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Review Article

Radioprotectants: basic concepts, current status and future directions

Somnath Singh, Alekha K. Dash*
*Department of Pharmacy Sciences, School of Pharmacy and Health Professions, Creighton University, 2500 California Plaza, Omaha, NE, 68178, USA. *Correspondence: email: adash@creighton.edu*

CONTENTS

Abstract

Uncontrolled exposure to radiation from possible nuclear battlefields, outer space travel or accidents at nuclear power plants presents the greatest threat and challenge to the civilized world today. The consequences of ionizing radiation, either from intentional or unintentional sources, on cells and tissues are complex phenomena. Death from radiation exposure is the result of sequences of events which occur within a fraction of a second to several weeks. One of the most challenging tasks of radiobiology today is the development of pharmacological agents that can protect, repair and regenerate the early damage produced in cells and tissues by ionizing radiation. Protection depends on the ability of chemical agents to reduce the intracellular concentration of free radicals and reactive oxygen species that are produced within the first millisecond after irradiation. The first-generation chemical agents used as radioprotectants are compounds with antioxidant and scavenging potential. Various novel compounds are currently under clinical investigation as radioprotectants that can accelerate recovery of tissue stem cells and their precursors after radiation exposure. Current treatment strategies also include the use of immunomodulators and natural products with free radical-scavenging capability and the ability to induce bone morrow recovery.

Introduction

A radioprotectant is an agent or a drug used to block the adverse effects of ionizing radiation exposure that occurs or is likely to occur inevitably, unconsciously or accidentally. Ionizing radiation is defined as any kind of electromagnetic radiation, such as x- or gamma rays or particulate radiation, such as neutrons or alpha particles, that has enough energy to ionize atoms or molecules. Individuals may be exposed to moderate doses (1-10 Gy) of ionizing radiation during various medical treatments or space travel, or to a higher dose during unintentional exposure such as during a nuclear plant accident; in a worst-case scenario, a dirty radiological bomb could expose a large population to damaging lethal radiation (1). In today's world, which faces the constant threat of a nuclear/biological or chemical hazard, there is a need to protect not only high-risk service groups but also the general population from the health hazards of ionizing radiation exposure (2).

One of today's unfulfilled dreams is to find a pharmacologically active agent that can be taken orally prior to radiation exposure to provide maximum protection without undue side effects. Such a drug has yet to be developed and approved for human use. Time, distance and shielding are the three major guiding principles for protection against radiation. Unfortunately, it is not uncommon to have a situation where these three principles are not adhered to and overexposure can result in injury to cells. Hence, regardless of the nature or circumstances of radiation exposure, additional types and forms of bodily protection would be exceedingly useful and necessary. These may include medicinal agents, dietary supplements and selective engineered genetic and physical devices (2).

The resulting radiation injury is due to a series of molecular, cellular, tissue and whole-body exposures (3). Death from radiation injury arises from a sequence of events that occur directly or indirectly, as shown in Figure 1, over a period of less than a billionth of a second to several weeks (4). The effects of radiation on the human body can be divided into two main classes: somatic and hereditary. Somatic effects arise from damage to the ordinary cells of the body and affect only the irradiated person. The hereditary effects, on the other hand, arise only in the offspring as a result of radiation damage to the

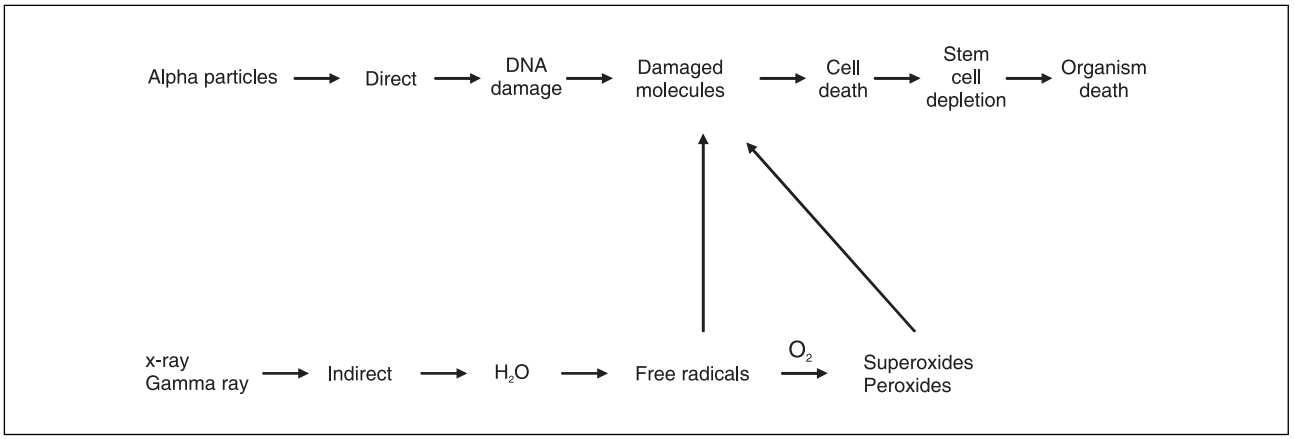

Fig. 1. Direct and indirect radiation effects on biological molecules leading to cell death (59).

germ cells in the reproductive organs. Radiation injuries from single-dose exposure are categorized into three syndromes (3) depending on the strength of the source. Cerebrovascular syndrome arises when exposure is greater than 100 Gy and death occurs within 24-48 h of exposure. Gastrointestinal syndrome is generally encountered when exposure is in the 5-12-Gy range and death occurs within 3-10 days. However, the victim may survive when exposure is at the lower end of this range. When exposure is in the 2.5-8-Gy range, survival is possible and this is categorized as the hematopoietic syndrome.

Mechanisms of action of radioprotectants

When a radiation beam passes through a living system, energy is transferred to tissues via photoelectric, Compton and pair production processes (5). The interaction of ionizing radiation with tissue water produces reactive free radicals, such as hydroxyl radicals, hydrogen radicals and hydrogen peroxide, which can damage or critically impair the normal functions of vital macromolecules like protein and DNA, leading to complex disease states collectively known as radiation injuries, which can be fatal depending upon the extent of radiation absorbed (6).

The development of chemical radioprotectants is based on antioxidants that can scavenge free radicals and thereby protect macromolecules from being altered/damaged due to radiation. Thiol radioprotectants consume oxygen in the cells by forming a byproduct, such as disulfide or hydrogen peroxide, which desensitizes the cells to radiation. The limitations of these radioprotectants are: they are short-acting, toxic and most effective when administered prior to radiation exposure. The second group of radioprotectants includes immunomodulators, which make radiation-exposed cells generate cytokines such as granulocyte–monocyte colony-stimulating factor (GM-CSF), erythropoietin, G-CSF, thrombopoietin and interleukins, which stimulate the activity of hematopoietic stem cells, thereby mitigating radiation-induced hematopoietic injury and reducing mortality (7).

Classification

A radioprotective agent is intended to ameliorate the side effects of radiation exposure when used either prophylactically or therapeutically (2). An ideal radioprotectant should have the following properties: 1) it can be easily taken orally without any undue side effects; 2) it must provide significant protection for the majority of organs against the damaging effects of radiation; 3) it must have an acceptable toxicity and stability profile; 4) it must be compatible with a wide range of concurrently administered drugs; 5) it must have a safe therapeutic window; and 6) it should be reasonably cheap. However, a single agent with all the above-mentioned properties has yet to be identified.

Chemicals used as radioprotectants differ widely in their structure and mechanism of action. It is difficult to categorize them solely on the basis of their pharmacological action or chemical structure. Therefore, they have been categorized here either on the basis of similarity of chemical structure or pharmacological action for the sake of simplicity of presentation. A list of currently used radioprotectants is shown in Table I.

Thiols

Many thiol antioxidants have been used as radioprotectants, but their application has been limited by their toxicity. There is some controversy regarding the role of antioxidants during radiotherapy. However, a majority of researchers support the conclusion that antioxidants do not interact negatively with radiotherapy used in the treatment of a wide variety of cancers, but in fact may help mitigate the adverse effects of such treatment (8).

Thiols were considered as the first generation of radioprotectants and contain a free or potential sulfhydryl group in their structure. One study compared the radioprotective efficiency of acetylcysteine amide (NACA) with a commonly used antioxidant, *N*-acetylcysteine (NAC). The protective effects of NACA and NAC were investigated using Chinese hamster ovary (CHO) cells irradiated

Table I: List of radioprotectants and their important features.

Category	Example	Mechanism of action	Salient points	Ref.
Thiol and synthetic radioprotectants	Amifostine	Free radical scavenging	FDA-approved, administered parentarally; better than *N*-acetylcysteine	1
	N-Acetylcysteine amide	Free radical scavenging		9
Nitroxides	Tempol	Free radical scavenging	Protects salivary glands but not tumor	15
Bisbenzimidazoles	Hoechst-33342	Compaction of chromatin and quenching of free radicals	Mutagenic and cytotoxic; synthesis of less toxic derivatives or administration transdermally is being tried	23
Superoxide dismutase and metal complexes	Mn–porphyrins, Orgotein®	Free radical scavenging	Short half-life, large molecular weight and potentially immunogenic	26, 27
Cytokines	Stem cell factor, Flt3 ligand, thrombopoietin and IL-3	Stimulation, maintenance and proliferation of hematopoietic stem and progenitor cells	Stem cell factor (SCF) and thrombopoietin work synergistically	29, 30
	ATP	Inhibits release of TNF-α; increases release of IL-10	Works even 3-6 h after radiation exposure	31
Immunomodulators	5-Androstenediol (5-AED)	Induction of cytokine release	Effective even when injected 2 h after irradiation	37
Herbal drugs	Liv-52	Reduce lipid peroxidation	Nontoxic	40
	Mace lignans	Antioxidant	Augmentation of apoptosis	44

with 6 Gy radiation by measuring various oxidative stress parameters, such as levels of reduced glutathione (GSH), cysteine and malondialdehyde (MDA), and activities of antioxidant enzymes such as glutathione peroxidase, glutathione reductase and catalase. The results indicated that NACA was capable of restoring GSH and malondialdehyde levels, caspase-3 and antioxidant enzyme activities to control levels. Moreover, NAC was found to be cytotoxic to cells at higher concentrations, whereas NACA was nontoxic at similar concentrations (9).

Aminothiols and their phosphorothioate derivatives have been the most extensively investigated radioprotectants. They supposedly act by scavenging free radicals, hydrogen transfer, inducing hypoxia or stabilizing DNA by direct binding (10). Amifostine (Ethyol®, WR-2721) is the most effective aminothiol drug and is in fact a prodrug, whereby the thioester bond is cleaved by membrane-bound alkaline phosphatase to release the free active metabolite WR-1065. WR-1065 provides radioprotection for a prolonged period by inducing superoxide dismutase (SOD) enzyme activity, which protects both nonmalignant and malignant cells from the harmful effect of radiation therapy. Although WR-1065 represents a novel approach for radioprotection in nonmalignant cells, persistently elevated radiation resistance in malignant cells is a potential concern in patients exposed to thiol-containing drugs (11).

Amifostine has been approved by the FDA as a radio- and chemoprotectant and has shown the highest dose reduction factor (DRF) in mice exposed to gamma radiation for 30 min. The DRF is defined as the ratio of the required radiation doses with drug/without drug to give equivalent response. Amifostine is administered intravenously (i.v.), although subcutaneous (s.c.) administration is reported to have some advantages over i.v. administration (1). This limits its application under emergency situations in the field. A biodegradable s.c. pellet of amifostine is reported to provide sustained blood levels of WR-1065 for 2 h after implantation, while s.c. injection showed a sharp peak in concentration at 30 min (12).

A combination of amifostine and 16,16-dimethyl-PGE_2 has been shown to have a synergistic effect, as the radioprotectant effect increased to a maximal DRF of 2.5 (13). The addition of nimodipine to WR-151327 also produced an additive radioprotective effect with DRF increasing from 1.46 to 1.67, and increased the magnitude and duration of the locomotor deficit compared with WR-151327 alone (14).

Nitroxides

Nitroxides work as radioprotectants via the mechanism of free radical scavenging. Tempol (4-hydroxy-2,3,6,6-tetramethylpiperidin-1-oxyl) is the most extensively studied nitroxide. It provided radioprotection of the salivary gland, but did not protect the tumor, which is hypothesized to be due to comparatively more rapid reduction of tempol to hydroxylamine (nonradioprotectant) in tumors than in normal tissues (15). Temporal treatment (i.p., i.v. or s.c.) 10 min before irradiation of the head of experimental mice significantly reduced the irradiation-induced decrease in salivary function, but i.m. administration was ineffective. However, in the same study, a mouthwash or a topical gel formulation was found to be effective (16). Clinical application of this agent is restricted because it produces hypotension and an increase in heart rate at the dose required for radioprotection. It also has a short time window of effect (17).

Tempol-H (hydroxylamine of the nitroxide tempol) was reported to have similar radioprotective efficiency as tempol, but is expected to be associated with a significantly

lower drop in blood pressure and a reduction in hemodynamic toxicity (18). Therefore, tempol-H may be further investigated as a potential candidate for clinical application to protect patients undergoing radiotherapy.

Ionizing radiation activates mitochondrial nitric oxide synthase (NOS) in the uroepithelial cells that line the urinary bladder, which leads to a cascade of events including inhibition of the respiratory chain, generation of excess superoxide, peroxynitrite production and nitrosative damage. It has been demonstrated that the presence of an NOS inhibitor in the bladder during irradiation is radioprotective (19). An NOS antagonist can be delivered to the bladder intravesically, but this is inconvenient. Therefore, systemic administration has been attempted, but was shown to cause adverse side effects such as hypertension or stomach motility. Mitochondrial targeting of these compounds conjugated with a peptide has been reported, which enhances their radioprotective effects and reduces the adverse complications. Moreover, these novel mitochondria-targeting peptides can be optimized for developing a sustained-release prodrug that can increase the duration of action significantly (20).

Bisbenzimidazoles

These compounds are capable of binding specifically with double-stranded DNA due to the presence of two benzimidazole groups and one phenol group. They show marked radioprotection through drug-induced compaction of chromatin and direct quenching of free radicals generated by radiation (21). Hoechst-33342 is an example of such a compound and showed a significant radioprotective effect in a mouse lung model when administered i.v. before irradiation (22). However, mutagenicity and cytotoxicity limit its use as a protector of normal tissues. Therefore, attempts were made to synthesize nontoxic analogues. Two of the analogues – one having two methoxy group substitutions and the other having a methoxy and a hydroxyl group – were reported to have significantly less toxicity and better radioprotective efficiency than the parent compound (23). Another approach to reduce toxicity could be to develop transdermal formulations.

Superoxide dismutase and metal complexes

The antioxidant enzyme SOD protects against the harmful effects of superoxide radicals by its free radical-scavenging activity. SODs convert superoxide to hydrogen peroxide, which is then removed by glutathione peroxidase or catalase. Thus, SODs prevent the formation of highly aggressive reactive oxygen species (ROS), such as peroxynitrite or hydroxyl radical (24). SODs are generally found to contain transition metals such as Cu, Zn or Mn.

The treatment of prostate cancer in mice with an Mn(III)-containing porphyrin complex has been shown to enhance the effectiveness of radiation therapy, as measured by the reduction in intratumoral hypoxia-inducible factor 1α (HIF-1α) and tumor necrosis factor α (TNF-α) (25). In another study, a comparison was made between two Mn–porphyrin complexes for their ability to protect lungs from radiation-therapy induced injuries. The compound with a lower lipophilicity was found to have better efficiency (26). In a study in 100 rectal cancer patients, Orgotein®, a Cu/Zn-containing SOD, was reported to reduce radiation injury specifically in the lower abdomen (27).

The potential clinical applications of SODs and their metal complexes are severely limited by their short half-life, large molecular weight and potential immunogenicity. Therefore, researchers are attempting to develop synthetic SODs having a long half-life, low molecular weight and reduced toxicity (28).

Cytokines

Ionizing radiation causes injury to hematopoietic tissues and progenitor cells, which reduces circulating blood cells, resulting in septicemia, hemorrhage, anemia and death. Therefore, one of the approaches for designing novel radioprotective agents could be the stimulation, maintenance and proliferation of hematopoietic stem and progenitor cells (HSPCs) from bone marrow, which are responsible for producing blood cells. Cytokines are known to stimulate hematopoietic stem cells (29).

Apoptosis is reported to play a major role in the death of the radiosensitive HSPCs soon after irradiation. Therefore, antiapoptotic cytokine combinations such as stem cell factor (SCF), Flt3 ligand, thrombopoietin and interleukin-3 (IL-3) may act as radioprotectants, particularly when these factors are administered early (29). A combination of SCF and thrombopoietin synergistically protected $CD34^+$ colony-forming units (CFU)-megakaryocytes against x-ray-induced death (30).

Proinflammatory cytokines are responsible for some of the complications in healthy cells due to the use of radiation in cancer patients. Adenosine triphosphate (ATP) is reported to inhibit the radiation-induced release of TNF-α but increase the release of IL-10, a cytokine synthesis-inhibitory factor. Therefore, ATP administration 3 and 6 h after irradiation alleviates radiation toxicity to blood cells by inhibiting radiation-induced inflammation and DNA damage (31).

Immunomodulators

Immunomodulators can induce cytokine release, which can stimulate the growth, differentiation and proliferation of HPSCs. β-Glucans are water-soluble polysaccharides that are reported to possess immunopharmacological activity and presumably act as biological response modifiers. Intraperitoneal injection of β-glucan was shown to greatly delay mortality in mice exposed to whole-body x-radiation and tumor growth in tumor-bearing mice. The radioprotective effect of β-glucan is reported to be partly due to its hematopoietic action, because leukocyte and lymphocyte numbers are increased after its administration. Moreover, both natural killer (NK) and lymphokine-

activated killer (LAK) cell activities are significantly increased by repeated doses of β-glucan, which may play a role in preventing secondary infection associated with irradiation, and probably contributes to the attenuated tumor growth in tumor-bearing mice through enhanced antitumor immunity (32).

Ginsan is a polysaccharide extracted from *Panax ginseng* that is known to have multiple immunomodulatory effects. A dose of 100 mg/kg of ginsan in mice before gamma radiation significantly increased the survival rate, with a DRF of 1.45, which may be due to increased viability of bone marrow cells against gamma radiation, as indicated by increased allogeneic $CD4^+$ T lymphocyte proliferation and IL-12 (33). This protective effect was also ascribed to increased mRNA expression levels of TNF-α, IL-1β, IL-6, SCF and GM-CSF observed with ginsan treatment (34).

Oxymetholone, an anabolic androgenic steroid, can stimulate bone marrow cells and increase blood cells in peripheral blood vessels. Therefore, it has been investigated for its potential radioprotectant effect. Oxymetholone was administered orally to mice 24 h prior to 8 Gy gamma radiation. The survival rate was found to be 75%, with a DRF of 1.14, in the groups receiving oxymetholone at a dose of 640 mg/kg *versus* only 15% in control groups after 30 days of irradiation (28).

The steroid 5-androstenediol (androst-5-ene-3β,7β-diol, 5-AED) is reported to enhance immune function and promote survival after whole-body exposure to ionizing radiation (35). Improvements in survival were observed when it was administered by s.c. injection to mice between 24 h before and 2 h after gamma radiation, with a DRF of 1.3. The mechanism involved was an increase in circulating neutrophils, platelets, NK cells and granulocyte–monocyte progenitors in bone marrow (36, 37). However, a recent study suggested that radioprotection is not dependent on the 5-AED concentration at the time of irradiation, but rather on events triggered during the first few hours after administration. Therefore, further studies are warranted to test the role of cytokines in the radioprotective effects of 5-AED (38).

In the homeopathic system of medicine, a high dilution of thymilin, a thymic hormone, and bursin, a tripeptide isolated from bursa fabricii in birds, was found to possess immunomodulating effects, which indicated the possibility of treating immunosuppressed animals by using high dilutions of endogenous molecules belonging to the immune system (39).

Herbal drugs

Liv-52, a well-known hepatoprotective product of Himalaya Herbal Healthcare (India), is reported to reduce gamma radiation-induced lipid peroxidation and increase glutathione concentrations 31 days following exposure in experimental mice (40). An aqueous extract of *Podophyllum hexandrum* (RP-1) has been reported to provide protection and more than 82% survival against whole-body lethal (10 Gy) gamma radiation in mice. Pretreatment with RP-1 was found to counter the radiation-induced decrease in IL-1, IL-3 and immunoglobulins in the serum of mice (41).

The aqueous and hydroalcoholic extracts of *Rhodiola imbricata* were investigated for protection against whole-body lethal gamma radiation (10 Gy)-induced mortality in Swiss albino strain "A" mice. Pre-irradiation administration of the aqueous extract produced > 90% survival, while the hydroalcoholic extract produced > 83% survival beyond the 30-day observation period. The number of CFU per spleen in irradiated mice was 1.91 ± 0.15, while in mice given the aqueous or hydroalcoholic extract 30 min before irradiation (10 Gy), it increased to 17.3 ± 0.67 and 15.6 ± 0.61, respectively (42).

The effect of various doses of a 50% ethanolic extract of Chyavanaprasha (an Ayurvedic rejuvenating herbal preparation) was studied on the survival of mice exposed to 10 Gy of gamma radiation. Treatment with Chyavanaprasha consecutively for 5 days before irradiation delayed symptoms of radiation sickness and the onset of mortality when compared with the untreated irradiated controls (43).

The lignans obtained from the aqueous extract of fresh nutmeg mace (aril of the fruit of *Myristica fragrans*) were found to possess antioxidant properties in cell-free systems and protected the PUC18 plasmid against radiation-induced DNA damage. These mace lignans protected splenocytes against radiation-induced intracellular ROS production in a concentration-dependent manner. Moreover, mace lignans inhibited the proliferation of splenocytes in response to the polyclonal T cell mitogen concanavalin A via cell cycle arrest in the G1 phase and increased apoptosis (44).

A new group of radioprotectants referred to as nutraceuticals has been investigated, including plant flavonoids such as orientin, genistein (45) and vitamin E (46). α-Tocopherol succinate was shown to be the best of all the tocopherols investigated (47). These natural radioprotectants provide a lesser degree of protection against radiation as compared to the synthetic aminothiols. However, these compounds have shown reduced toxicity compared to the aminothiols.

Novel radioprotectants

Radioprotectants under investigation or recently reported that have potential for future development are shown in Table II along with important features.

Antioxidants

Naturally occurring antioxidants, such as vitamin E and selenium, are less effective radioprotectants than synthetic thiols, but may provide a longer window of protection against lethality and other effects of low-dose exposure (8). Some natural antioxidants have antimutagenic properties that need further investigation with respect to long-term radiation effects. Furthermore, modulation of endogenous antioxidants, such as SOD, may

Table II: List of novel radioprotectants and their salient features.

Radioprotectant	Salient features	Ref.
Melatonin	Relatively nontoxic, optimum dose not known	49, 50
Eckol	Decreases proapoptotic p53 and Bax; increases antiapoptotic Bcl-2	54
Resveratrol	Suppresses antiapoptotic proteins c-FLIP and Bcl-xL	55
Vitamin E and selenium	Less effective but longer time window	8
Nimodipine, propranolol and methylxanthines	Can be combined with phosphorothioates	48
Anti-TGF-β antibody	A target pathway for further investigation	51
TAT–BH4 complex	Protects against apoptosis even when administered after irradiation	53
Polyamino acids modified with sugar	Biodegradable and biocompatible, nontoxic	56
Benzylstyryl sulfones (ON-01210; Onconova Therapeutics)	Arrest normal cells at the G1 and G2 stage of the cell cycle; safe	57, 58

be useful in specific radiotherapy protocols. Some drugs (nimodipine, propranolol and methylxanthines) that have antioxidant properties in addition to their primary pharmacological activity may have utility as radioprotectants when administered alone or in combination with phosphorothioates (48).

The main secretory product of the pineal gland of the brain, melatonin, is reported to ameliorate oxidative injury due to ionizing radiation, although the optimum dose has yet to be determined (49). Administration of 25 μg melatonin/100 g body weight/day for 4 weeks to the Indian tropical male squirrel *Funambulus pennant* was found to protect the hematopoietic system and lymphoid organs following 2.06 Gy x-ray exposure. It was found that total leukocyte count, lymphocyte count, SOD activity and total antioxidant status were reduced, and lipid peroxidation and apoptosis were increased more in saline-treated squirrels (controls) than in melatonin-treated squirrels (50).

Cytokines

Administration of a single i.p. dose of 1.0 mg/kg of an anti-TGF-β (transforming growth factor β) antibody was associated with decreased morphological changes, inflammatory response and expression and activation of TGF-β in lungs 6 weeks and 6 months after 40 Gy irradiation to the right hemithorax (51). Therefore, it appears that targeting the TGF-β pathway may be a useful strategy to prevent radiation-induced lung injury.

The direct effect of ionizing radiation on hematopoietic tissues reduces neutrophil count and platelet number. Their reduction in the circulation may lead to septicemia, hemorrhage, anemia and death. One of the strategies currently used in the development of novel radioprotectants is the identification of an agent or agents that can stimulate, maintain and induce the proliferation of progenitor cells from bone morrow. Combined use of different cytokines has been shown to enhance neutrophil and platelet recovery and survival after irradiation (7).

TNF-α, a pleiotropic inflammatory cytokine, has been implicated in radiation-induced toxicity to healthy cells, which limits the delivery of high-dose radiation to tumors. In one study investigating the role of the TNF-α pathway in tumor radiotherapy, mouse lung was irradiated with various doses of radiation and assessed for TNF-α production at various time points. Two types of mice were used, C57BL and BALB/c mice, in which the TNF-α receptor (TNFR1) was inhibited by the genetic knockout method and therapeutic silencing with antisense oligonucleotide administration, respectively. The results indicated that inhibition of TNFR1 tended to preserve lung function without compromising lung tumor sensitivity to radiation. Therefore, inhibition of the TNFR1 pathway could be a novel strategy for radioprotection of healthy normal cells (52).

Antiapoptotic agents

The BH4 peptide domain of the antiapoptotic protein Bcl-xL can be covalently attached to the TAT peptide transduction domain (TAT–BH4), which can be delivered to cells and protect them from radiation-induced death. Isolated human lymphocytes treated with TAT–BH4 were protected against apoptosis when exposed to 15 Gy radiation. Splenocytes and thymocytes in mice treated with TAT–BH4 either before or after exposure to 5 Gy radiation were found to be protected from radiation-induced apoptotic cell death (53). Therefore, by targeting steps involved in the apoptotic signaling pathway, it could be possible to develop even postexposure treatments to protect tissues from radiation injuries.

Some natural compounds have been found to possess radioprotective effects due to their antiapoptotic activity. Eckol is a component of the seaweed *Ecklonia cava* which has been investigated for its cyto- and histoprotective effects on lymphocytes and the intestine against the damage caused by whole-body irradiation. It was found that eckol protected lymphocyte viability and rescued intestinal cells from radiation-induced apoptosis by decreasing the amount of proapoptotic p53 and Bax and increasing the amount of antiapoptotic Bcl-2 (54).

Conversely, apoptotic agents have been investigated for the sensitization of cancer cells to radiotherapy.

Resveratrol, a polyphenolic phytoalexin, inhibits STAT3 (signal transducer and activator of transcription 3) and NF-κB-dependent transcription in melanoma cells, which results in suppression of c-FLIP and Bcl-xL expression involved in inhibition of the apoptotic signal, while activating the MAPK- (mitogen-activated protein kinase) and the ATM/Chk2/p53 pathways mediating apoptosis. Resveratrol also upregulates TRAIL (TNF-related apoptosis-inducing ligand) promoter activity and induces TRAIL surface expression in some melanoma cell lines, resulting in rapid development of apoptosis. It has been found that sequential treatment of melanoma cells first with gamma radiation to upregulate TRAIL-R surface expression and then with resveratrol to suppress the antiapoptotic proteins c-FLIP and Bcl-xL and induce TRAIL surface expression had dramatic effects on the upregulation of apoptosis in some melanoma lines, such as SW1 and WM35 (55).

Polyamino acids

A U.S. patent has been issued for using water-soluble polyamino acids and/or their derivatives modified with sugar components for protection against radiation (56). The polyamino acids used in the invention could be either natural or synthetic, and they could be used in various forms, such as films, sheets, coating and paste in cosmetic products. They are biodegradable, biocompatible and do not cause antigenicity, and they could therefore be investigated for delivering other radioprotectants as transdermal or implant formulations.

Synthetic compounds

ON-01210 is a chlorobenzylsulfone derivative developed by Onconova Therapeutics with potential for mitigating the effects of accidental or intentional exposure to life-threatening levels of radiation (57, 58). This compound is uniquely specific to target mammalian cells and has the ability to arrest normal cells at the G1 and G2 stage of the cell cycle. Alkaline comet assays showed that exposure of cells to ON-01210 before irradiation results in significant protection from DNA damage in comparison to untreated cells. Furthermore, toxicological studies did not show any marked difference in the behavior and physiological state of ON-01210-treated and untreated animals. Thus, this compound is expected to be pharmacologically safe (57).

Future research recommendations

Injury from radiation exposure, irrespective of the source, is due to a series of molecular, cellular, tissue and whole-body processes. There still exists a gap in our understanding of the mechanisms of ionizing radiation in humans. Further research is required in the areas outlined below, which will help in bridging this gap and in developing novel radioprotectants in the future (3).

- Determine the genetic and epigenetic mechanisms governing individual susceptibility to radiation
- Develop the methods to characterize genomic and proteomic markers quantifying radiation exposures in the moderate range of radiation exposure
- Develop acute and long-term *in vivo* studies to determine the consequences of radiation-induced stem and parenchymal cell dysfunction
- Establish long-term organ and animal toxicity studies of ionizing radiation alone and in combination with radioprotectants
- Determine molecular targets that can be used for intervention in radiation-induced injury
- Determine the role of oxidative stress and antioxidants in the cellular and tissue response to ionizing radiation

Despite the extensive research conducted over the last few years to develop an effective radioprotective drug, there is no approved drug of this class available to date. Amifostine is the only FDA-approved drug used for the prevention of xerostomia encountered during radiation. The first-generation radioprotectants, particularly aminothiols, present unacceptable side effects at doses needed for radioprotection. Therefore, the search for less toxic radioprotectants continues. More research needs to be conducted if we are to develop effective radioprotectants against high-LET (linear energy transfer) radiation. Development of these new-generation radioprotectants requires a clear understanding of the mechanisms of radiation injury at the molecular and cellular levels. Identifying these mechanisms and finding ways to deliver the newer generation radioprotectants efficiently to the target site by novel drug delivery approaches will definitely stimulate cells and tissues to protect them against this injury.

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