days following fractionated upper hemibody irradiation prevents neutropenia induced by 15 doses of 3 Gy/d (300 rad/d) (160). Administration of G-CSF to tumor-bearing rats also maintains the white blood cell counts above critical levels, enabling completion of the irradiation schedule and better tumor response. Thus G-CSF may be useful with conventional XRT schedules.

Similar results have been reported in sublethally and lethally irradiated dogs, where G-CSF reduced the severity and duration of neutropenia and the duration of thrombocytopenia (161). G-CSF increases the survival of lethally irradiated animals by inducing earlier recovery of neutrophils and platelets. GM-CSF also decreases the severity and duration of neutropenia in dogs exposed to 2.4 Gy (2400 rad) TBI, but does not influence monocyte or lymphocyte recovery (162), indicating its expected selective action.

If G-CSF and GM-CSF can prevent neutropenia caused by XRT, their use may allow more intensive XRT to be given to cancer patients. In cancer patients, the selectivity factor has further relevance because cytokines can stimulate tumor growth. *In vitro* experiments show that some human myeloid malignancies and solid tumors produce myeloid growth factors, proliferate in response to growth factors, or express growth factor receptors. However, studies using G-CSF and GM-CSF have shown no protective effect against a variety of tumors (163). Another potential concern is that G-CSF and GM-CSF, in stimulating the proliferation of BM progenitors, could sensitize the progenitors to the effects of cytotoxic agents. Some clinical data support this concern (44) and further investigation is needed for the optimal sequence administration of these agents relative to the cytotoxic therapy. An additional concern is that G-CSF and GM-CSF could act to promote carcinogenesis or tumor progression if given close to the time of irradiation.

Clinically, GM-CSF or G-CSF have been used to accelerate recovery after chemotherapy and total body or extended field irradiation, situations that cause neutropenia and decreased platelets, and possibly lead to fatal septic infection or diffuse hemorrhage, respectively. G-CSF and GM-CSF reproducibly decrease the period of granulocytopenia, the number of infectious episodes, and the length of hospitalization in such patients (152), although it is not clear that dose escalation of the cytotoxic agent and increased cure rate can be reliably achieved. One aspect of the effects of G-CSF and GM-CSF is that these agents can activate mature cells to

function more efficiently. This may, however, also lead to the production of cytokines, such as TNF- α, that have some toxic side effects. In general, both cytokines are reasonably well tolerated. The side effect profile of G-CSF is more favorable than that of GM-CSF. Medullary bone pain is the only common toxicity.

The role of cytokine therapy in the management of radiation accident victims has been summarized (152). In Goiânia in Brazil in 1987, eight radiation accident victims were treated with GM-CSF one month after radiation exposure. Marked increases in granulocyte production were induced in five persons, although this did not prevent death.

3.2. Interleukin-1 α and β

IL-1 has radioprotective activity toward BM and other tissues (151, 164). IL-1 is produced in response to endotoxin, other cytokines, and microbial and viral agents, primarily by monocytes and macrophages. Other nucleated cells can also produce it. IL-1 appears to play an important role in the regulation of normal hemopoiesis directly by stimulating the most primitive stem cells and indirectly by stimulating other hemopoietic factors, including G-CSF, GM-CSF, M-CSF, and IL-6.

Although IL-1 protects a number of normal tissues against radiation injury, its effects on BM are the best characterized. IL-1 provides varying degrees of protection, depending on the timing; $\sim$20 h prior to irradiation is optimal (164). Synergy has been found with other cytokines, most notably TNF- α, IL-6, and SCF. Multiple daily injections of IL-1 α preceding irradiation are more effective than single doses in promoting both BM progenitor cell survival and granulocyte recovery (165). Protection may involve a number of mechanisms. One is the stimulation of BM progenitor cells such that more of these are in the radioresistant S-phase of the cell cycle (151). Another is the induction by IL-1 of a number of radioprotective substances such as prostaglandins (PGs), metallothionein, scavenging acute-phase proteins, GSH, and SOD, as well as other hemopoietic growth factors (151). The protective effects of preirradiation IL-1 in murine BM cells and human cell lines correlate closely with the induction of MnSOD (126), and growth factors induced from accessory cells that constitute the hemopoietic microenvironment can enhance repopulation of the immune and hemopoietic systems after irradiation. Possible effects of IL-1 on radiation-induced apoptosis in tissues have yet to be reported, but cytokines can clearly affect the tendency of cells to undergo this form of death. The ability of IL-1 to accelerate the reconstitution of murine BM following lethal doses of radiation, which may be through stimulating production of CSFs, can also impact survival. Although IL-1 can protect mice from acute lethality and from CFU-GM damage caused by TBI, it has no significant effect on immediate CFU-GM survival or on the level of radiation-induced DNA strand breaks in BM cells (166).

A critical step in radioprotection involves the IL-1 receptors. Monoclonal antibodies to the type 1 IL-1 receptor block IL-1-induced radioprotection (167). Although this receptor is not present on BM cells, it is present on fibroblasts, which suggests that the effects of IL-1 on stem cells may be largely indirect and mediated by stromal cell activation (168). Anti-IL-1 receptor (type 1) also sensitizes normal mice to the effects of TBI, which suggests that endogenous IL-1 has an intrinsic radioprotective role. IL-6 induction by IL-1, but not CSF levels, is inhibited, which supports the concept that G-CSF and GM-CSF are insufficient by themselves at radioprotecting stem cells and indicates a contributory role for IL-6. Anti-IL-6 antibody blocks IL-1 and TNF-induced radioprotection and also decreases the intrinsic radioresistance of mice, as does anti-TNF- α (169).

Anti-SCF antibody similarly abrogates lipopolysaccharide- and IL-1-induced radioprotection (170) and sensitized mice to radiation. Such effects are not obtained using anti-IL-3, anti-IL-4, or anti-GM-CSF antibodies. SCF, IL-1, IL-6, and TNF- α have acknowledged interactive roles in the normal development of BM stem cells, and their radioprotective activities seem consistent with these roles, as is the ability of CSFs to promote further hemopoietic development at higher hierarchical levels.

Clinical use of IL-1, IL-6, and TNF- α, is limited by associated systemic toxicity. SCF seems better tolerated. It may be possible to develop derivatives of other cytokines that are less toxic. The synthetic nonapeptide

VQGEESNDK (position 163–171 of human IL-1 β) (see Amino acids; Protein engineering) increases the survival of mice when injected 20 h before or immediately after exposure to 8.5 Gy (850 rad) TBI (171). Although the nonapeptide is less effective than IL-1 β, it does not exhibit the IL-1-like inflammatory side effects of the whole molecule.

IL-1 can radioprotect murine and human BM progenitors *in vitro* as well as *in vivo* (126). Also, BM cells from donor mice treated with IL-1 *in vitro* prior to irradiation show an increased ability to rescue irradiated recipient mice following BM transplantation, which suggests that IL-1 exerts its protective effects directly on the hemopoietic cells and protects both short-term and long-term repopulating stem cells (126). In fact, most hemopoietic growth factors protect their respective target cells from the effects of irradiation *in vitro,* a phenomenon that may reflect the tendency of the cells to apoptose in the absence of growth factor.

The effects of IL-1 in accelerating recovery of BM hemopoiesis in mice have been characterized (172). Injection of IL-1 20 h prior to sublethal irradiation promotes an earlier CFU-S/CFU-GM recovery in the BM and spleen, and markedly affects BM cellularity and mobilization of progenitor cells (172). Differences have been found between strains and administration protocols, especially with respect to BM CFU-GM numbers.

Synergy has been reported between IL-1, IL-3, and SCF, in enhancing the survival of lethally irradiated mice engrafted with 2×10^6 BM cells and immediately given cytokines once daily for five days (173). SCF alone does not enhance survival, and IL-1 or IL-3 has limited effect. Pretreating mice with thymopentin, a synthetic pentapeptide derivative of thymopoietin, enhances the protective effect of IL-1 α, as indicated by 30-day survival (174).

Extrapolation of experimental observations using cytokines to the clinical setting, ie, to patients with altered physiological conditions, may not be possible (175). Whereas IL-1 administered 24 h prior to a lethal dose of TBI increases 30-day survival of normal mice, it does not protect tumor-bearing animals known to have altered hemopoiesis. The accelerated repopulation of eight-day CFU-S, CFU-GEMM, and CFU-M, following sublethal TBI seen in control mice, has not been seen in tumor-bearing animals. The failure of IL-1 to protect tumor-bearing animals is not a result of elevated plasma prostaglandin (PG) levels caused by the tumor. On the positive side, doses of IL-1 that protect BM and oral mucosa do not protect the RIF-1 and SCCVII mouse tumors (176) which indicates a degree of selectivity in effects.

In addition to BM, IL-1 protects murine intestinal crypt cells (177, 178), oral mucosa (176), and lung (179) against radiation injury. Optimal protection of colony-forming duodenal crypt cells was observed when IL-1 was given $\sim 13 - 25$ h before irradiation, although protection was observed up to 20 h after irradiation, presumably involving accelerated recovery of crypt cells (177). The mechanism by which IL-1 protects GI and hemopoietic tissues appears to be different. Higher doses of IL-1 are required for the protection of the BM than for that of the intestine, perhaps reflecting different IL-1 receptor densities. Protection of the murine intestine by IL-1 is dependent on the mouse strain, the IL-1 dose, and the method used to assess protection (178).

Because the GI syndrome after TBI includes a hemopoietic component, the observed protection of the jejunum may partly be a result of the IL-1 protection of the BM. IL-1 given before abdominal irradiation also increases survival, which suggests that protection against GI syndrome by IL-1 is at least partially independent of its effect on BM (178). IL-1 also protects mouse duodenal crypt cells against fractionated irradiation (176). There is one report that IL-1 given ip prior to abdominal irradiation increases the incidence of peritoneal adhesion formation (180), which indicates that the effects of cytokines may be site- and time-dependent.

IL-1 increases the 24-h thymidine labeling index, with or without localized irradiation, in several normal mouse tissues, including the lip and tongue mucosal basal cell layers, crypt cells of the duodenum, alveolar cells of the lung, hepatocytes, and basal skin cells, but not in the RIF-1 tumor (176).

3.3. Stem Cell Factor

Stem cell factor (SCF), also known as c-kit ligand, is the ligand for a tyrosine kinase-associated receptor encoded by the c-kit protooncogene, and stimulates primitive multipotential hemopoietic stem cells. It does not act as a colony-stimulating factor (CSF) for these cells but rather primes cells to respond to other cytokines, such as IL-1. Mutations in white spotting and steel mice that affect hemopoiesis have been influential in characterizing SCF. These strains display an increased sensitivity to lethal doses of irradiation, which is thought to be the result of a lack of the apoptosis-suppressing effects of SCF, in addition to a lack of proliferative effects (181). SCF is radioprotective in mice on its own (182) and in concert with other cytokines.

Mice treated with SCF show improved long-term survival, more rapid hemopoietic recovery after irradiation, and a much reduced incidence of septicemia. The optimum SCF schedule, which involves both pre- and post-irradiation treatments, protects by $\sim$1.3−fold. Administration of IL-1 and SCF to mice 18 h before lethal TBI results in synergistic radioprotection in terms of both survival and recovery of $c-kit^+$ BM cells (183). Anti-SCF antibody inhibits IL-1-induced radioprotection, which indicates that endogenous SCF is necessary for radioprotection by IL-1. In turn, radioprotection by SCF is reduced by anti-IL-1-receptor antibody, which indicates that endogenous IL-1 contributes to radioprotection by SCF. SCF, unlike IL-1, does not induce hemopoietic CSFs, IL-6, or MnSOD, which suggests that SCF and IL-1 may protect BM cells by different pathways. The c-kit mRNA and SCF binding to BM cells is elevated within 2–4 h of IL-1 administration. Thus, the synergy between SCF and IL-1 may depend on IL-1 and SCF-induced increases in numbers of $c-kit^+$ stem and progenitor cells that survive lethal irradiation.

SCF increases absolute colony number and surviving fraction of CFU-E, CFU-G, and CFU-GM in irradiated human BM. An increase in the fraction of $CD34^+$ cells in the radioresistant S-phase has been noted, which suggests a possible mechanism (184). A cautionary note has been sounded about attempting to predict interactions between SCF and CSFs in hemopoietically deprived individuals (185). Although SCF synergizes with GM-CSF or GM-CSF and IL-3 to increase CFU-GM *in vitro,* no such effect has been found *in vivo.*

SCF protects mice from GI death after irradiation and increases the number of surviving mucosal crypt stem cells (186) in a manner similar to IL-1.

3.4. Other Lympho/Hemopoietic Cytokines

IL-3 shares a common signaling receptor chain with GM-CSF, but stimulates the proliferation, differentiation, and function of a less mature, multipotential, myeloid progenitor cell population. The broader activity of IL-3 suggests that it may be of greater benefit than the more lineage-restricted G-CSF and GM-CSF in accelerating BM recovery after injury. Administration of IL-3 to sublethally irradiated mice induces cell recovery in the thymus (187), and in primates, IL-3 effectively decreases the period of thrombocytopenia after drug or radiation-induced BM aplasia, a feature that G-CSF or GM-CSF do not possess, although IL-1 and IL-6 do. Because of IL-3's broader activity, a combination of IL-3 and later-acting CSFs is expected to be efficacious at enhancing post-irradiation recovery of platelets and neutrophils. The sequence of administration is critical for combined effects of IL-3 and GM-CSF in primates receiving 4.5 Gy (450 rad) (188).

Administration of IL-12 before lethal γ-irradiation of mice protects against hemopoietic death and increases the number of BM cells at six days after irradiation, but sensitizes for GI injury (189). The protective effects are abrogated by anti-IL-1 receptor or anti-SCF antibodies but not by anti-IFN- γ antibodies. The sensitizing effect of IL-12 may be due to its ability to prime for TNF and IL-6, and can be abolished by anti-IFN antibody.

TNF- α also protects mice against the lethal effects of radiation (164). TNF- α given before sublethal irradiation reduces the decline of neutrophils and total blood counts and accelerates the recovery of peripheral blood cells (190). TNF- α also alters the radiosensitivity of murine GI progenitors (191).

3.5. Other Cytokines

Basic FGF (b-FGF or FGF-2) belongs to a class of heparin-binding growth factors associated with growth and differentiation of a number of cell types, including endothelial cells and fibroblasts, and a number of *in vivo* processes, including angiogenesis and wound healing. Its presence is required for endothelial cell culture *in vitro* and protects against the ability of radiation to induce apoptosis (192). Basic FGF has no effect on the repair of SSBs or DSBs but is required for the shoulder on the survival curve, in essence allowing repair of potentially lethal damage. Protection appears to be mediated by PKC activation. Radiation induces b-FGF production by these cells, allowing a survival-associated positive autocrine loop to develop.

Basic FGF protects the endothelial cell lining of the pulmonary microvasculature after iv injection into mice receiving lethal doses of whole-lung irradiation (192). b-FGF given immediately before and within the first two hours after irradiation prevents the clinical syndrome of radiation pneumonitis, presumably by modulating apoptosis in the endothelial cells (192). In contrast, b-FGF fails to protect against classical radiation pneumonitis in two strains of mice having different susceptibilities to lung injury (193). Furthermore, there is little evidence of radiation-induced apoptosis in the lungs of either strain. The reasons for this discrepancy are not clear, but the lung-irradiation conditions, including the doses and the fields, are different in the two studies (193). Minor, but possibly significant, differences exist between the mouse strains (192, 194).

Basic FGF can also stimulate murine hemopoietic progenitors *in vitro.* It is synergistic with hemopoietic growth factors such as GM-CSF, EPO, and Meg-CSF and has radioprotective activity *in vivo,* increasing the number of day-9 and day-12 CFU-S from lethally irradiated animals (195). Furthermore, b-FGF combined with GM-CSF protects against the killing of murine and human CFU-GM exposed to radiation *in vitro* (195).

Another member of the FGF family, FGF-4, protects against radiation-induced cell killing and enhanced the G_2 arrest when overexpressed in a human adrenal cortical carcinoma cell line (196). This effect is again manifested as the appearance of a shoulder on the survival curve, although neither the control nor the transfected cells undergo radiation-induced apoptosis. No differences in the yield or repair of either SSBs or DSBs have been observed.

Whereas epidermal growth factor (EGF) enhances the radiosensitivity of human squamous cell carcinoma cells *in vitro* (197), addition of EGF to hormone-deprived MCF-7 breast cancer cells prior to irradiation results in increased radioresistance (198). An anti-EGF-receptor monoclonal antibody blocks the ability of EGF to enhance growth and radioresistance. Tumor cells, the growth of which is stimulated by EGF, appear to be protected; those where growth is inhibited are sensitized (198).

3.6. Other Agents

Many agents that nonspecifically enhance immunological and hemopoietic responses can also function as radioprotectants. Immunomodulatory substances such as endotoxin, glucan, muramyl dipeptide, bacillus of Calmette and Guerin (BCG), and OK-432 given to mice prior to, and in many cases after, irradiation result in an increased survival beyond 30 days. Such substances are believed to protect by activating macrophages and inducing the production of endogenous cytokines such as IL-1, TNF-α, G-CSF, GM-CSF, and IFN in irradiated animals that subsequently act synergistically to stimulate the proliferation of the target cells. Although it is likely that there are differences in the pattern of cytokines that are expressed following administration of these agents, and in their mechanisms of action, in general these are poorly described. In some cases radioprotection may be attributed in part to the mobilization of stem cells from BM to spleen, as opposed to stimulating proliferation *in situ* (164). Others may modulate oxidative processes, and some exhibit both prooxidant and antioxidant properties.

3.6.1. OK-432

A lyophilized preparation of an attenuated strain of *Streptococcus haemolyticus* available commercially as Picinabil, OK-432, is a cytokine inducer having strong immunomodulatory activity. OK-432 protects *in vivo* against BM suppression in medulloblastoma patients receiving whole-axis irradiation and in mice given TBI (199). Protection of mice has been observed when the drug is given ip from 48 h before to 24 h after irradiation. Treating irradiated mice using OK-432 accelerates recovery of neutrophil count as well as improving host defense systems, which may lead to the prevention of bacteriemia. OK-432 also protects 10-Gy-irradiated murine BM CFU-GM *in vitro* when the drug is added from 24 h before to 3 h after irradiation (199). It was concluded that OK-432 stimulates BM cells to produce GM-CSF *in vitro* by a direct contact effect. Immediate post-irradiation OK-432 also increases the 30-day survival of mice (200). Because the use of antibiotics (qv) to prevent bacteriemia can be suboptimal in immunocompromised animals, the activity of a concomitant immunomodulator, ie, OK-432, and the antibiotic aztreonam has been examined. Post-irradiation OK-432 plus aztreonam greatly increases the 30-day survival of lethally irradiated mice compared to OK-432 alone (200), presumably reflecting a cooperative action of the bactericidal effect of aztreonam and improved host defense by OK-432. Thus, combining an immunomodulator with a broad-spectrum antibiotic may be a useful strategy for treating patients having radiation injuries.

3.6.2. Ammonium Trichloro(dioxoethylene-O,O′)tellurate

AS101, ammonium trichloro(dioxoethylene-*O,O′*)tellurate, is a minimally toxic synthetic organotellurium compound that stimulates the production of a variety of cytokines and is radioprotective when injected into mice prior to sublethal and lethal irradiation (201). The compound appears to act partly by inducing hemopoietic progenitors into the radioresistant S-phase, and partly by stimulating CFU-S to proliferate and self-renew. AS101 stimulates DNA repair replication in spleen and BM cells of mice *in vivo* and in spleen cells *in vitro* (201). It protects cells from DSB induction and enhances their ability to rejoin DSBs. AS101 also enhances the ability of irradiated splenocytes, both *in vitro* and *in vivo,* to repair DNA damage, possibly by increasing DNA polymerase activity.

3.6.3. Tetrachlorodecaoxide

Tetrachlorodecaoxide (TCDO) administered iv from days 4–11 after TBI using γ-rays protects rats against the acute lethal effects of TBI, mainly by accelerating the regeneration of the hemopoietic system and by preventing severe hemorrhage in the lungs and the GI tract. Hair loss is also reduced (202). TCDO also protects against colon damage even when administered days or weeks after irradiation, probably by stimulating the regeneration of colon epithelial cells. TCDO (1 mL/kg) given iv daily within a week after 3 Gy (300 rad) TBI promotes recovery of BM and spleen cells of sublethally irradiated mice (203). TCDO greatly affects spleen weight and cellularity, as well as endogenous CFU-S. These mice are protected from lethality caused by TBI by 1.12–1.18-fold and from endogenous CFU-S depletion by 1.4–1.5-fold. TCDO may enhance regeneration of hemopoietic stem cells by stimulating the immune system with the associated release of cytokines such as IL-1 and TNF. It may also protect partly by suppressing PG production (203). TCDO given to mice as single or multiple iv injections one day before or after irradiation actually radiosensitizes a murine fibrosarcoma tumor, whereas TCDO given on days 17–21 after irradiation protects against leg contracture, which suggests a therapeutic advantage.

Not only is TCDO a potent therapeutic agent in acute radiation syndrome, but treatment using TCDO from days 4–11 after TBI increases the survival rate in rats for up to one year, protects against the development of late GI ulcers, and also reduces the development of γ-ray-induced leukemias and malignant epithelial tumors, but not sarcomas (202). The anticarcinogenic effect of TCDO may be related to the inhibition of PGs, which promote carcinogenesis, or to immunostimulation, which may result in a more effective elimination of malignant cells.

3.6.4. Endotoxin and Muramyl Dipeptide Derivatives

Bacterial cell wall constituents such as the lipopolysaccharide endotoxin and muramyl dipeptide, which stimulate host defense systems, show radioprotective activity in animals (204). Although endotoxin is most effective when given ∼24 h before irradiation, it provides some protection when administered shortly before and even after radiation exposure. Endotoxin's radioprotective activity is probably related to its lipid component, and some of its properties may result from PG and leukotriene induction (204).

Muramyl tripeptide phosphatidylethanolamine (MTP-PE), a synthetic analogue of muramyl dipeptide and an effective systemic macrophage activator, induces a variety of cytokines such as IL-1, IL-6, and TNF, as well as PGE_2 (205). Preirradiation treatment of mice using MTP-PE encapsulated in liposomes, which can intensify radioprotective ability, stimulates the monocyte/macrophage system and accelerates the recovery of hemopoietic cells. The recovery phase is associated with BM granulocyte hyperplasia, accelerated erythropoiesis in the spleen, and recovery of granulocyte counts in the peripheral blood. The drug has no immediate effect on the number of day-8 exogenous BM CFU-S, but stimulates the regeneration of the surviving endogenous CFU-S and BM CFU-GM (205). Optimum survival of both endogenous CFU-S and mice (∼1.17−fold protection) has been observed with 200 μg MTP-PE given ip 24 h before irradiation. Some protection has been observed when the drug is injected 8 h after irradiation. Combining MTP-PE (24 h) and indomethacin (INDO) (24 and 3 h) prior to irradiation exerts an additional radioprotective effect (205).

3.6.5. Glucan and Derivatives

Glucan (β, 1-3 polyglucose), an immunomodulator and hemopoietic stimulant isolated from *S. cerevisiae,* enhances resistance to lethal irradiation in animals. This effect is mediated primarily by an enhanced host resistance to life-threatening opportunistic infections and accelerated hemopoietic regeneration. Glucan protects a variety of indexes for damage to the murine hemopoietic system (206). Glucan and several glucan derivatives protect against the induction of micronuclei in polychromatic erythrocytes of the mouse BM. Significant protection by iv glucan is observed when the drug is given 1 h after irradiation (207).

Carboxymethylglucan, a soluble glucan derivative (208), enhances hemopoietic recovery in sublethally irradiated mice and increases the survival of lethally irradiated animals when given 24 h prior to γ-irradiation. Post-irradiation treatment using carboxymethylglucan also improves survival when used in combination with preirradiation cystamine. Carboxymethylglucan in combination with diclofenac, an inhibitor of PG production, when given to mice 1 day before γ-irradiation demonstrates at least additive radioprotective effects on hemopoietic recovery and mouse survival. These effects may be a consequence of increased hemopoietic cell proliferation as a result of the concomitant inhibition of PG production and the release of growth factors.

Significant protection of mice by several polysaccharides other than glucan isolated from *S. cerevisiae* has been described (209). A 2.16-fold protection in the $LD_{50/30}$ assay is observed for one modifier, MNZ, when given 15 min prior to irradiation. Glucan protects 2.25-fold in this same protocol. Many of these polysaccharides may act through activation of the complement system, rather than directly on cells.

3.6.6. Broncho-Vaxom

The bacterial extract Broncho-Vaxom (BV) protects murine hemopoietic tissue by 1.18-fold in the $LD_{50/30}$ assay. Maximum protection is achieved when the drug is given 24 h before irradiation (210). Pretreating mice with BV before sublethal irradiation increases the number of endogenous CFU-S. The optimal CFU-S survival again is observed when BV is given 24 h before irradiation. BV does not affect day-9 BM CFU-S survival immediately after irradiation. However, 5–12 days after irradiation, the number of day-9 CFU-S is ∼twofold higher in BV-treated mice. During this period BV-treated mice also show increased BM cellularity and accelerated BM CFU-GM regeneration (210). Combining BV (24 h) and INDO (24 and 3 h) prior to irradiation causes an additional radioprotective effect (210). BV given 24 h before irradiation also accelerates peripheral blood recovery, but does not affect thymus recovery.

BV, like many immunostimulatory radioprotective agents, activates macrophages (206). It has been suggested that most immunomodulators that affect macrophages affect radioprotection not only by direct macrophage activation and enhancing the nonspecific host defense mechanisms, but also by inducing the release of cytokines that directly or indirectly enhance additional hemopoietic and immunologic activities (206). Therefore, a possible mechanism of radioprotection is BV-induced secretion of IL-1 and PGs. *In vitro* studies have shown an increased IL-1 and PG production, reaching the maximum within 24 h of BV administration. If a similar situation occurs *in vivo,* then the peak period of IL-1 and PG production coincides with the maximal protective effect.

3.6.7. Lactobacillus Casei Preparations

Lactobacillus casei LC9018, prepared from heat-killed Lactobacillus casei YIT9018, exerts radioprotective effects in a variety of systems. Given to mice immediately after TBI, LC9018 causes a sustained increase in serum colony-stimulating activity followed by an enhanced repopulation of CFU-GM in the femoral BM and spleen (211). The numbers of blood leukocytes, erythrocytes, and platelets are increased earlier in the treated mice than in the controls, and survival is increased significantly. A single sub-q injection of LC9018, given before or after irradiation, increases the survival of mice given 8.5 Gy TBI. Similar protection is observed when LC9018 is administered between 2 d before to 9 h after irradiation, although the preirradiation treatment is slightly better. Increases in the weight of the spleen and in the number of endogenous 8- and 12-day CFU-S suggest that the radioprotective effect is based on enhanced recovery of hemopoietic tissues.

LC9018 proves to be an effective agent for adjuvant immunotherapy when combined with XRT in a randomized, controlled, comparative study of 228 patients with stage IIIB cervical cancer (212). Not only does LC9018 enhance tumor regression by XRT, but the combination therapy also prolongs survival and the relapse-free interval compared to XRT alone. Side effects of combined LC9018 include fever and skin lesions at the injection site, but no severe symptoms are noted. Radiation-induced leukopenia is also less severe in the LC9018-combined group than in the XRT-alone group.

3.6.8. Other Immunostimulatory Agents

Other immunomodulatory substances having demonstrated radioprotective activity include Shigoka (*Acanthopanax senticosus* Harms), Bestatin, Ivastimul (an extract from chlorococcal algae), trehalose dimycolate (TDM) and a synthetic analogue (S-TDM), dextran sulfate (a synthetic heparinoid polyanion), the bacterial immunomodular Nocardia delipidated cell mitogen (NDCM), the lipopeptide lauroyl-L-Ala-γ-D-Glu-L,L-A2pm (LtriP), AM5 (a protein-associated polysaccharide present in the biologic AM3), thrombopoietin, Thymex L (a thymic preparation), adenochrome monoaminoguanidine methanesulfonate (AMM) (an activator of CFU-GM), and serum thymic factor (FTS). A clinical protocol combining vitamin A, IFN, and XRT for advanced cervical cancer produces severe proctitis that necessitates dose reductions. However, in mice treated with vitamin A (100 μg/d) and IFN (3×10^4 units/d) for five days before TBI, there is a modest (1.1–1.4-fold) protection of the bowel, assessed by maintenance of body weight and colon crypt cell survival (213).

3.7. Arachidonic Acid Metabolites

3.7.1. Prostaglandins

Various bioactive lipids, especially the prostaglandins (qv) (PGs) and leukotrienes (LTs), the principal eicosanoid products of the arachidonic acid cascade, are radioprotective. Indeed, protection of hemopoietic tissue by eicosanoids can approach that achieved using WR-2721 (see Table 1) (214, 215). Most attention has focused on the PGs, which are synthesized via the cyclooxygenase pathway and are among the most promising of the newer radioprotective agents for clinical use during cancer treatment (215). The mechanisms by which eicosanoids protect against radiation damage are obscured by their wide range of both pathological and normal physiological effects on tissues and animals. Protection by PGs is seen only when administered prior to

irradiation. WR-2721 protects in mg quantities, whereas PGs protect in μg quantities. Thus the PGs are probably not direct protectors; rather, they induce secondary changes within cells that lead to protection (215). Many eicosanoids are vasoactive and some are proinflammatory, although these effects may or may not be observed at radioprotective concentrations. Receptor-mediated mechanisms are clearly important, and many cell types in various tissues express eicosanoid receptors. Eicosanoid receptor expression may be regulated by cellular differentiation, which may in turn influence the location of protection within a tissue. For the E-series PGs, the GC receptor appears to be important (215). Loss of PG-induced protection in cultured cells appears to correlate with the loss of these PG receptors. Membrane effects may be involved in PG-induced protection (215). Whether or not hypoxia plays a significant role in eicosanoid radioprotection is a contentious issue (216, 217). PGs may also increase intracellular cyclic adenosine monophosphate (cAMP) levels (218), although elevated cAMP alone does not lead to PG-induced radioprotection of bovine aortic endothelial cells (219). Some eicosanoids may act as free-radical scavengers.

3.7.1.1. PGE_2 and Derivatives. PGE_2 and several related PGs protect against radiation injury in the rodent intestine with respect to both crypt clonogen survival and $LD_{50/6}$ (215). Protection of hemopoietic tissue has also been reported using the exogenous CFU-S assay (215), and 40 μm of 16,16-dimethyl PGE_2 (DMPG) given 30 min preirradiation increases $LD_{50/30}$ by $\sim$1.7$-$fold (220). Both systemic and topical DMPG given 1 h prior to irradiation protect mice from γ-ray-induced alopecia after single doses. Similar changes are observed using fractionated XRT and repeated drug administration prior to each fraction (215). Topical DMPG and WR-1065 protect by 1.25–2.0-fold against alopecia resulting from fractionated XRT (221). Post-irradiation hair regrowth is increased by both WR-2721 and eicosanoids. It is unclear whether increased regrowth is solely the result of initial radiation protection of hair follicles, other cell populations and/or vasculature, or also occurs through accelerated repair processes. Although PGs are radioprotective when given either systemically or topically, the former is slightly more effective. Topical PGs or WR-1065 may, however, be clinically more useful in protecting against scalp alopecia, and perhaps radiation dermatitis/mucositis of the oral cavity, rectum, and bladder, when these tissues lie within the treatment field. Studies using topical viprostol, another PGE_2 analogue, also suggest that the use of selected vehicles may lead to negligible blood concentrations of isotope-labeled compound (222).

A 2-h incubation with another PGE_2 analogue, nocloprost (9β-chloro-DMPG) protects normal human fibroblasts but has no effect on the survival of colon adenocarcinoma cells exposed to 10 Gy (1000 rad) (218). Nocloprost protects against radiation-induced DSBs in normal cells but not in tumor cells. Moreover, incubation using nocloprost for 2 h after irradiation enhances the rate of DSB rejoining in fibroblasts but not in adenocarcinoma cells. These data possibly reflect a different distribution of PG receptors on the plasma membrane of the two cell types.

3.7.1.2. PGE_1 and Derivatives. Synthetic PGE_1 analogues such as misoprostol actually protect better than the naturally occurring compounds (215). Misoprostol is an effective radioprotector of clonogenic cells in the rodent intestine, BM, and hair follicle (215). Given sub-q 2 h prior to irradiation, it protects against the killing of Syrian hamster embryo (SHE) cells exposed *in utero* to x-rays by $\sim$1.5$-$fold, and protects $\sim$20$-$fold against oncogenic transformation, compared to 4-6-fold for WR-1065 (216). Although the mechanisms by which misoprostol modulates transformation are unknown, arachadonic acid regulates *ras* proto-oncogene function (223). Some PGs stimulate *ras*-GAP (guanosine triphosphatase (GTPase) activating protein) and thereby switch off the gene product, p21 *ras*.

A clinical trial to evaluate misoprostol as a protector of normal tissue during a course of XRT in cancer patients suggests a reduction in acute normal tissue injury (215). A randomized, prospective, double-blind study indicates that topical misoprostol, administered as an oral rinse $\sim$15 $-$ 20 min before irradiation using conventional 2-Gy (200 rad) fractions, five days a week over 6–7 weeks, significantly protects the oral mucosa from radiomucositis, a frequently observed normal tissue complication during XRT for head and neck cancer (215).

Topical misoprostol directly administered into the operatively exteriorized intestinal lumen of rats 30 min prior to 11 Gy (1100 rad) of x-rays reduces the severity of acute radiation effects, as evidenced by the increased numbers of surviving crypt cells and mucosal height five days after irradiation (224). Topical misoprostol does not reduce blood flow. The attractive possibility of combining misoprostol with antioxidants has been reported (225). Two novel antioxidants, the 21-amino steroid U-74500A and the vitamin E-like compound U-78518F, as well as some nonsteroid antiinflammatory drugs (NSAIDs) and methylprednisolone, are radioprotective for the rat intestine in these same assays when given by the lumenal route. Misoprostol alone, WR-2721 alone, or the combination of the two agents, also increases the survival of intestinal clonogenic cells and animal survival following exposure to neutrons (225). PGE_1 analogues also protect the arterial wall of rabbits irradiated with single or repeated doses up to 10 Gy (1000 rad) (226). Administration of either PGE_1 or its active metabolite 13,14-DH-PGE_1 (5 μg/kg either 6 h pre- or post-irradiation) reduces radiation-induced mitotic activity and extracellular matrix and glycosaminoglycan formation.

3.7.2. Leukotrienes

Leukotrienes, products of the lipoxygenase pathway, are generally less radioprotective than the PGs, with the exception of LTC_4, which is among the most potent of the naturally occurring eicosanoids (214). LTC_4 radioprotects V79 hamster cells *in vitro* and mouse CFU-S and intestinal crypt cells *in vivo* (215, 227). Protection factors of 1.65 for exogenous CFU-S and 2.01 for CFU-GM are obtained with mice given 400 μg/kg LTC_4 (214). In addition, 200 μg/kg LTC_4 given 5 min before irradiation protects 1.45 fold in the $LD_{50/30}$ assay (228). In all cases, protection is observed only when the LTC_4 is administered prior to radiation exposure. The mechanism of LTC_4 radioprotection is unknown, although *in vitro* data suggest that binding to a specific LTC_4 receptor is involved. Differences in the time course for protecting CFU-S versus $LD_{50/30}$ imply different mechanisms of action. LTC_4 may enhance the survival of mice partially via physiological changes such as induction of hypoxia (217). However, LTC_4 mediates a number of physiological changes, including inflammatory responses and cardiovascular changes, that may also be important.

3.7.3. Other Bioactive Lipids

Linoleic acid [60-33-3] (*cis*-9,*cis*-12-octadecadienoic acid), an essential fatty acid, can act as a radioprotective agent of BM while being toxic to certain tumor cells. Both irradiation (0.5 Gy (50 rad)) and exogenous linoleate generate increased levels of the oxygenated product of linoleate, 13-hydroxyoctadecadienoate 13-HODE), by macrophages (229), most likely through a 15-lipoxygenase-mediated pathway. This pathway seems to serve not only as a means of dealing with free radicals that are generated in cells but also as a mediator of oxidative stress responses.

Nontoxic doses of γ-linolenic acid [506-26-3] (6,9,12-octadecatrienoic acid) protect against radiation injury to pig skin (230). Pigs receiving 3 mL/d orally of an active oil containing 9% γ-linolenic acid for four weeks before and for 16 weeks after localized β-irradiation of the skin show less severe (1.13–1.24-fold) acute reactions, ie, erythema and moist desquamation, than pigs receiving linoleic acid or pigs that are only treated prior to irradiation. A similar reduction in the severity of acute skin injury is seen in pigs treated for 10 weeks after irradiation. Late skin damage, ie, late erythema or dermal necrosis, is also decreased by 1.14–1.51-fold.

Mice fed a diet containing the hexaisoprenoid cholesterol precursor squalene [111-02-4] (2,6,10,15,19,23-hexamethyl-2,6,10,14,18,22-tetracosahexaene) for 14 days prior to and 30 days after 6–8 Gy (600–800 rad) TBI also show some cellular and systemic radioprotection, including prolonged survival (231).

3.8. Eicosanoid Blocking Agents

A number of studies have documented the radioprotective effects of eicosanoid blocking agents such as the NSAIDs (215). Prophylactic administration of INDO, an inhibitor of PG synthesis, delays or reduces radiation mucositis owing to head and neck and thoracic XRT and experimental radiation esophagitis (232, 233). Whereas

INDO radiosensitizes murine solid tumors that have a high level of eicosanoid production, apparently by stimulating antitumor immune reactions, it has little effect on tumors having low eicosanoid production (233). In contrast, pretreatment of mice using INDO has no effect on the radiosensitivity of hair follicles, jejunum, and soft tissues of the extremities, whereas it protects hemopoietic tissue in both the $LD_{50/30}$ and endogenous CFU-S assays and lung (233). It also protects against carcinogenesis (233). Protection of hemopoietic tissue is not a result of an effect of INDO on the radiosensitivity of, or on the number of, BM stem cells, although the number of stem cells in the spleen is increased. Rather, removal of immunosuppressive PGs by INDO may augment immune functions and release IL-1 and other cytokines that can stimulate the proliferation of hemopoietic stem cells in the spleen. Enhanced hemopoietic recovery is also reported in mice given INDO after irradiation (234). INDO decreases indexes of early radiation effects on pulmonary endothelial cell function *in vivo* 7–8 h after exposure of rabbits to 30 Gy (3000 rad) to the chest (235).

In contrast to these protective effects, acute high dose (25 mg/kg) INDO injected ip 30 min prior to γ-irradiation decreases the LD_{50} of mice from ~6.5 Gy (650 rad) to ~4.5 Gy (450 rad) (232).

The ability of various NSAIDs to protect against mortality owing to radiation pneumonitis in mice receiving 19 Gy (1900 rad) to the thorax (bilaterally) has been examined (236). Treatments are continuous from 10 weeks after irradiation. Each inhibitor has the expected effect on arachidonate levels in the lungs. The 5-lipoxygenase inhibitor diethylcarbamazine and the LTD_4/LTE_4 receptor antagonist LY171883 markedly reduce mortality. The cyclooxygenase inhibitors piroxicam and ibuprofen are marginally protective, whereas INDO accelerates mortality, and aspirin reduces mortality.

Nordihydroguaiaretic acid, a lipoxygenase inhibitor, also protects against radiation-induced effects on hemopoiesis in mice (237). INDO and the lipoxygenase inhibitor esculetin show different effects on hemopoiesis (238). Whereas INDO augments the *in vitro* proliferation of BM CFU-GM from irradiated mice, esculetin is inhibitive. Similarly, post-irradiation INDO stimulates CFU-S and CFU-GM in lethally irradiated mice, whereas esculetin inhibits CFU-GM. When given ip 1 h before 5 Gy (500 rad), INDO enhances the post-irradiation recovery of CFU-S, CFU-GM, peripheral blood granulocytes, and nucleated BM cells, whereas esculetin has no effect or even inhibits recovery. These effects are not the result of changes in the intrinsic radiosensitivity of the CFU-S or CFU-GM. These results suggest that the balance between PG and leukotriene production may be important in determining the regulatory role of arachidonic acid metabolites in hemopoiesis in irradiated organisms. Such effects may be mediated through the control of cytokine production.

3.8.1. Steroids and Glucocorticoids

Glucocorticoid (GC) steroids, potent antiinflammatory and immunosuppressive agents, inhibit the hydrolysis of membrane phospholipids by phospholipase A2, which is the initial step in the generation of both lipoxygenase and cyclooxygenase products of arachidonic acid. GCs are used clinically to treat radiation pneumonitis, although the response rate is variable (239). Steroids can prevent death from radiation pneumonitis in animals, but their withdrawal prior to the end of the usual period of pneumonitis results in accelerated mortality (240). Conflicting observations on the ability of GCs to prevent radiation injuries may be partly related to how these drugs are administered after irradiation and to drug dosage (241).

Continuous low dose post-irradiation administration of the synthetic GC dexamethasone (DEX) in the drinking water is beneficial in treating radiation-induced lung and kidney injuries, deleterious for spinal cord injury, and of little value against hepatic injury (241, 242). DEX delays the development of anemia and uremia, the impairment of glomerular filtration, and mortality resulting from renal failure after partial-body or local-kidney irradiation (241). Protection against lethal nephropathy is unexpected considering earlier reports that radiation nephropathy is enhanced by post-irradiation GCs. Chronic low dosage DEX therapy probably delays mortality after kidney irradiation in part by suppressing acute and chronic inflammation reactions and by stimulating physiological responses that compensate for the physiological derangements which develop after radiation exposure of this organ, and may be an effective therapeutic approach to delaying the development

of lethal radiation nephropathy. In a more detailed study, various DEX treatments in the post-irradiation drinking water have increased survival times and delayed the development of kidney dysfunction. The most effective treatment was 94 μg/L DEX for 88 days (242). GCs such as DEX can down-regulate inflammatory eicosanoids and cytokines such as TNF-α, and thereby suppress the radiation-induced inflammatory response that perpetuates radiation damage in some tissues. Knowledge of the dose of GC and timing needed for suppression of radiation-induced cytokine responses is becoming available (243) and this may help to explain some of the discordant observations. Clinically, prolonged treatment using high doses of DEX may offset some beneficial effects of this steroid.

In an attempt to better define the mechanisms of delay of the development of lethal radiation nephropathy by DEX, the effect of post-irradiation DEX on the kidneys of rats receiving 20 Gy intraoperative bilateral local kidney irradiation has been examined (244). DEX significantly protects against loss of body weight, renal mass, renal function, and the development of anemia, but has no effect on edema or fibrosis. DEX-treated irradiated kidneys have significantly less damage to the glomeruli and tubules. The results suggest that DEX prevents the destruction of the nephron.

GCs such as methylprednisolone can delay radiation-induced lung injury in rodents, even when given well after lethal lung irradiation (236, 240). DEX also protects against lung injury (30–90 day lethality after partial-body irradiation) (241), possibly by preventing the increase in capillary permeability and consequently the leakage of protein and fluid into the pleural cavity. This is because steroids do protect against radiation-induced vascular injury. In a rat model, ip administration of methylprednisolone three times weekly during weeks 3–8 after irradiation suppresses pneumonitis, delays the rise in tissue mast cell number, does not affect fibrosis at 20 weeks, supporting the dissociation of the pneumonitis and fibrosis phases (245). In contrast, WR-2721 protects better against late fibrosis than against pneumonitis (246). This dissociation may arise because the two different responses are mediated by different target cell populations (239). The observation that pneumonitis can be suppressed almost completely when GCs are administered after the development of significant interstitial edema may be important clinically, and combining steroids and WR-2721 may alleviate the entire course of the radiation-induced lung syndrome (245).

As regards GC treatment of central nervous system (CNS) radiation injury, short-term high dose DEX therapy of spinal-cord-irradiated animals delays the progression of injury to complete paraplegia and results in a transitory improvement in motor function of paralyzed animals (247). In contrast, chronic low dose DEX shortens the latent period between spinal cord irradiation and paralysis without affecting the tolerance dose (241), which suggests that DEX promotes the development of CNS radiation injury without changing the sensitivity of the target cells. The difference between these two studies may result from the equivalent daily GC doses used, which were much ($\sim$1000 times) larger in the former study. High doses may be needed to completely suppress inflammatory responses. On a more cautious note, administration of prednisolone acetate, a synthetic GC, after 2.84-Gy (284 rad) TBI results in an increased incidence of myeloid leukemias in mice. However, corticosterone, a GC secreted by cells, shows no such activity (248).

3.9. Miscellaneous Radioprotective Agents

Steroid hormones have been extensively studied for their ability to protect against infertility in males receiving XRT (see Hormones). A variety of results have been reported (249). Pretreatment using a GnRH antagonist protects spermatogonial stem cell function from single doses of x-rays. Pretreatment using testosterone also protects spermatogonial stem cells from four daily x-ray fractions, whereas sub-q medroxyprogesterone and testosterone pretreatments protect against a single 3-Gy (300-rad) dose of x-rays. Similarly, 1.5–2.2 fold protection of spermatogonial stem cells has been reported in rats implanted with encapsulated testosterone and estradiol six weeks prior to irradiation. Such protection perhaps involves alterations in oxygen levels, GSH levels, and DNA repair activity in the stem cells. In contrast, no protection against damage to spermatogenesis by pretreatment using either testosterone or estrogen is observed after single γ- or x-ray doses.

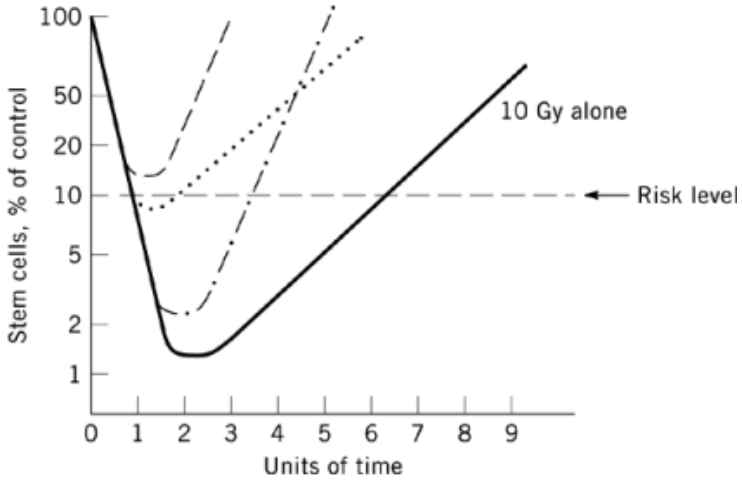


Fig. 1. Hypothetical kinetics of depletion of bone marrow (BM) stem cells following exposure to a lethal total-body irradiation (TBI) of 10 Gy (1000 rad) of x-rays (—) in the absence of radioprotective agents; (···) when treated with WR-2721 30 min prior to irradiation; (—·—) when treated with IL-1; and (– – –) when treated with WR-2721 and IL-1.

The potential for normal brain tissue injury is one of the limiting factors in the use of XRT for brain tumors. Pentobarbital is a cerebral radioprotectant in rodent and primate models after single doses, but is associated with significant risks. Of alternative barbiturates, thiopental given to rats receiving 70-Gy (7000-rad) whole-brain irradiation in a single fraction enhances the 30-day survival similarly to pentobarbital, whereas ethohexital and phenobarbital show no radioprotective activity (250).

4. Combinations of Biologics and Thiols or Other Agents

Significant clinical promise lies in the concept of combining low (nontoxic) doses of radioprotective drugs having different, but complementary, mechanisms of action to achieve better protection than using either agent alone. In particular, combining an agent such as IL-1 that has the potential to stimulate stem cell proliferation before or after irradiation with an agent such as WR-2721 that protects the individual stem cells is appealing. This is illustrated in the diagram in Figure 1. The critical level below which the integrity of the tissue, and thus the viability of the animal, may be compromised is also shown. The balance between the gradual removal of the aging mature cells and their replacement from the regenerating stem cell pool determines whether the tissue remains sufficiently intact to maintain its integrity. An important general observation is that a combined effect can be seen only following radiation doses that do not eradicate most of the stem cells. Several biologics have been shown to exhibit additive or synergistic effects in combination with aminothiols, and may thus be potentially useful for reducing the risks associated with myelosuppression induced by XRT.

4.0.1. Glucan Plus WR-2721/Aminothiols

Combinations of WR-2721 and glucan, given prior to irradiation, are additive in protecting hemopoietic tissue (206). Glucan can be administered up to $\sim$1 h after irradiation. Whereas WR-2721 and glucan alone protect $\sim$1.37−fold and $\sim$1.08−fold, respectively, the combination of WR-2721 and glucan results in $\sim$1.52−fold protection. Repopulation of hemopoietic cells in mice treated with both agents appears to occur faster. Combining selenium, Se; glucan; and WR-2721, which protect by different mechanisms, also gives an additive or synergistic radioprotection of endogenous CFU-S and accelerates BM and splenic CFU-GM regeneration (2). At least

additive protection is also observed with the combination of preirradiation po cystamine and post-irradiation ip glucan in mice receiving sublethal and lethal TBI, as evidenced by enhanced hemopoietic recovery and survival (251).

4.0.2. G-CSF Plus WR-2721

Because glucan is a potent inducer of several hemopoietic cytokines, it has been suggested that specific hemopoietic cytokines may also stimulate hemopoiesis and synergize with WR-2721 (252). Based on $LD_{50/30}$ values, combining WR-2721 (4 mg/mouse ip 30-min preirradiation) and G-CSF ($2.5\mu g$/(moused)) sc from days 1–16 post-irradiation) protects by 1.64 fold, which is greater than the additive effects of G-CSF (1.06-fold) and WR-2721 (1.44-fold) alone (252). BM and splenic CFU-S and CFU-GM recoveries are also accelerated in mice treated with WR-2721 plus G-CSF, and these animals exhibit the fastest recovery of mature functional blood elements (252). Thus, G-CSF does accelerate hemopoietic reconstitution from WR-2721-protected stem and progenitor cells, further increasing survival. WR-2721 and G-CSF also show additive effects in dogs receiving TBI, which has led to its evaluation in leukemia patients (253).

4.0.3. Other Immunomodulating Agents Plus WR-2721/Thiols

Combining WR-2721 (200 mg/kg ip 30-min preirradiation) and BV (25 mg/kg ip 24-h preirradiation) demonstrates at least additive radioprotection in the $LD_{50/30}$ assay (254). Protection is optimal when WR-2721 is given 30 min before irradiation and BV 24 h before or 4–8 h after irradiation. The combined treatments are also more effective in accelerating BM CFU-GM recovery. Endotoxin produces an additive effect when combined with the aminothiol AET (204). IL-1 enhances survival ($LD_{50/30}$) when given in combination with WR-2721 at radiation doses of 15–16 Gy (1500–1600 rad) causing hemopoietic and GI injury (2). Whereas IL-1 alone is not protective when given 30 min before irradiation, simultaneous administration of IL-1 and WR-2721 30 min before irradiation gives greater than additive protection. Synergistic protection in the mouse $LD_{50/30}$ assay is reported for combinations of WR-2721 and 5-hydroxytryptamine, as well as for WR-2721 plus DMPG, whereas combinations of DMPG with cysteine, glucan, GSH, or 5-hydroxytryptamine are only additive (255).

4.0.4. Prostaglandins Plus WR-2721

Results on the efficacy of combining PGs and aminothiols are variable. Although combining WR-2721 and DMPG has no further effect on the radiosensitivity of mouse jejunal crypt cells beyond that observed using WR-2721 alone, six-day survival is increased in a greater than additive fashion (215). This drug combination is slightly less than additive in the 30-day survival assay (2). Misoprostol effectively protects intestinal clonogenic cells in combination with WR-2721 and increases the survival of intestinal clonogenic cells and mice exposed to neutrons (225). In contrast, WR-2721 followed by DMPG prior to neutrons does not improve the survival of mice over those receiving only WR-2721 (256).

4.0.5. Indomethacin Plus Thiols

Preirradiation INDO, which stimulates hemopoietic stem cell proliferation, and WR-2721 combines to give a greater-than-additive protection of endogenous CFU-S and increases survival (257). WR-2721 does not compromise the ability of INDO to potentiate local tumor control in mice bearing FSa tumors, nor does INDO compromise the ability of WR-2721 to protect against hair loss or leg contracture. Clearly, the combination of INDO and WR-2721 may be valuable if hemopoietic toxicity is the limiting factor in XRT. Combined preirradiation INDO and cystamine also synergistically enhances the recovery of hemopoiesis in sublethally irradiated mice (258). These effects, however, do not translate into an increased survival, apparently because of the GI toxicity of the drug combination. For less toxic combinations, such as diclofenac and WR-2721, additive protection of survival has been observed (258).

4.0.6. Bryostatin and Hemopoietic Growth Factors

Bryostatin 1 is a macrocyclic lactone PKC activator that, in combination with hemopoietic growth factors, exerts a variety of effects on radiosensitivity both *in vitro* and *in vivo* (259). A concomitant 24-h preirradiation treatment using GM-CSF-bryostatin 1 or other PKC activators enhances *in vitro* radioprotection of day-14 CFU-GM. Treating cells using GM-CSF-bryostatin 1 immediately after irradiation also results in some radioprotection. GM-CSF-bryostatin 1 pretreatment also protects enriched ($CD34^+$) progenitors. In contrast to the results with GM-CSF, preincubation of cells using bryostatin 1 and the GM-CSF/IL-3 fusion protein PIXY 321 does not lead to increased radioprotection of total day-14 CFU-GM. However, the combination treatment selectively augments the radioprotective capacity of the hybrid cytokine toward noneosinophilic elements. Bryostatin 1 is itself radioprotective in lethally irradiated mice and can augment the radioprotective capacity of GM-CSF, although marked strain differences have been observed.

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