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## Radioprotection

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### Abstract

Over 40% of cancer patients will require radiation therapy during management of their disease. Although radiation therapy improves the survival of a significant number of cancer patients, both acute radiation toxicity (that which manifests during a course of clinical radiotherapy or shortly thereafter), and late toxicity (developing months to years after completion of radiotherapy) compromise overall outcomes for successfully treated cancer patients.

### Keywords

radiation damage; protector drugs

### Introduction

Despite improvements in the development of clinical radiotherapy treatment planning and treatment delivery technologies (Table I), there remains a significant toxicity of radiotherapy to normal tissues and organs.(1–3) Improved local control of cancer by radiotherapy dose escalation in patients with, for example, lung, esophagus, colorectal, pancreas or pelvic malignancies is associated with significant acute toxicity and normal tissue damage; however, higher radiation doses which are likely to be more effective cannot be used in these patients owing to acute toxicities occurring during the clinical course of radiotherapy.(4–7) These acute toxicities are associated with the tissue inflammatory response and are not always limited to the normal tissue in the irradiation beam. Acute toxicity can extend beyond the treated area. Examples include esophagitis (difficulty swallowing) and pneumonitis (cough, fever, lung fluid accumulation) in the lung cancer patient, and intestinal and rectal irradiation-induced inflammation (diarrhea, cramps, abdominal pain) in the colorectal cancer patient. Acute toxicities are usually transient and symptoms resolve weeks after completion of treatment. Indeed, acute side effects may limit the patient's capacity to comfortably complete a treatment course. Furthermore, there is renewed concern, about the occurrence of late-manifesting toxicities (defined as those appearing months to years after completion of a successful treatment course) in patients treated with radiotherapy. Late toxicity is usually limited to tissues treated and does not usually affect survival; however, late effects including fibrosis (scarring) and organ functional failure may ensue depending on the volume of tissue treated and dose of irradiation delivered.(5–7) Therefore, to ameliorate these toxicities and thereby improve the therapeutic ratio (that is ratio of cancer cell killing to normal tissue toxicity caused by a given dose), radioprotective drugs are receiving significant interest.(1–2,8–11)

The molecular pathways that are utilized for radiation protection follow on current knowledge regarding the molecular biological mechanisms of ionizing irradiation induced cell killing at the level of single cells, tissues and organs (Figure 1). Ionizing irradiation, hits oxygen and

water molecules in cells and results in production of radical oxygen species (ROS) such as superoxide and hydroxyl radical which deplete cellular antioxidant stores, most prominently glutathione.(8,9) Replacement of cellular antioxidant capacity by increasing levels of the enzyme Manganese Superoxide Dismutase is an example of one radioprotector strategy at the cellular level.(10–11) Both dying and surviving cells within an irradiated tissue cell release inflammatory cytokines which can act as cytotoxins at both the local tissue level and also through the blood circulation, can affect distant sites *via* action on specific cellular surface receptors.(3–4) Agents which limit cytokine binding or action at the cellular receptor level include the TLR5(12) receptor agonist and provide another strategy for radiation protection. Therefore, recognition of potential radioprotective pathway targets is based on understanding the underlying molecular biology of irradiation cellular killing.

This article will address current and future strategies for development of radioprotective agents, both systemically delivered and organ specific targeted radioprotectors. Radioprotectors are being developed for the purpose of both reducing acute radiotherapy side effects and minimizing late chronic radiation toxicity in the cancer patient.

## Ionizing Irradiation Clinical Effects

Ionizing irradiation causes significant toxicity at the single cell, tissue and organ level, and the clinical effects of therapeutic irradiation depend upon the dose delivered and the volume of tissue exposed.(1,3,6–7) For example, if a tumor volume is large, this necessitates ionizing irradiation delivery to a significant volume of normal tissue. There is a non-linear relationship between dose of irradiation and cell death.(3) Cell phenotype within a tissue and tissue specific differences in irradiation response determine the shape of the cell killing curve. For example lymphoid tissues such as the thymus are relatively radioresponsive compared to skeletal muscle tissue. Dose rate (the quantity of irradiation delivered per minute), fraction size (dose delivered per treatment session) and level of oxygenation of the tissues treated directly increase cell death.(3) Irradiation not only kills tumor cells, but also proliferating normal cells. Both normal and tumor tissue contain a subset of dividing cell populations, and quiescent or non-dividing subsets. Quiescent cells are relatively resistant to ionizing irradiation killing.(3) Rapidly dividing normal and tumor cell populations are more susceptible than those cells which are either slowly proliferating or non-proliferative. However, unlike normal tissues, rapidly dividing tumor cell subsets can outdistance their blood supply and become hypoxic.(3,13,14) Since oxygen is a main molecular target for irradiation production of ROS, hypoxic cells in tumors show relative radioresistance.(13–14 )

Release of cytotoxic inflammatory cytokines from irradiated tissue can also recruit inflammatory cells including lymphocytes, macrophages and polymorphonuclear leukocytes which then infiltrate tissues and cause further normal cell killing(4,15–16) through the generation of yet other inflammatory cytokines and byproducts, including more ROS.(17–18) The cellular and tissue specific pathways involved in irradiation killing are shown in Figure 1.

## Ionizing Irradiation Toxic Effects

### Acute Effects of Ionizing Irradiation

Depending on the anatomic site treated (Figure 2) acute effects may include: nausea and vomiting, tiredness, fatigue, diarrhea, headache, as well as normal tissue swelling, skin erythema, cough, difficulty swallowing and difficulty breathing.

The acute effects of irradiation are based on both normal tissue response and tumor cell killing following on the underlying molecular biological effects of ionizing irradiation. Within tissues

and organs, ionizing irradiation kills dividing cells by both stochastic (random) and determinative (microenvironment induced) mechanisms.(19) Dividing cells in the DNA synthetic or (S) phase of the cell cycle are relatively less sensitive to radiation killing compared to those in mitosis (M) or in the second gap (rest phase between DNA synthesis and mitosis). (3) Those cells in metabolic quiescence (non-dividing) and those in relatively hypoxic areas are less sensitive to irradiation killing.(3,13) Direct irradiation killing leads to elimination of those cells from the tissue and organ. Both direct and indirect (mediated by cytokine and ROS release from dying cells) irradiation killing effects are significant in influencing the magnitude and duration of acute side effects. In cells that repair irradiation damage and survive, release of inflammatory cytokines including transforming growth factor  $\beta$ 1 (TGF $\beta$ 1), Interleukin 1 (IL-1), and tumor necrosis factor  $\alpha$  (TNF $\alpha$ ) act both locally within the irradiated tissue/tumor, and enter the systemic circulation where tissues outside the irradiation beam can experience cell killing.(4,15,20) Inflammatory cytokine binding to specific receptors on sublethally irradiated or unirradiated cells (outside the irradiation volume), leads to cell death through the apoptosis (programmed cell death) pathway(17–18,21), autophagy(22) and necrosis(4) death pathways.

Cells and tissues recover from irradiation acute effects in a variety of ways. Surviving cells within irradiated tissue and those in adjacent unirradiated tissue, particularly in the primitive or stem cell compartments are induced to proliferate and repopulate areas in the tissue that were depleted by irradiation killing.(3) In addition, stem cell or progenitor populations from outside the irradiated tissue migrate into the irradiated volume and facilitate repopulation or replenishment of tissue function.(23–25) Stem cell populations involved in regeneration of irradiated tissue (epithelial progenitor cells for example in the irradiated oral cavity, esophagus or intestine) include those which home to sites in the vacated tissue microenvironment, depleted by irradiation killing. For example, endothelial cell progenitors of bone marrow origin migrate in and repopulate blood vessel endothelial cells killed by irradiation.(25) Repair and replenishment of irradiated tissue is also facilitated by migration into the irradiated area of lymphocytes, macrophages and neutrophils which elaborate reparative cytokines including vascular endothelial growth factor I (VEGF-1), hepatocyte growth factor (HGF), fibroblast growth factor (FGF) and epidermal growth factor (EGF).(26)

Thus, recovery from acute irradiation effects occurs at the cellular level by restoration of antioxidant pools through biochemical synthesis of glutathione and upregulation of antioxidant enzymes(8,17–18), and at the tissue level by stem cell mediated repopulation through both proliferation of *in situ* cells and by migration into tissues *via* the circulation of progenitor cells from distant sites.(3,23–25)

### Chronic Effects of Ionizing Irradiation

Chronic irradiation effects are critically important in all patients, but particularly in those who receive total body irradiation (TBI). Total body irradiation is utilized in some cancer therapies particularly for patients who require a bone marrow transplant.(27–29) TBI is delivered either in single fraction or in multiple fractionated courses designed in part to clear space in the bone marrow by causing apoptotic death of a sufficient number of hematopoietic stem cells and their progeny for homing and proliferation of donor hematopoietic stem cells that are injected intravenously. Observing responses to total body irradiation in both experimental animal models and in clinical radiotherapy patients demonstrates multiple chronic effects including features common to premature aging such as hair graying, skin thinning and dryness, formation of cataracts, early myocardial fibrosis, myocardial infarction, neurodegeneration and osteopenia/osteomalasia.(3,30) In pediatric and adult long term cancer survivors, who received total brain irradiation, neurocognitive defects have been detected.(3,31–33) Radiation may induce life shortening by decreasing endocrine function through glandular cell death or by

system wide depletion of antioxidants which are exhausted by perpetual response to persistent ROS production in tissues.(34–35) During aging, ROS production is persistent, so the requirements for continual replenishment of antioxidant stores including glutathione, superoxide dismutase, catalase and glutathione peroxidase increase.(34) Furthermore, accumulation of increased levels of p53, p21 and BAX in naturally aging cells or those prematurely aging after irradiation may slow proliferation and return more cells to quiescence. (30,35) Therefore, long-term consequences that follow the cellular, tissue and organ responses to ionizing irradiation, are common to those of aging.

Chronic or late effects depend upon the organ treated, but are also directly related to irradiation dose and volume treatment(3) (Figure 2). Response to irradiation acute effects may contribute towards the manifestation of chronic effects.(3) After recovery from the acute effects of irradiation, tissues appear normal *in situ* and under the microscope.(3,30,35) There may be no obvious sign of irradiation injury. Current experimental evidence suggests that both endothelial cells in blood vessels recovering within irradiated tissues and those supplemented by endothelial progenitors which migrate into the tissues from bone marrow origin are depleted of intracellular levels of thrombomodulin.(36) Irradiated tissues which recover from the acute effects continue to produce ROS which may continually deplete thrombomodulin.(20,36) Upregulation of cell-destructive proteases can also initiate a second wave of apoptosis.(36) Endothelial cell death has been associated temporally with accumulation in irradiated tissues of fibroblast progenitor cells migrating in from the bone marrow microenvironment.(37) The signals that elicit this migration are unknown, but accumulation of fibroblast progenitor cells leads to their proliferation in irradiated tissues, and functional inactivation in that organ by replacement of functioning areas with scar tissue.(4,15,20)

Mechanistic models for irradiation chronic effects have been aided by observations in animal models. For example, there is a relative decrease in histopathologic evidence of late irradiation fibrosis, in animal strains genetically altered to mask the expression of inflammatory cytokines such as (TGF $\beta$ )(38–40), or deleted for expression of enzymes required for generation of free radicals (nitric oxide synthase-1, neuronal NOS,) as in mitochondrial NOS knockout mice. (41) Animals deficient in the signaling response to irradiation induced TGF $\beta$  (SMAD3-deficient mice) demonstrate decreased irradiation-induced late fibrosis in skin and lung.(38–39) Chronic side effects are not limited to fibrosis, but include abnormal blood vessel formation called telangiectasias(19), ulcerations and organ failure.(42–44)

A prominent chronic effect of ionizing irradiation is carcinogenesis/leukemogenesis. The irradiated tissue microenvironment can prevent apoptosis of damaged proliferating cells by cell to cell contact.(30,45–53) Irradiation induces cell cycle growth delay by both a G1 (gap in the cell cycle between mitosis and DNA synthesis) and a G2 (block in the cell cycle following mitosis) growth arrest.(3) Holding cells in growth arrest creates a condition of quiescence.(3, 16) Prolonged production of ROS in cells of the microenvironment of the irradiated lung(54) and bone marrow(55–56) months to years after irradiation can potentially induce genetic change in other quiescent cells. Furthermore, migration of a stem cell population from distant sites into an irradiated microenvironment can expose those homing stem cells to ROS released from irradiated stromal cells causing mutations and even malignant transformation.(24–25, 57–58) Therefore, the persistent elaboration of both ROS and humoral cytokines by surviving cells within an irradiated tissue/organ facilitate chronic interaction with other cells that are attempting to repopulate and restore that tissue and organ.

**Systemic Effects**—Systemic effects of ionizing irradiation have been well described in subtotal body as well as total body irradiated experimental animals and in humans.(3,27–30) Systemic effects include both acute and chronic effects as described above, but with several unique features. In particular, systemic effects include symptoms in areas that were not

irradiated including overall tiredness and easy fatigability, and are probably caused by the persistent elaboration through the circulation of inflammatory cytokines.(4,16,20)

Systemic effects apply to the response to subtotal body or regional irradiation such as a thoracic, abdominal or pelvic irradiation volume, as well as total body irradiation effects and are described as syndromes. The experience from clinical radiotherapy, principally total body irradiation to prepare patients with leukemia, lymphoma or disseminated cancer for a life saving marrow transplant, led to description of several syndromes of radiation toxicity.(59) A common principle with many of these syndromes is that partial body shielding greatly decreases the severity and the experience of the syndrome. A second basic principle is that protection from each syndrome is associated directly with reduced radiation dose rate and total dose.(3)

The **central nervous system syndrome** is associated with doses above 800 cGy total body dose or higher doses to the head and presents with signs and symptoms of brain swelling including nausea and vomiting, headache, sweating, rapid heart rate and rapid death. The **gastrointestinal syndrome** associated with TBI doses above 500 cGy presents with nausea, vomiting and diarrhea, and is associated with destruction of intestinal crypt and endothelial cells in the intestine, dehydration, severe abdominal pain, infection and blood loss.(69) The **hematopoietic syndrome** is associated with TBI doses above 300 cGy and a decrease in peripheral white blood cell count, platelet count, red blood cell count, and in the absence of source of bone marrow transplantation to replace damaged hematopoietic stem cells, may lead to death from infection, hemorrhage, weakness and fatigue.(59) The **immunosuppression syndrome** is associated with TBI doses as low as 100 cGy. Lymphocytes are the most radiosensitive cells in the peripheral blood, and thus a basic radiological biomarker dose sustained involves the magnitude of a decrease in slope of a peripheral blood lymphocyte count. Lymphocyte decrease can be associated with immunosuppression and susceptibility to infection, weakness and fatigue.(3)

Other systemic clinical effects are associated with partial body irradiation, such as the high dose irradiation induced **cutaneous syndrome** skin burns (beta burns) caused by local high doses above 30 Gy by electron irradiation or from the accumulation of radioactive isotopes on the skin. This syndrome is associated with erythema/redness, ulceration of the skin, heat loss, extravasation of fluids, lymphedema, hemorrhage and secondary infection.(3)

Details of the Hematopoietic Syndrome reveal many important radiobiologic principles. It is a collection of symptoms and signs associated with suppression of bone marrow function. This results in reduction of the number of peripheral blood red cells, platelets, and leukocytes (white cells). Individuals experience tiredness associated with anemia (low red cell count), propensity for bleeding (associated with low platelets), and inability to fight infections (associated with decreased white blood cell count). Shielding of as little as 10% of the bone marrow volume during total body irradiation can result in successful repopulation of the entire hematopoietic system by bone marrow stem cells that were in the protected microenvironment and can ameliorate or even prevent the Hematopoietic Syndrome.(3,59) The production of inflammatory cytokines including TNF $\alpha$ , TGF $\beta$ 1 and IL-1 correlates with the severity of suppression of hemopoiesis.(4,20) Within the dose range required to cause the hematopoietic syndrome in humans (200 – 600 cGy total body dose) there are individuals who show reduced severity of depression of hemopoiesis (less of a decrease in white cell, platelet and red cell counts as well as immunosuppression by reduction of T-cell and B-cell numbers). These individuals may have less systemic cytokine production by irradiated tissues compared with others that develop severe hematopoietic depression.(59) The reason for individual patient variation in susceptibility to the Hematopoietic Syndrome is unknown, but genetic factors tend to make some individuals more sensitive. These include individuals with ataxia, telangiectasia (60–63), Fanconi anemia (64–66), Werner's Syndrome(67), Blooms Syndrome(67–68) and



other categories of radiation sensitive individuals also termed “hyper-responders”(61) with no known genetic defect, but with an exacerbated response to irradiation doses compared to other individuals.

With all syndromes, genetic predisposition to irradiation toxicity can shift the radiation dose response curve to the left in effect giving the individual a greater chance of experiencing the toxicity at a lower sustained dose of irradiation. Other conditions known to increase sensitivity of individuals to side effects of irradiation include those associated with DNA strand break repair such as ataxia telangiectasia(60), Werner’s syndrome(67), Bloom’s Syndrome(68) and Fanconi Anemia.(66) Of importance to the physician, there are subsets of individuals with no genetic markers, but with greater sensitivity to ionizing irradiation called “hyper-responders”. (3,61) Whether these individuals have a greater irradiation induction of inflammatory cytokines or defect in regulation of endothelial cell function is unknown. Patients likely to develop pulmonary complications of lung irradiation include those with increased serum levels of TGF $\beta$  detected within the first weeks of radiotherapy.(15)

Acute, chronic and systemic responses to ionizing irradiation illustrate many common pathways in normal cellular, tissue and organ tissue repair. Knowledge of the underlying molecular biological pathways initiated by irradiation-induced DNA strand breaks, cellular apoptosis, and cell to cell interaction, including the elaboration of inflammatory cytokines, helps define several pathways for development of radioprotective agents.

**Radioprotective Pathways**—The mechanistic/biological basis for development of a radioprotective strategy necessitates an understanding of the molecular biology underlying the mechanism of the cellular, tissue and organ specific radiation damage response. Examples of the pathways for focus are shown in Figure 3 and include: nuclear DNA strand breaks, communication of nuclear stress responses through the cell cytoplasm to mitochondria, mitochondrial response to nuclear signaling, and mitochondrial initiation of apoptosis.(47–48,69) Finally other cells respond to the inflammatory cytokine cascade that follows cell killing in a second wave of cell death.(3) This second wave may slowly persist or may occur in a delayed but severe fashion leading to the rapid onset of what is called chronic effects described above.

Agents delivered prior to the initiation of radiotherapy would be the ones expected to target critical biochemical pathways in cells yet to be exposed to irradiation, and to either decrease the magnitude of a response pathway or convert the response to an alternate biochemical pathway.(70) Use of such an agent would be critically dependent on time of delivery, specificity of uptake in the tissues to be protected and delivery to the intracellular sites of interest. Organ specific targeted delivery of an antioxidant therapy is one example of such an agent.(11,55) Intraorgan administration of MnSOD-PL to the oral cavity, esophagus, lung, bladder and intestine has been shown to be a potentially successful approach to localized radioprotection.(71) Other antioxidant agents which can be delivered locally or systemically are listed in Table II.

There are several possible targets for design and application of a radioprotector. These are shown in Figure 3.

### **Blocking nuclear DNA damage and its communication to the mitochondria**

Overlapping pathways of cellular protection from ionizing irradiation, ultraviolet irradiation and heat have been revealed in the discovery of damage repair genes, genes for induction of antioxidant proteins(72–76), free radical scavengers, and by study of the evolution of heat shock proteins.(72) A common pathway in defense against ionizing irradiation involves protection of single and double strand nuclear DNA breaks, which lead to induction of the self-

destructive pathways of apoptosis, autophagy and mitotic arrest(3,73) as well as delayed mutations. There is evidence that all phyla in both the plant and animal Kingdoms maintain common genetic functions for adjusting to conditions of low level ionizing irradiation.(72–76) A radioprotector could well be one that protects against DNA strand breaks.

### **Mitochondrial Stabilization**

Development of radioprotectors has also followed on knowledge of the intrinsic radiation resistance of specific transgenic mice that display overproduction of a mitochondrial localized antioxidant protein.(77) Also of importance was the observed relative radiosensitivity of a knockout strain of mice deficient in production of an antioxidant radioprotective protein such as MnSOD.(70–71) Agents which increase the cellular antioxidant pool anticipating large quantities of irradiation induced ROS, thus anticipate the need to neutralize these molecules. Such radioprotective agents include MnSOD transgene therapy(70–71,78), and small molecules MnSOD mimics.(79) Other strategies to elevate cellular antioxidant stores, would be to deliver the immunostimulant TLR5-Flagellin(11) or another biological agent or derived product that elicits a stress response in cells including upregulation of MnSOD gene transcription and its protein production to achieve the goal of increasing the cellular antioxidant response capacity. Yet other relevant approaches would include small molecules that could act as ROS scavengers.(80–83) (Table II)

Other examples of therapeutic agents which have been developed along the lines of protecting the mitochondria in cells from initiating apoptosis do so by elevating antioxidant levels in response to irradiation such as WR2721 (Amifostine) which was designed as a ROS scavenger molecule.(83–85) (Table II)

### **Blocking caspase activation and poly ADP-ribosyl-polymerase (PARP) cleavage**

These are two theoretical targets for future research, based upon knowledge of the post-mitochondrial events in single irradiated cells.(86) (Figure 3)

**Decreasing systemic cytokine mediated cell death**—Experimental approaches to ameliorate late irradiation effects have been identified in animal model systems administration of novel counteracting cytokines, anti-cytokines and immune stimulation with or without stem cell transplantation as well as dietary antioxidant strategies.(15,20,87)

**Radiosensitizers**—Reversing the strategy described for radiation protectors could result in development of radiosensitizers or agents that increase the cellular capacity to respond to ionizing irradiation. This strategy has been utilized in the development of tumor radiosensitizers designed to deliver specifically drugs that would sensitize the tumor relative to normal tissue.(3) An agent which specifically sensitizes tumor cells can also appropriately affect the therapeutic ratio (greater tumor toxicity compared to normal tissue toxicity). Such tumor radiosensitizing agents include: Bromo-deoxyuridine, BUDR, Taxol, Cis-Platin, Cytosin and analogs, and recent anti-angiogenic drugs designed to target tumor vasculature or tumor cells.(3,88–89) A major challenge for the development of tumor radiosensitizers has been the difficulty in finding tumor cell specific targets that do not overlap significantly with normal tissue functions. Currently available radiosensitizers have exploited tumor cell deficiencies or their overexpression of specific radiation damage response proteins.(90) The overlap between normal tissue and tumor cells has been significant and application of these new agents to experimental models or clinical trials has met with significant normal tissue toxicity.

## Strategies and issues of concern

Long term side effects of radioprotectors have been a concern. Since the irradiation response of cells and tissues cause many cells to remain in a quiescent state protected from cell division by their residence in the microenvironment, there is a possibility that uptake of a radioprotective agent in those cells might alter their biological behavior while in the quiescent state. During the transition from recovery from the acute irradiation effect, radioprotective agents could have a second function that might be deleterious. For example, a small molecule capable of neutralizing ROS might be metabolized intracellularly to another molecule that could function as a carcinogen. Recent data indicates that MnSOD-PL administration systemically for protection against the hematopoietic syndrome, leads to an increase in survival of C57BL/6J mice with no detectable increase in carcinogenesis.(35)

New areas of research are showing particular promise including an understanding of the difference in redox chemistry and metabolism of oxygen between hypoxic regions within tumors and surrounding normal well oxygenated tissue. Oberley and colleagues(91–97) first described the difference in redox chemistry between tumor cells and normal tissues. Particularly in patients with large greater than 1 cm diameter squamous cell tumors of the lung, head and neck region, esophagus, and other bulky tumors of the pelvis such as cervical and endometrial cancer, and large abdominal tumors such as pancreas cancer or colon cancer, there has been appreciation of a shift in tumor cell metabolism from oxic to hypoxic conditions. Any unregulated growth of cancer cells beyond blood vessels produces areas of hypoxia and anoxia leading to necrosis.(3) Delivery of radioprotector drugs by the intravenous route may not reach a significant portion of the tumor volume. In addition, delivery of antioxidant drugs including some compounds that scavenge free radicals including superoxide, hydrogen peroxide, and peroxytrite may halt the production of hydrogen peroxide products in normal cells.(94) Normal cells have an increased capacity to neutralize hydrogen peroxide through catalase and glutathione peroxidase while tumor cells, particularly in hypoxic or anoxic regions show down-regulation of these enzymes.(91–92)

Entry of antioxidant agents into tumor cells which result in the generation of hydrogen peroxide, can produce additional tumor selective toxicity through limited capacity for metabolism of hydrogen peroxide.(94–97) Furthermore, limited effectiveness of cancer blood vessels, as well as altered tumor redox metabolism in the cancer cells, may enhance relative levels of antioxidant drug delivery to normal tissues for radioprotection in the cancer patient. (88) An increased understanding of the metabolic differences between tumor cells and normal tissue with respect to capacity to activate radioprotectors could lead to the same strategy used in the development of the hypoxic cell cytotoxin Tirapazamine.(98) With this drug, normal oxygen concentration metabolizes the drug into a non-toxic moiety while hypoxic regions of tumors suffer the toxic effects of the unmodified drug.

The administration of a radioprotective agent to a target tissue must take advantage of the time course for reaching target cells at risk and must include a delivery system designed to penetrate deep enough into the tissue to reach the proliferating stem cell populations. For example, in the prevention of irradiation-induced esophagitis, or oral cavity mucositis, intravenous administration of a radioprotector drug might be expected to reach all tissues, but may not provide a high enough level of uptake in the stem cell populations in those critical target tissues. In contrast, intra-oral or intra-esophageal localized administration using a delivery system known to penetrate several cell layers into a particular tissue, should provide a higher concentration of drug or transgene product to that site.(23) In contrast, the goal of protection of all normal tissues from total body irradiation might require intravenous administration or transdermal administration with effective absorption such that blood levels would reliably achieve effective systemic levels within the appropriate time prior to irradiation.(35)



Overproduction of a radioprotective antioxidant transgene product, or DNA repair enzyme, if administered too far in advance of irradiation can lead to compensatory down regulation of other antioxidants or DNA repair pathways and potentially remove the desired radioprotective effect.(82) Similarly, the administration of a radioprotective agent after radiation exposure might be ineffective due to the overwhelming amount of ROS produced by the oxidative stress of irradiation.(70–72) A list of representative radiation protectors, radiation damage mitigators and radiation damage treatment agents currently under consideration or development is shown in (Table II).

## Conclusions

The success in development of radioprotective agents will depend increasingly on an understanding of the molecular biology of radiation damage, cellular, tissue, organ responses to irradiation, the effect of comorbid factors, and differences between tumor and normal cell biology. Strategies for developing tumor radiosensitizers and normal tissue radioprotectors have in the past relied upon known differences in tumor specific vs. normal cell biology in terms of cell cycle, expression of specific growth factor receptors, cell surface adhesion molecules, or other biological or immunological characteristics. Molecular targets of new radioprotectors should concentrate on the mechanisms of action on irradiation-induced damage, after nuclear DNA strand breaks are repaired, focusing instead on distal steps in the cellular response, including nuclear to mitochondrial transport of signaling molecules, and steps in induction of the cell death pathways including autophagy, apoptosis and necrosis. New strategies to identify metabolic differences between normal tissue and tumor cells will also be critical to the design of new classes of radioprotectors for clinical use.

Of equal importance is the concern of potential delayed deleterious effects of radioprotective agents in preventing the removal of irradiation damaged cells the survival of which may lead to an increase in unacceptable chronic side effects including organ failure and carcinogenesis.

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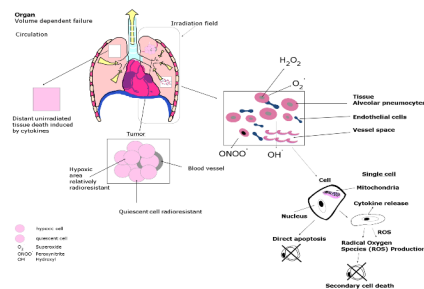
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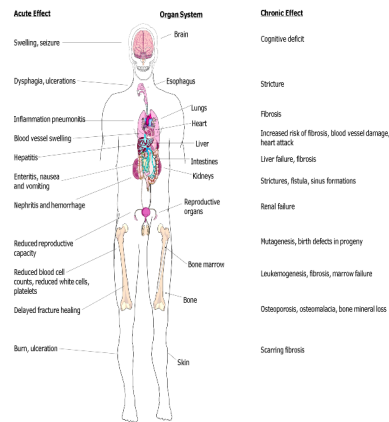
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**Figure 1. Cell, tissue and organ specific pathways of ionizing irradiation toxicity**

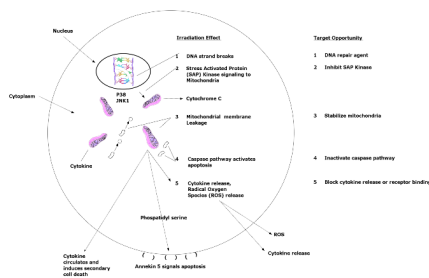
Subtotal lung irradiation is shown as an example. Single alveolar pneumocytes from an area within lung tissue are shown relative to total lung irradiation. Individual cells experience ionizing irradiation-induced production of radical oxygen species (ROS) including superoxide, hydroxyl radical, nitric oxide and peroxynitrite from the interaction of ionizing irradiation with oxygen and water. ROS interaction with pyrimidine and purine bases nuclear DNA produces single and double strand breaks, initiation of DNA repair, communication of DNA damage through the cell cytoplasm to the mitochondrial membrane *via* (stress activated protein (SAP) kinases) and then translation of pro-apoptotic, BCL2 family members from nucleus to mitochondria.(86) Then follows mitochondrial membrane permeability, cytochrome c disassociation from cardiolipin, and cytoplasmic leakage of cytochrome c which leads to activation of the caspase-3 pathway and apoptosis.(17–18,86) Both dying and irradiated but recovering cells release ROS and inflammatory cytokines including IL-1, TNF $\alpha$  and TGF $\beta$ , which directly (through cell to cell contact with other cells in the tissue), and indirectly (*via* the circulation to cells at distant sites), produce acute local tissue and systemic effects respectively. Within the irradiated tissue differences in radiosensitivity of different cell phenotypes (endothelial cells, alveolar pneumocytes, alveolar macrophages and bronchopulmonary stem cells) contribute to the magnitude of tissue damage. The volume of tissue within the lung that is in the irradiation beam determines as does irradiation dose the magnitude of acute and chronic normal tissue effects.



**Figure 2. Organ specific acute and chronic radiation toxicities**

Acute tissue toxicity experienced during a radiotherapy treatment course or shortly thereafter is described as symptoms and signs of tissue damage for each organ (left side). Chronic radiation side effects occurring months to years later are also shown (right). Severity and duration of both acute and chronic side effects depends on radiation dose, dose rate, quality of irradiation (greater for high LET radiation beams – See Box 1) and volume of tissue treated.





**Figure 3. Targets for development of radioprotector agents based on molecular pathways of the irradiation response**

The molecular mechanism of irradiation damage in single cells (effects #1 – 4) and released inflammatory cytokines (effect #5) is defined by several target points where efforts for development of radioprotector drugs can focus. Radioprotectors could target the DNA damage step(121–122): (1); molecular translation of the DNA damage event to the mitochondria through the cytoplasm, (2); mitochondrial stabilization by preventing membrane permeability and leakage of cytochrome c, (3); activation of caspases to cause apoptosis(123), (4); or intracellular communication of cellular and tissue damage by elaboration of cytokines, (5). Examples of radioprotector drugs currently under development or in clinical trial are correlated to each irradiation effect and are shown in Table I.

**Table I**

## Radiotherapy Technologies Currently Utilized

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1	External beam radiotherapy (Intensity modulated radiation therapy – IMRT, Image Guided Radiotherapy – IGRT)
	A. X Irradiation (Photon Beam Therapy)
	B. Electron Beam (Beta Irradiation)
	C. Proton Irradiation, High Linear Energy Transfer (LET) Particles
2	Stereotactic Radiosurgery
	A. Gamma Knife
	B. Linear Accelerator Mediated Frameless Stereotactic Radiosurgery
	C. Robot Arm Controlled X Irradiation Delivery System
3	Radioisotope Radiotherapy for Organ Specific or Cancer Cell Specific Uptake
4	Radioisotope Bound to Monoclonal Antibody for Tumor Targeted Radiotherapy
5	Brachytherapy (Interstitial or Intracavity) High Dose Rate Radiation Source implantation
6	Permanent Radioactive Seed Implantation for Organ Specific Dose Delivery

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**Table II**

## Categories of Radiation Dose Modifying Agents

<b>Protectors</b>	<b>Examples</b>	<b>References</b>	<b>Irradiation Effect Target Opportunity In Fig. 3</b>
Sulfhydryl Compounds	Cysteine WR2721	(1,83,85) (84–85) (2)	1–3 1–3
Antioxidants	Tempol		3
Targeted Mitochondrial Agents	MnSOD Mimics mn – porphryn based GS-Nitroxides, Eukaryon-134-SOD mimic Molecules	(79,99) (80–81) (99)	3 3 3
Cryoprotective Agents	DMSO	(100)	
Immunomodulators	Histamine H2 receptor Antagonists Polysaccharides Heat killed lactobacillis Synthetic chemicals Flagillin B-Glucan Prostaglandins	(101)  (102–103) (103) (102) (12) (104) (106)	5 5 4–5 1–5 3–5 3–5 3–5
Plant extracts	Curcumin Orjentin Viciden Ngella sativa Podophyllum hovandrum	(105) (105) (105) (103) (107)	3 3 3 3 3
Vitamins	Vitamin E Vitamin C	(108) (109)	3 3
Cytokines	IL-1 Stem Cell Factor G-CSF	(110) (111) (112)	5 5 5
Gene Therapy Delivered			
Antioxidants	MnSOD-PL	(70–71)	3
<b>Mitigators</b>	<b>Examples</b>	<b>References</b>	
ACE Inhibitors	Captopril	(113–115)	5
All Type-1, Type-2	Clanipril	(113–114)	5
Receptor Antagonists	Penicillamine Pentoxiphylline	(105) (116)	5 5
Endothelial Cell Infusion	Vascular endothelium	(45–46)	5
<b>Treatments</b>	<b>Examples</b>	<b>References</b>	
Pentoxifylline		(117)	5
a-tochoferol			
caloric restriction		(118–120)	5