Chapter 11

PERSPECTIVES IN RADIOLOGICAL AND NUCLEAR COUNTERMEASURES

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INTRODUCTION

Medical Management of Radiation Events

Threat from a nuclear event can occur due to a radiologic (dispersal or use of radioactive material) or nuclear (improvised nuclear device) exposure. A comprehensive response plan to meet such events can be found on the Web site http://www.remm.nlm.gov, and was summarized by Coleman et al.[1] The components of this response plan consist of underpinnings from basic radiation biology, tailored medical responses, delivery of medical countermeasures for postevent mitigation and treatment, referral to expert centers for acute treatment, and long-term follow-up. The emphasis of this plan is emergency management of a nuclear event.

Protection of First Responders

Radiation countermeasures have been classified as radioprotectants (administered before radiation exposure), mitigators (given during or shortly after exposure, before overt symptoms appear), and treatments (given after overt symptoms appear).[2] One important application of radiation countermeasures is to protect first responders deployed in a radiation exposure field for rescue and other military operations. This is an urgent need for the military and for US Department of Homeland Security scenarios involving nuclear terrorist threats. Radiation exposure can result in short-term lethality and long-term consequences, like cancer and pulmonary fibrosis. Currently, there are no countermeasures against these threats that can be used in humans, which is a serious capability shortfall. This is a critical issue for commanders in planning and executing military operations. Developing radiation countermeasures for use prior to exposure has been identified as one of the highest priority areas for research.[3] Postirradiation treatment is also an important aspect of radiation countermeasure development, but that is beyond the scope of this chapter and is discussed elsewhere in this volume.

Historically, studies on radiation countermeasures began in 1949, testing the radioprotective efficacy of cysteine in mice.[4] Since that time, many diverse compounds have been shown to have protective characteristics (Table 11-1). More recently, several medical protocols have been proposed,[5] but a safe and effective radiation countermeasure is not available for acute radiation syndrome (ARS). The one approved radiation countermeasure (to be given in a clinic setting before therapeutic irradiation), amifostine (see Radiation Countermeasures, Aminothiols and Other Thiol Derivatives, below), causes several toxic manifestations[6] that could impair task performance, which is critical for military and first-responder operations. Radiation countermeasure development has focused on protecting against acute, high-dose radiation injury and protecting the normal tissues of cancer patients who are undergoing radiotherapy. Additional areas that need to be studied involve protecting against low-dose and chronic radiation exposure scenarios, such as in potential terrorist events using nuclear devices ("dirty bombs" or improvised nuclear devices) and during extra-vehicular activity associated with space missions, including proposed manned flights to Mars by the National Aeronautics and Space Administration.

With new advances in immunology, biochemistry, radiobiology, and pharmacology, the development of a safe and effective radiation countermeasure may be at hand. Over the longer term, newer concepts and techniques in molecular biology may provide exciting approaches for developing specific and effective means to prevent, mitigate, or treat radiation injury. The primary objective of prophylactic studies is to develop an agent or combination of agents that will substantially increase survival and enhance the postincidence effectiveness of first-responder military personnel on a nuclear battlefield. These treatments must be easily self-administered shortly before or after radiation exposure to reduce early molecular, cellular, and tissue damage. This chapter briefly reviews the relevant radiobiological concepts, presents strategies and mechanisms, and discusses some of the more promising agents being investigated.[7]

RADIATION INJURY

To understand the various strategies being used to prevent, mitigate, and treat ionizing radiation injury, it is first necessary to define ionizing radiation and to consider the events that occur in the development of ARS (also see Chapter 2, Acute Radiation Syndrome in Humans).

Ionizing Radiation

Ionizing radiation can be defined as any type of electromagnetic radiation (such as gamma or X-rays) or particulate radiation (such as neutrons or alpha particles) that has sufficient energy to ionize atoms or mol-

TABLE 11-1

SELECTED RADIATION COUNTERMEASURE AGENTS

Compounds	Protective Efficacy (scale of 1–4, 4 being the best)	Probable Mechanism of Action
Aminothiols		Free-radical scavenging, hydrogen donation
Cysteine[1]	2	
WR-2721[2–4]	4	
N-acetylcysteine[5]	3	
Diethyl dithiocarbamate[5]	2	
Immunomodulators		Hematopoietic system regeneration
Glucan[6,7]	3	
Trehalose dimycolate[8]	3	
Endotoxin[9]	3	
5-AED*[10,11]	3	
Cytokines		Hematopoietic system regeneration
Interleukin 1[12]	3	
Tumor necrosis factor[12]	2	
Antioxidants/Nutraceuticals		Free-radical scavenging
Vitamin E[13,14]	3	
Vitamin A (β-carotene)[15]	2	
Superoxide dismutase[16,17]	3	
Selenium[18,19]	2	
γ-tocotrienol[20–22]	4	
Eicisanoids		Uncertain
$DiPGE_2$[23]	3	
Iloprost, Misoprostol[24]	3	
Unknown/Proprietory		
BIO-300*†	2	
Ex-RAD*‡[25]	2	Antiapoptotic
CBLB502*§[26]	3	TLR agonist
17-DMAG (geldanamycin derivative)[27]	2	Antiapoptotic

*Approved by US Food and Drug Administration as investigational new drug
†BIO-300 is manufactured by Humanetics Corporation (Eden Prairie, MN).
‡Ex-RAD is manufactured by Onconova Therapeutics, Inc (Newtown, PA).
§CBLB502 is manufactured by Cleveland BioLabs, Inc (Buffalo, NY).
5-AED: androst-5-ene-3beta,17beta-diol (5-androstenediol)
$DiPGE_2$: 16,16-dimethyl prostaglandin E_2
17-DMAG: 17-(dimethylaminoethylamino)-17-demethoxygeldanamycin
TLR: toll-like receptor

Data sources: (1) Patt HM, Tyree E, Straube RL, Smith DE. Cysteine protection against X-irradiation. *Science* 1949;110:213–214. (2) Yuhas JM. Biological factors affecting the radioprotective efficiency of S-2-(3-aminopropylamino)ethylphosphorothioic acid (WR-2721): $LD_{50(30)}$ doses. *Radiat Res.* 1970;44:621–628. (3) Glover DJ, Glick JH, Weiler C, Hurowitz S, Kligerman M. WR-2721 protects against the hematologic toxicity of cyclophosphamide: a controlled phase II trial. J C*lin Oncol*. 1986;4:584–588. (4) Weiss JF, Kumar KS, Walden TL, Neta R, Landauer MR, Clark EP. Advances in radioprotection through the use of combined agent regimens. *Int J Radiat Biol*. 1990;57:709–722. (5) Landauer MR, Davis HD, Dominitz JA, Weiss JF. Comparative behavioral toxicity of four sulfhydryl radioprotective compounds in mice: WR-2721, cysteamine, diethyldithiocarbamate, and N-acetylcysteine. *Pharmacol Ther.* 1988;39:97–100. (6) Patchen ML, Brook I, Elliott TB, Jackson WE. Adverse effects of pefloxacin in irradiated C3H/HeN mice: correction with glucan therapy. *Antimicrob Agents Chemother*. 1993;37:1882–1889. (7) Patchen ML, MacVittie TJ, Weiss JF. Combined modality radioprotection: the use of glucan and selenium with WR-2721. *Int J Radiat Oncol Biol Phys*. 1990;18:1069–1075. (8) Madonna GS, Ledney GD, Elliott TB, et al. Trehalose dimycolate enhances resistance to infection in

(*Table 11-1* **continues**)

Table 11-1 **continued**

neutropenic animals. *Infect Immun*. 1989;57:2495–2501. (9) Ainsworth EJ. From endotoxins to newer immunomodulators: survival-promoting effects of microbial polysaccharide complexes in irradiated animals. *Pharmacol Ther*. 1988;39:223–241. (10) Whitnall MH, Villa V, Seed TM, et al. Molecular specificity of 5-androstenediol as a systemic radioprotectant in mice. *Immunopharmacol Immunotoxicol*. 2005;27:15–32. (11) Whitnall MH, Wilhelmsen CL, McKinney L, Miner V, Seed TM, Jackson WE III. Radioprotective efficacy and acute toxicity of 5-androstenediol after subcutaneous or oral administration in mice. *Immunopharmacol Immunotoxicol*. 2002;24:595–626. (12) Neta R. Role of cytokines in radioprotection. *Pharmacol Ther*. 1988;39:261–266. (13) Srinivasan V, Jacobs AJ, Simpson SA, Weiss JF. Radioprotection by vitamin E: effects on hepatic enzymes, delayed type hypersensitivity, and postirradiation survival of mice. In: Prasad KN, ed. *Modulation and Mediation of Cancer by Vitamins*. Basel, Switzerland: Karger; 1983: 119–131. (14) Kumar KS, Srinivasan V, Toles R, Jobe L, Seed TM. Nutritional approaches to radioprotection: vitamin E. *Mil Med*. 2002;167:57–59. (15) Seifter E, Rettura G, Padawar J, Stratford F, Weinzweig J, Demetriou AA, Levenson SM. Morbidity and mortality reduction by supplemental vitamin A or beta-carotene in CBA mice given total-body-radiation. *J Natl Cancer Inst*. 1984;73:1167–1177. (16) Petkau A. Radiation protection by superoxide dismutase. *Photochem Photobiol*. 1978;28:765–774. (17) Srinivasan V, Doctrow S, Singh VK, Whitnall MH. Evaluation of EUK-189, a synthetic superoxide dismutase/catalase mimetic as a radiation countermeasure. *Immunopharmacol Immunotoxicol*. 2008;30:271–290. (18) Davis TA, Clarke TK, Mog SR, Landauer MR. Subcutaneous administration of genistein prior to lethal irradiation supports multilineage, hematopoietic progenitor cell recovery and survival. *Int J Radiat Biol*. 2007;83:141–151. (19) Patchen ML, MacVittie TJ, Weiss JF. Combined modality radioprotection: the use of glucan and selenium with WR-2721. *Int J Radiat Oncol Biol Phys*. 1990;18:1069–1075. (20) Kumar KS, Ghosh SP, Hauer-Jensen M. Gamma-tocotrienol: potential as a countermeasure against radiological threat. In: Watson RR, Preedy VR, eds. *Tocotrienols: Vitamin E Beyond Tocopherols*. Boca Raton, FL: CRC Press; 2009: 379–398. (21) Ghosh SP, Kulkarni S, Hieber K, et al. Gamma-tocotrienol, a tocol antioxidant as a potent radioprotector. *Int J Radiat Biol*. 2009;85:598–606. (22) Berbée M, Fu Q, Boerma M, Wang J, Kumar KS, Hauer-Jensen M. Gamma-tocotrienol ameliorates intestinal radiation injury and reduces vascular oxidative stress after total-body irradiation by an HMG-CoA reductase-dependent mechanism. *Radiat Res*. 2009;171:596–605. (23) Walden TL Jr, Patchen M, Snyder SL. 16,16-Dimethyl prostaglandin E, increases survival in mice following irradiation. *Radiat Res*. 1987;109:440–448. (24) Kumar KS, Srinivasan V, Palazzolo D, Kendrick JM, Clark EP. Synergistic protection of irradiated mice by a combination of iloprost and misoprostol. *Adv Exp Med Biol*. 1997;400B: 831–839. (25) Ghosh SP, Perkins MW, Hieber K, et al. Radiation protection by a new chemical entity, Ex-Rad: efficacy and mechanisms. *Radiat Res*. 2009;171:173–179. (26) Burdelya LG, Krivokrysenko VI, Tallant TC, et al. An agonist of toll-like receptor 5 has radioprotective activity in mouse and primate models. *Science*. 2008;320:226–30. (27) Fukumoto R, Kiang JG. Geldanamycin analog 17-DMAG limits apoptosis in human peripheral blood cells by inhibition of p53 activation and its interaction with heat shock protein 90 kDa after ionizing radiation. *Radiat Res*. 176:333-345, 2011.

ecules; that is, to eject electrons from their outer orbits.

In considering the effects of radiation on biological systems, it is important to distinguish the different types of ionizing radiation according to their linear energy transfer (LET). This term describes the amount of energy deposited by a particular type of radiation per unit of path length. Low-LET radiation (gamma and X-rays) is sparsely ionizing because it causes few ionizations per micron of path length, whereas high-LET radiation (neutrons and alpha particles) is densely ionizing because it produces many ionizations per micron of path length. Generally, high-LET radiation produces more biological damage than low-LET radiation.[7,8]

Biological Damage

Death from radiation injury is the result of a sequence of events that occurs over a period of less than a billionth of a second to several weeks (Figure 11-1).[9,10] The first step in this sequence is the transfer of radiation energy from the photon or particle to atoms and molecules in its path through a process of direct (eg, alpha or beta particles) or indirect (eg, X-rays, gamma rays, or neutrons) ionization. This results in the ejection of a particle (such as an electron) that causes the first discrete lesion in the sequence: direct or indirect damage to macromolecules that are critical for biological function. Direct and indirect ionization are separate from, and occur prior to, direct or indirect damage to macromolecules (see below). If a critical biological molecule is in the radiation path, it can become chemically altered by direct interaction by radiation energy (direct damage). If that molecule is not in the radiation path, it can still become chemically altered indirectly via reactions with free radicals, reactive oxygen species, and reactive nitrogen species produced primarily from the radiolysis of water, and by interactions of free radicals.[9] Although the importance of membrane damage is still being evaluated, damage to deoxyribonucleic acid (DNA) and proteins are important factors in cell death, with DNA strand breaks commonly thought to be the primary lesions.[9,10]

Reactive oxygen species are important in the overall scheme of radiation injury because their lifetime in solution is sufficiently long to allow them to diffuse and extend the damage beyond the primary path of radiation. In this way, the effects of ionizing radiation within the cell are greatly amplified. Most radiation injury from low-LET radiation is the result of indirect damage, while that from high-LET radiation is from direct damage.[11] The net effect of direct and indirect damage is the disruption of molecular structure and function, leading to dysfunctional cells and organ systems and resulting in altered cell division, cell death, depletion of stem-cell pools, and, if the radiation dose is high enough, death of the organism.

Types of Radiation Injury

ARS (sometimes called acute radiation *sickness*) develops after exposure of the whole body or a major part of the body to ionizing radiation with doses in excess of 1 to 2 Gy. A useful concept for understanding ARS is the 50% lethal dose, or LD_{50}. This is the radiation dose that will lead to death of 50% of uniformly exposed individuals, assuming no medical intervention.[12] In reality, the lethal dose is influenced by a number of confounding factors, such as the type of radiation, uniformity of radiation exposure, dose rate, penetration, combined injury with biological or chemical damage, and health status of the exposed individual. Supportive therapy exerts a substantial influence on survival after radiation exposure. Hence, the LD_{50} in humans is about 3.5 to 4.0 Gy when no or only minimal supportive care is provided. On the other hand, with the use of standard supportive therapy, the LD_{50} is estimated to be in the 6 to 7 Gy range.[12,13] With optimal pretreatment, availability of an appropriate bone marrow match, and successful bone marrow transplantation, doses in the 9 to 14 Gy range may be survivable.[14] Partial shielding of the active bone marrow, such as occurs when the exposure is nonuniform, also exerts a major effect on survival. For example, shielding of just 10% of the active bone marrow will lead to close to 100% survival after a total-body dose that is otherwise at the LD_{50}.

Several systems have been proposed to classify ARS according to severity and prognosis based on the radiation dose received. For example, the Radiation Injury Severity Classification was proposed by an international group in 2008.[15] Another system, published by the International Atomic Energy Agency, classified ARS in five categories: (1) mild (1–2 Gy), (2) moderate (2–4 Gy), (3) severe (4–6 Gy), (4) very severe (6–8 Gy), and (5) lethal (more than 8 Gy).[16] It should be noted, however, that exposed individuals may survive doses up to 12 Gy for 6 to 12 months with optimal supportive therapy.

Clinically, ARS after exposure to whole-body irradiation generally progresses through four phases. The prodromal period is characterized by nausea, vomiting, and, at higher radiation doses, diarrhea. A latency period of variable duration comes next. The third phase of radiation illness includes various manifestations, depending on the radiation dose received. Last is the period of recovery or demise.

ARS affects, at increasing doses, the hematopoietic, gastrointestinal, cardiovascular, and central nervous systems (CNS). It is common practice to divide ARS into subsyndromes depending on the organ systems that are predominantly responsible for the symptoms.

Hematopoietic Subsyndrome

The bone marrow is the most important organ of the hematopoietic system, but several other organs, such as the thymus, lymph nodes, and spleen, contribute to maintaining homeostasis of the immune responses. The hematopoietic system contains pluripotent and multipotent stem cells that give rise to lineage-committed progenitor cells and subsequently to mature

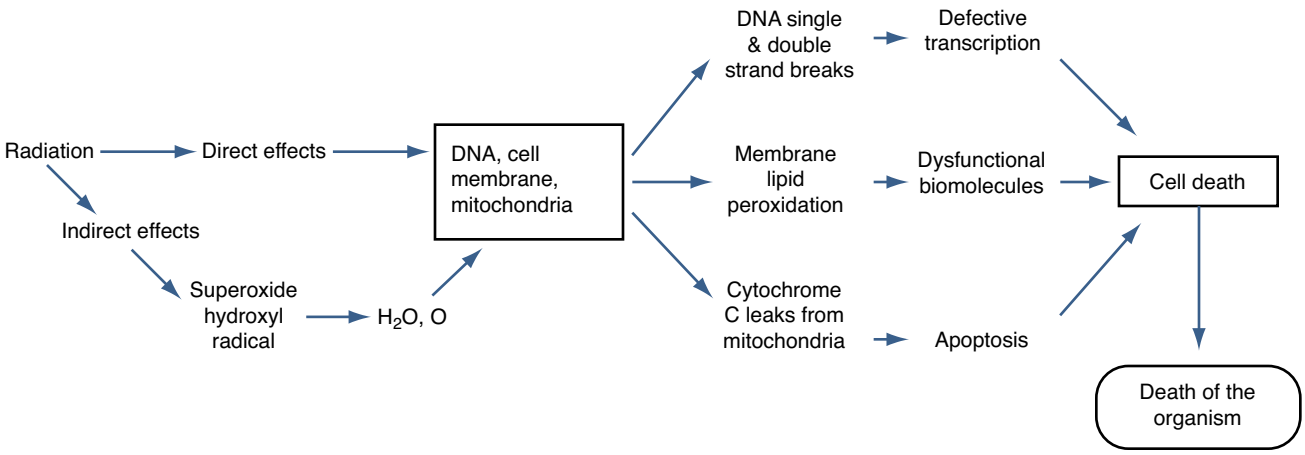

Figure 11-1. Direct and indirect radiation effects on key biological molecules leading to cell and organism death.
DNA: deoxyribonucleic acid
H_2O: water
O: oxygen

peripheral blood cells. The hematopoietic stem cell is central to maintaining hematopoiesis and in recovery after exposure to ionizing radiation. While previously considered a single "target cell," it has now become increasingly recognized that, rather than viewing hematopoietic stem cells in isolation, they should be considered in context with their microenvironment. Hence, the stem cell niche consists of multiple cell types, tissue matrix, and paracrine factors, as well as metabolic products that play essential roles in the ultimate regulation of stem-cell survival, proliferation, and differentiation.[17,18] The entire hematopoietic and immune systems can be regenerated from hematopoietic stem cells. While the majority of hematopoietic stem cells are located in the bone marrow, a few also circulate in the body. The exact role of these circulating stem cells and the extent to which they home to specific locations is still unclear.

All cells of the immune system originate from bone-marrow–derived hematopoietic stem cells. It is customary to classify the immune system into primary, secondary, and tertiary organs. The thymus is the production site of naive T cells that subsequently migrate to the secondary lymphoid organs, such as lymph nodes, spleen, and Peyer's patches in the intestine. Once activated, lymphocytes can enter tertiary, nonlymphoid sites, such as the skin and intestinal mucosa, and contribute to infection clearing. The immune system of the intestine is the largest in the body, containing 50% to 80% of all the body's immunoglobulin-producing cells and 40% of its T cells.

Because of the rapidly proliferating hematopoietic progenitor cell compartment in the bone marrow, the hematopoietic system is extraordinarily radiosensitive. Radiation doses as low as 0.5 to 1 Gy elicit clear changes, and significant hematopoietic and immune system dysfunction occur after radiation doses in excess of 2 Gy. Clinically, hematopoietic injury is characterized by decreased numbers of white cells, red cells, and platelets in the peripheral circulation.

The temporal development of hematopoietic radiation injury is well known.[19] As a general rule, lymphocytes are depleted within hours of radiation exposure, granulocytes and platelets over days, and erythrocytes over weeks. Small lymphocytes, although they do not divide, are extremely radiosensitive and are known to undergo apoptosis (acute cell death, described later in this chapter) after exposure to radiation doses as low as 0.2 to 0.3 Gy. In fact, how fast and low the lymphocyte count drops after radiation has been proposed as a way to predict the level of exposure.[19] Granulocytes and platelets also have rather short life spans; thus granulocytopenia and thrombocytopenia develop early after radiation exposure.

Death from infectious and bleeding complications generally occurs after acute radiation exposure (because of granulocytopenia and thrombocytopenia) within 14 to 28 days after irradiation. Successful treatment depends almost entirely on the ability to enhance the recovery of the hematopoietic stem and progenitor cells within a reasonable period of time. Immune system dysfunction is another important part of the hematopoietic subsyndrome. Naive T cells may take up to a year to regenerate, which puts the patient at increased risk for infections.

Gastrointestinal Subsyndrome

The epithelial lining of the intestine covers an area roughly 200 times that of the surface of the skin and is the most rapidly renewing cell system in the body. Epithelial cells proliferate in the crypts, migrate along the villi, and eventually get shed into the intestinal lumen. The cell cycle time in the human intestine is approximately 30 hours.[20] Therefore, radiation injury to the intestine becomes clinically manifest within days of exposure. In unirradiated humans, intestinal villus cells are replaced by proliferating progenitor crypt cells, which originate from the bottom of the villi. But on radiation exposure, villus cells are no longer replaced by crypts, since crypt cells undergo clonogenic (mitotic) death or apoptosis. The relative importance of clonogenic death versus apoptosis of intestinal crypt cells in the context of the gastrointestinal subsyndrome is unclear. It appears that, while the propensity of the intestinal microvascular endothelium to undergo apoptosis affects the intestinal radiation response,[21] apoptosis of intestinal crypt cells does not play a major role.[22]

The gastrointestinal tract plays a prominent role in the response to total-body irradiation in several ways. First, it is responsible for the prodromal symptoms (nausea, vomiting, and diarrhea) seen even after very low (1 Gy) radiation doses. These symptoms present within minutes to hours of radiation exposure, before structural injury occurs. The time to onset, severity, and duration of the prodromal symptoms are considered a reasonably reliable indication of the radiation dose received. However, because of a high false-positive rate, prodromal symptoms as predictors of radiation dose should be used with caution.[23] Second, the classical gastrointestinal subsyndrome, as described by Quastler, develops in humans after exposure to radiation doses in excess of 6 Gy.[24] It is associated with extensive destruction of the mucosa and characterized by severe diarrhea with pronounced

loss of fluids and electrolytes, leading to dehydration and electrolyte imbalance. Treatment with electrolytes and fluids may postpone death, but there are few specific therapeutic options available and survival is extremely unlikely with full-fledged gastrointestinal radiation subsyndrome. Death occurs 3 to 14 days after exposure, usually before day 10, and mostly around day 5 to 7. Although bacteremia does occur in the classical gastrointestinal subsyndrome, it is infrequent and antibiotics do not generally reduce lethality. Third, and perhaps most importantly, gastrointestinal injury plays a prominent role in the response to radiation doses in the hematopoietic dose range (2–6 Gy in humans). Radiation doses in this range do not result in development of full-fledged gastrointestinal subsyndrome. However, breakdown of the mucosal barrier converts the intestine into a large proinflammatory organ that releases cytokines and other inflammatory mediators into the circulation. Moreover, translocation of bacteria from the bowel lumen to the systemic circulation and remote organs occurs, and sepsis from enteric microorganisms (usually Enterobacteriaceae) is an important cause of death after exposure to radiation in this dose range.

Neurovascular Subsyndrome

The mature CNS consists of neurons, glial cells, astrocytes (oligodendrocytes), and blood vessels. Mature neurons are postmitotic (ie, specialized cells that are unable to divide). In contrast, most glial cells retain their capacity to divide under specific circumstances, albeit with slow turnover rates.[25] Microglia, so named because they were once classified as glial cells, develop from monocytes and have phagocytic properties similar to macrophages elsewhere.

Despite the fact that neurons and neuroglial cells are resistant to irradiation in terms of cell death, and that the neurovascular syndrome develops only after very high radiation doses, it is interesting to note that changes in neurological function occur after very low radiation doses. For example, electroencephalographic abnormalities are detectable after doses as low as 0.01 Gy.[26] True neurovascular subsyndrome occurs after exposure to more than 50 Gy, with an expected survival time of generally less than 48 hours. The symptoms of acute CNS injury include disorientation, apathy, and ataxia. Seizures, triggered by minimal external stimuli, are also common. Death results from meningomyeloencephalitis and acute vascular leakiness, resulting in increased fluid accumulation and pressure on critical structures. Cerebral and brainstem edema, caused by fluid leakage, may also result in increased pressure on critical structures, in turn affecting essential physiological functions, such as blood-pressure regulation, respiration, and temperature regulation. Therapy-resistant cardiovascular shock ("radiogenic shock") sometimes develops in individuals exposed to doses in this range. The mechanism underlying the inability to maintain blood pressure under these circumstances appears to involve a combination of factors, such as massive fluid extravasation, endothelial apoptosis and disruption of tight junctions between endothelial cells, autonomic nervous system dysfunction with loss of blood-pressure control, vasodilatation because of histamine release and other vasoactive mediators by mast cells, and other factors.[27]

The exact pathogenesis of the neurovascular subsyndrome remains unclear, and the issue of whether the target is vascular, parenchymal, or a combination is still unresolved. The prevailing notion at this time is that endothelial cell apoptosis, rather than oligodendrocyte apoptosis, is the primary event responsible for the acute disruption of the blood-brain barrier after irradiation, while oligodendrocyte apoptosis occurs as a secondary consequence.

Radiation-Induced Multiple Organ Dysfunction Syndrome

To convey principles of radiation toxicity in a particular organ effectively, it is useful to consider the radiation response of that organ separately. Moreover, after exposure to total-body irradiation, depending on the radiation dose received, symptoms that can be ascribed to specific organ systems predominate, hence the terms hematopoietic, gastrointestinal, and neurovascular subsyndromes. However, it is important to recognize that reference to the individual subsyndromes of ARS simply indicates that toxicity in those organ systems predominate clinically, but that the pathophysiological manifestations depend heavily on interactions among multiple cell types and organ systems in the body.

In other words, to develop a proper understanding of acute radiation toxicities in response to total-body irradiation, it is imperative that this reductionistic view be supplemented with pertinent principles based on systems biology. The importance of these interacting factors has led to the concept of radiation-induced multiple organ dysfunction syndrome.[28] Hence, total-body irradiation affects all tissues and organ systems in the body, and there are critical interactions among many of these tissues and organ systems. For example, although intestinal irradiation is necessary and sufficient to produce what is commonly referred

to as the gastrointestinal subsyndrome (in fact, surgical removal of the exposed bowel prevents the syndrome from occurring),[29] it is firmly established that lethality from bowel toxicity is heavily influenced by radiation injury to other organ systems, such as the hematopoietic system.[30] Conversely, it is also well known that intestinal injury, even after radiation doses in the hematopoietic dose range, influences lethality from hematopoietic and immune system failure.

PROTECTION, MITIGATION, AND TREATMENT

Characteristics of a Radiation Countermeasure

An ideal radiation countermeasure must have several characteristics that are necessary for its applicability to first responders. It must

- be stable at ambient temperature,
- be easily administered either as an intramuscular injection or orally,
- be free from toxic side effects that will compromise behavior and performance, and
- be free from abuse potential, and
- lack toxicity on repeat administration.

In addition to these characteristics, it is necessary to consider any countermeasure's therapeutic index. The therapeutic index, as used here, refers to the ratio between the toxic LD_{50} and the protective drug dose used to produce a specific dose reduction factor (DRF). It would also be advantageous to include information on acute side effects produced by potential agents at protective doses.

Several strategies have been developed to obtain a radiation countermeasure with these desirable characteristics to reduce radiation injury and mortality. These strategies are based on the mechanisms of pharmacological agents to protect against indirect damage, repair damage once it occurs, or stimulate the regeneration of depleted cell populations (Figure 11-2).

Spanning these strategies are new genetic approaches that are just beginning to be used in the development of advanced pharmacological agents. Combinations of agents that exploit the operative mechanisms in at least two of these strategies may substantially improve drug effectiveness. Barring the conventional physical approaches of time, distance, and shielding, almost nothing can be done pharmacologically to protect against the initial transfer of radiation energy to either water or critical biological molecules. The transfer occurs too rapidly (within 10^{-14} seconds after irradiation) and is a purely physical process.[9]

The failure of radioprotective agents to protect against direct damage to critical molecules indicates an inherent upper limit to the degree of protection that can be achieved pharmacologically. Because injury from high-LET radiation is due primarily to direct damage, and because the relative yields of radiolytic products of water and reactive oxygen species decreases with increasing LET, protection against high-LET radiation injury with free-radical scavengers will be less effective.[7]

The earliest point at which a protective effect from pharmacological agents can be detected is around 10^{-12} seconds after irradiation.[10] At that time, pharmacological agents can begin to prevent chemical damage by directly scavenging the free radicals produced by radiolysis of water or by interaction among themselves.[9] The next level of protection can occur by repairing the chemical damage produced in critical biological molecules and also by reacting with the chemical intermediates that indirectly damage these molecules.

Mechanisms

The damage induced by the products of radiation and water interactions can be reduced either by inhibiting the formation of these reactive radical intermediates or by eliminating them from the cellular environment. This can be accomplished using agents that induce hypoxia or scavenge toxic products.

Hypoxia

The formation of reactive oxygen species can be inhibited by the induction of hypoxia. The extent of radiation damage in a tissue is directly related to the degree of oxygenation of that tissue; agents capable of reducing oxygenation will mitigate the injury.[7,31] Many of these chemical agents are known to induce transient systemic or localized hypoxia.[7,8] Systemic hypoxia can be achieved in several ways: through induction of hemodynamic cardiovascular alterations, interference with hemoglobin function, increased tissue oxygen use, and depressed respiratory-center function. At the cellular and molecular levels, localized hypoxia can be achieved by agents that take part in the chemical and biochemical reactions that use oxygen.

Induction of hypoxia is a widespread protective mechanism that accounts, at least in part, for the protective action of many different chemicals, drugs, and physiological mediators. In spite of that, the usefulness

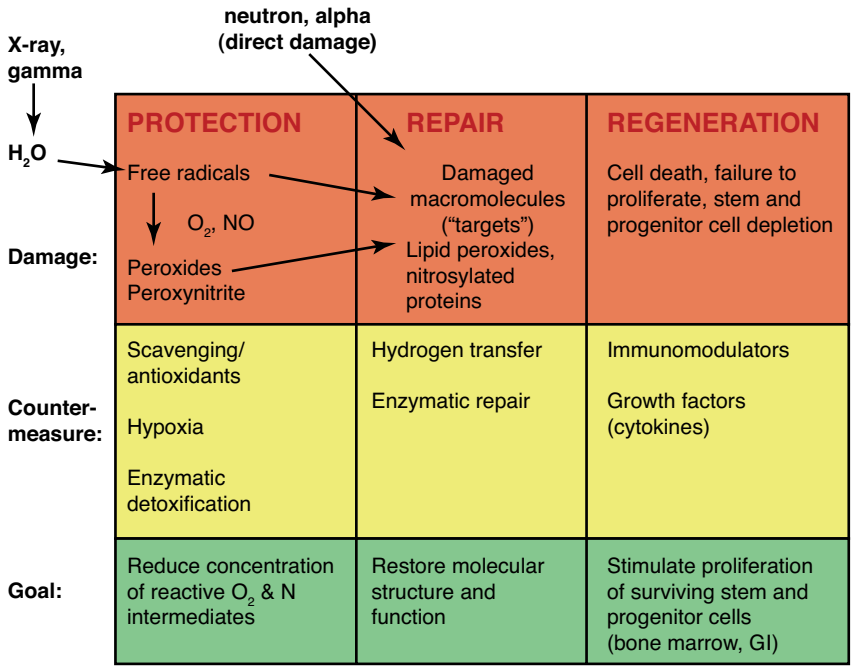

Figure 11-2. Three major possible mechanisms of radiation countermeasures. **Protection**: Preventing damage by scavenging free radicals (eg, $H^{\bullet}$, $OH^{\bullet}$, $O_2^{\bullet}$) or reducing formation of reactive oxygen or nitrogen intermediates such as hydrogen peroxide and peroxynitrite. Representative agents: aminothiol compounds (eg, amifostine) and antioxidants (such as γ-tocotrienol). **Repair**: Repairing molecular damage caused by free radicals. Representative agents: aminothiols. **Regeneration**: Stimulating function or proliferation of stem cells and progenitor cells in organs that rely on stem-cell proliferation for normal functioning, especially the hemopoietic system. Representative compounds: immunomodulators and cytokines. Cytokines are generally given after radiation; immunomodulators before. Steroid immunomodulator 5-androstenediol and antioxidant γ-tocotrienol stimulate cytokine expression and enhance survival after radiation.
GI: gastrointestinal
N: nitrogen
NO: nitric oxide
O_2: oxygen

of this mechanism must be considered with caution because of the potential effects of hypoxia on normal physiological function. This caution may apply more to agents that induce a systemic hypoxic state than to those that create localized hypoxia.

Scavenging

Free-radical scavenging and enzymatic detoxification refer to the ability of chemicals and endogenous enzymes to remove products of water radiolysis and highly reactive oxygen species before they can damage molecules of biological importance.[32,33] In essence, these are competitive reactions between protective agents and biological molecules. In aqueous solutions, protective agents and enzymes react with free radicals and oxygen species to form relatively stable, nontoxic end products, thereby reducing the concentration of these reactive species and sparing the biological target. Many protectants are very efficient scavengers of water-derived free radicals.

Chemical Repair by Hydrogen Transfer

Radiation damage to a critical biological molecule results in the transformation of that molecule into an organic free radical. In this form, the molecule can then react with oxygen or other free radicals and become permanently chemically altered. However, if a suitable hydrogen donor is in the vicinity of the damaged molecule, it can compensate for the damage by donating or transferring a hydrogen atom.[7,33] Hydrogen atom transfer can be thought of as an instant repair process in which the original molecular structure is restored before the damaged critical molecule becomes permanently altered by further chemical

reaction. Many of the agents that function as free-radical scavengers, particularly sulfhydryl agents, can also donate a hydrogen atom (eg, the aminothiols).[7]

Genetic Repair

Similar chemical alterations may also be induced by natural biological processes and disease states that generate free radicals. In the case of DNA, mammalian cells have evolved an elaborate and remarkably efficient system of enzymes that continually repair lesions in that critically important molecule. This is a complex system involving a number of different enzymes and a variety of regulatory molecules that control their synthesis and activity. One of the potentially useful features of this system is that it is inducible; that is, the synthesis of the repair enzymes and regulatory factors is activated when the need arises. Strains of prokaryotic organisms exist that are capable of surviving very high doses of radiation. One that has received attention is *Deinococcus radiodurans*, which is an extremely radioresistant strain of bacteria.[34] Although a study of these relatively simple prokaryotic systems may provide some insight into the genetic mechanisms involved in radiation sensitivity, relatively little progress has been made to unravel the radioprotective mechanisms in these bacteria to exploit for radiation countermeasure drug development.

Antiapoptotic Mechanisms

Much of the tissue injury occurring after exposure to ionizing radiation is due to apoptosis, either of mature cells (eg, lymphocytes), or progenitor cells necessary for tissue replenishment.[35–37] The two classes of progenitors that have received the most attention in countermeasure development are those in bone marrow responsible for regenerating blood cells and platelets and those in gastrointestinal crypts responsible for regenerating the gastrointestinal mucosa.[38,39] Since much radiation-induced apoptosis takes place in the hours after exposure, it has been recommended that delivery of antiapoptotic countermeasures should take place as early as possible.[40–43]

Radiation-induced apoptosis is caused by signaling pathways in the cell triggered by damage to macromolecules, or sensors that respond to radiation-induced free radicals. These signaling pathways comprise networks of interacting molecules that can alter the balance between repair and survival on one hand and programmed cell death on the other. The goal of antiapoptotic strategies is to activate or inhibit signaling molecules in such a way as to alter this balance in favor of survival.[44] In some cases, blocking apoptosis could make populations of cells more vulnerable to specific challenges. For example, inhibition of apoptosis with pifithrin improved survival in mice exposed to radiation doses that cause hematopoietic syndrome. However, in animals exposed to higher radiation doses, deletion of protein 53 (p53) was associated with increased mitotic catastrophe in the gastrointestinal mucosa and decreases in survival compared to vehicle-injected irradiated mice.[45]

Radiation-induced signal transduction pathways leading to apoptosis have been reviewed elsewhere.[44,46,47] The primary event is usually considered to be DNA damage detected by sensing proteins, which leads to activation of the ataxia telangiectasia mutated protein (ATM), which triggers both proapoptotic and prosurvival pathways. A central signal in the proapoptotic pathway is p53, which activates protein 21 (p21), cell cycle arrest, and eventual DNA repair and survival or apoptosis. Protein-53–independent proapoptotic pathways are also activated by irradiation,[48] and these pathways lead to effector caspases. In addition, ATM activates nuclear factor κ-light-chain-enhancer of activated B (NFκB) cells, a prosurvival factor. NFκB induces or activates a number of target genes that promote resistance to ionizing radiation, including cytokines; human epidermal growth factor receptor 2 (HER-2); manganese superoxide dismutase (MnSOD); cyclins; 14-3-3 proteins; growth arrest- and DNA-damage–inducible, alpha gene (GADD45β); human inhibitor of apoptosis protein-1 (HIAP-1); Ku (a protein involved in nonhomologous end joining of DNA); B-cell lymphoma 2 (Bcl-2); B-cell lymphoma-extra large (Bcl-XL); X-linked inhibitor of apoptosis protein (XIAP); and caspase 8 and fas-associated protein with death domain-like apoptosis regulator (c-FLIP).[49] Many of the radiation countermeasures under development inhibit p53 and/or activate NFκB. For example, growth factors and cytokines activate NFκB and inhibit apoptosis, and are themselves induced by NFκB.[50] Countermeasures that activate toll-like receptors also inhibit apoptosis via induction of NFκB.[51] Ex-RAD (Onconova Therapeutics, Inc, Newtown, PA) down regulates proapoptosis proteins such as p53 and its downstream regulators p21, Bcl-2–associated X protein (BAX), c-Abl, and protein 73.[52] Glycogen synthase kinase (GSK) 3 promotes cell death caused by the mitochondrial intrinsic apoptotic pathway, and GSK inhibitors have been proposed as radiation countermeasures.[53] Octadecenyl thiophosphate (OTP), a mimic of the proapoptotic signal lysophosphatidic acid (LPA), has also been shown to protect against radiation injury.[54] Another lipid pathway considered a possible target for mitigating radiation-induced apoptosis is the acid sphingomyelinase (ASMase)/ceramide pathway.[22]

Less is known about radiation-induced pathways triggered by events other than DNA damage. Reactive oxygen species and reactive nitrogen species inhibit protein tyrosine phosphatase, which can result in increased activation of signaling molecules, including receptors that promote activation of mitogen-activated protein kinase (MAPK) and phosphatidylinositol 3-kinases (P13K) pathways.[55,56] Some have proposed that oxidized proteins constitute a more important factor than DNA damage in radiation injury.[57] Recently, there has been interest in the possible role of oxidized proteins in the endoplasmic reticulum (ER), inducing autophagy or apoptosis in irradiated cells (ER stress, or unfolded protein response).[47,58] Unfolded proteins in the ER are detected by the sensors protein kinase RNA-like endoplasmic reticulum kinase (PERK), inositol-requiring 1 (IRE1), and activating transcription factor 6 (ATF6). These sensors in turn can activate downstream proapoptotic signals, such as controlled amino acid therapy/enhancer binding protein homologous protein (CHOP), c-Jun N-terminal kinases (JNK), and Bcl-2 proteins. Unfolded protein response can also lead to autophagy via these sensors. Activation of the PI3K/Akt/mammalian target of rapamycin (mTOR) pathway can promote survival via effects on autophagy and apoptosis.[47,59] There are indications of a balance between these two modes of cell death such that inhibition of apoptosis may lead to autophagy and vice versa.[47] Whether these signaling pathways and their effects on apoptosis and autophagy will have any influence on the long-term consequences of radiation is not clearly known. Importantly, blocking apoptosis can actually lead to an increase in radiosensitivity related to a concomitant promotion of autophagy.[60] An understanding of these relationships will be essential to developing radiation countermeasures based on inhibition of apoptosis or autophagy.

Regeneration After Radiation Injury

The aim of this strategy is to increase survival by stimulating the function and regeneration of stem and progenitor cell populations that have decreased in number due to radiation injury. Conceptually, this strategy can be applied to any organ system (such as the hematopoietic and gastrointestinal systems) that relies on stem-cell proliferation to provide mature differentiated cells for proper functioning. Only regeneration of the hematopoietic system is discussed here. Regeneration is a feasible strategy for mitigating radiation injury at doses below the threshold dose that would result in 100% death of hematopoietic stem cells. Exactly which cell type becomes stimulated depends on the type of agent involved. Nonspecific immunomodulators are exogenous agents that can bind to and stimulate a variety of different cell types. These agents are thought to induce the stimulated cells to release a variety of peptides (cytokines) that act specifically on immunopoietic and hematopoietic progenitor and stem cells to stimulate their growth and differentiation into mature, functional cells.[61]

Figure 11-3 examines hematopoietic progenitor cell survival as measured by the number of colony-forming units (CFUs) found in the spleens (endogenous CFU [e-CFU]/spleen) of irradiated mice. Some of the mice were treated with the regenerating agent glucan. In the radiation-control animals that were not given glucan, the number of e-CFU/spleen decreased with increasing radiation dose. Similarly, the effectiveness of glucan in increasing the survival of these cells also decreased with increasing radiation dose. This indicates that the effectiveness of these agents depends on the number of surviving progenitor cells. Above the threshold radiation dose that results in 100% progenitor-cell death (greater than 8.5 Gy in Figure 11-3), regeneration becomes ineffective.

Partial-Body Irradiation and Regeneration

The contribution of these protective measures was evident in the Chernobyl accident victims, in whom bone-marrow grafts apparently failed. These failures were due, at least in part, to host-versus-graft reactions

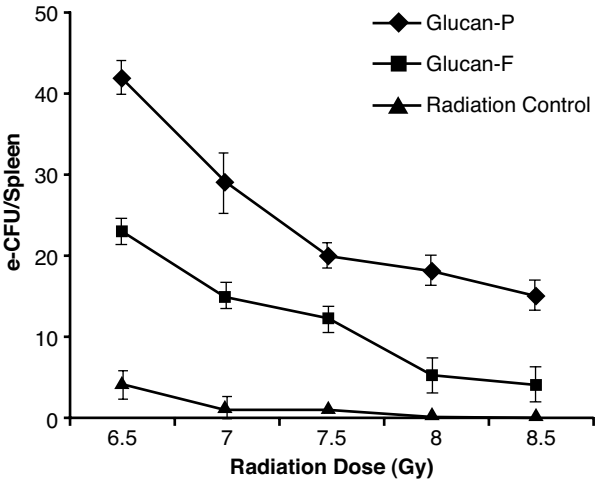

Figure 11-3. Hematopoietic progenitor cell survival as a function of radiation dose treated or not treated with glucan, a radiation countermeasure agent that promotes progenitor cell regeneration in irradiated mice. Glucan efficacy decreases as increased numbers of progenitor cells are killed by higher doses of radiation.
e-CFU: endogenous colony-forming unit

TABLE 11-2

SUPPORTIVE THERAPY IN SURVIVAL OF IRRADIATED PRIMATES*

	No Supportive Therapy	Antibiotics, Fluids, Platelets	Allogeneic Bone Marrow Transplant†	Partial Shielding‡
Total primates	4	4	5	4
Survivors	0	0	5	4
Mean survival (days)	12.5	16.3	> 30	> 30

*Irradiated with a dose of 8 Gy
†Also given antibiotics, fluids, and platelets
‡Less than 1% surviving stem cells
Data source: Giambarresi L. Prospects for radioprotection. In: Walker RI, Cerveny TJ, eds. *Medical Consequences of Nuclear Warfare*. In: Zajtchuk R, Jenkins DP, Bellamy RF, Ingram VM, eds. *Textbook of Military Medicine*. Washington, DC: Department of the Army, Office of The Surgeon General, Borden Institute; 1989. Chap 11: 245–273.

initiated by surviving stem cells, even in patients who were exposed to doses of radiation much greater than that expected to completely deplete stem cells.

The effectiveness of minimal local shielding in protecting even small numbers of stem cells is demonstrated in experiments done with monkeys (Table 11-2).[62] Supportive therapy (fluid, platelets, and antibiotics) significantly increased the dose of radiation expected to cause death to 50% of an exposed population within 30 days ($LD_{50/30}$) of irradiated animals. In monkeys exposed to a lethal dose (8 Gy) of whole-body cobalt-60 radiation, supportive therapy extended survival for a few days but had no effect on 30-day survival rates because the radiation dose completely depleted the stem-cell population. However, when the tibias of these animals were shielded so that less than 1% of their bone-marrow stem cells survived, regeneration occurred and many of the animals survived.

RADIATION COUNTERMEASURES

Single Agents

Some of the agents currently under various stages of research as candidates for protection are given in Table 11-1.

Aminothiols and Other Thiol Derivatives

Aminothiols make up the vast majority of agents that have been developed and tested in laboratory models for their ability to increase survival after irradiation.[63] These compounds are chemical analogues of cysteine, the sulfur-containing amino acid. Like cysteine, they have a sulfhydryl group separated by two or three carbon atoms from a strongly basic nitrogen group. As a group, the aminothiols are very effective protectants and they must be present in the system during irradiation. Optimal protection in laboratory animals is generally obtained by intraperitoneal injection 15 to 30 minutes before irradiation. The aminothiols function primarily through free-radical scavenging[9] and hydrogen-transfer mechanisms.[64,65] Hypoxia induction may also play a part in their functioning.[8,64]

One of the most significant events in the development of radioprotective agents was the synthesis of an aminothiol derivative in 1969 known as amifostine (previously known as WR-2721).[66] This drug was developed through a program sponsored by the Walter Reed Army Institute of Research and is the most thoroughly studied of over 4,000 compounds developed and tested to date. Amifostine has reportedly shown a high degree of protection, with a radiation dose factor of 2.7 when given to mice intraperitoneally 30 minutes before exposure to gamma radiation.[67,68] This is the highest DRF against mouse lethality at 30 days reliably reported for a single injection of a conventional radioprotectant.

In addition to providing radioprotection, amifostine significantly reduces the toxicity of the tumor chemotherapeutic agents cyclophosphamide and cisplatin,[69,70] apparently without altering their chemotherapeutic effectiveness. There are also reports indicating that amifostine preferentially protects normal tissues but not solid tumors against radiation.[68] For these reasons, amifostine is used under clinical supervision as an adjunct to tumor radiation and chemotherapy.

Amifostine remains unavailable as a field-useable radioprotective agent because it induces nausea, vomiting, and hypotension.[71,72] Although no cumulative or irreversible toxicity has been observed in humans

or experimental animals receiving this drug (even at relatively high doses), the animals did show significant performance degradation after its parenteral administration.[73,74] Another problem that must be overcome is the drug's poor oral bioavailability, due primarily to first-pass metabolism by the intestinal mucosa during absorption.[75] In addition, the drug is hydrolyzed in the acidic environment of the stomach, a factor that is aggravated by its ability to slow gastric emptying.[76] Because amifostine is a hypocalcemic agent, another clinical side effect of this drug is inhibition of parathyroid hormone secretion.[6] Due to these limitations, amifostine is not a drug of choice for radioprotection of first responders or astronauts in whom performance decrement is not acceptable. Although a DRF of about 1.2 has been obtained with amifostine administered intraperitoneally to mice at a dose that produced no observable side effects or performance degradation,[74] an equivalent dose in large animals and humans had unacceptable side effects.

Several other radioprotective derivatives of amifostine were developed through the Army's program. WR-3689 and WR-151327 were the most effective among these thioates (WR-2721 is considered the gold standard for radiation protection studies in mice). However, none of them was free from toxicities. Some studies indicate the efficacy of WR compounds against high-LET radiation, such as neutrons, either by radiation alone[77,78] or when combined with infection.[79] Other thiol compounds that have shown radioprotective effect include mercaptopropionyl glycine (MPG) and N-acetyl cysteine. Effective doses of these drugs for significant protection were close to the maximum tolerated dose.[80] Some of the thiols, such as aminoethyl thiouronium bromide (AET), are protective against high-LET radiation.

Nutraceuticals, Antioxidants, and Endogenous Antioxidant Systems

Certain naturally occurring compounds function as antioxidants, such as vitamins and minerals, enzymes, and enzyme mimetics. These are part of a natural biochemical defense system that has evolved to protect cells against free radicals and reactive oxygen species arising from normal metabolic processes. This defense can be divided into two components: (1) compounds of low molecular weight that scavenge free radicals, and (2) enzymes that detoxify reactive oxygen species.[81]

The low-molecular–weight compounds that function as free-radical scavengers in this defense system include vitamins A and E, which are lipophilic, and ascorbic acid (vitamin C), which is hydrophilic. The enzymatic arm of this system includes superoxide dismutase, which catalyzes the conversion of superoxide anions to hydrogen peroxide and molecular oxygen. The hydrogen peroxide produced by this reaction is removed from the system by two other enzymes: catalase and glutathione peroxidase. Selenium contributes to this scheme in that it is a cofactor for glutathione peroxidase.

Vitamin E has been shown to increase survival after irradiation when mice were fed a diet supplemented with three times the normal daily mouse requirement of vitamin E (dl-alpha-tocopherol) for 1 week before an 8.5 Gy dose of cobalt-60 gamma radiation and for 30 days after exposure. This regimen provided a survival protection of 90% and resulted in a decrease in radiation-induced, delayed-type hypersensitivity.[82] A single subcutaneous injection of vitamin E provided greater protection than administration in the diet.[83] Topical treatment of exteriorized intestine or oral treatment of rats with vitamin E increased the survival of intestinal crypts.[84] Both vitamin E and ascorbic acid reduced radiation-induced micronucleus formation and chromosomal aberrations in mice; vitamin E was more efficacious than ascorbic acid.[85]

Tocotrienols are superior to α-tocopherol in their radioprotective efficacy, perhaps because they are better antioxidants than α-tocopherol. Another effective option is γ-tocotrienol, a radioprotectant with a DRF of 1.3 that protects mice from hematopoietic failure, gastrointestinal injury, and lethality (Figure 11-4).[86–88] Unpublished results indicate that δ-tocotrienol is almost as effective as γ-tocotrienol.

Vitamin A also increases postirradiation survival when fed to mice as a dietary supplement.[85] In these experiments, mice were maintained on a diet containing various levels of vitamin A or β-carotene, and the mice fed on supplemented diets displayed better survival after irradiation than those fed the basal diet. Vitamin A fed to mice for 3 days before partial-body irradiation can substantially reduce the effects of localized (hind limb) X-irradiation.[89] In addition to its radioprotective ability, vitamin A or β-carotene may also be able to promote recovery from burn injury by reversing postburn immunosuppression.[90] This point is significant because burns are expected to be one of the collateral injuries on the nuclear battlefield.

Selenium is protective when administered either orally or parenterally. When given orally as sodium selenite in drinking water (4 ppm) or injected (1.6 mg/kg) 24 hours before exposure to 9 Gy of cobalt-60 radiation,[91] selenium provided slight but significant increases in survival. The real potential for using selenium as a radioprotective agent lies in its ability to act synergistically with other agents. Selenium was shown to decrease the toxicity of amifostine and

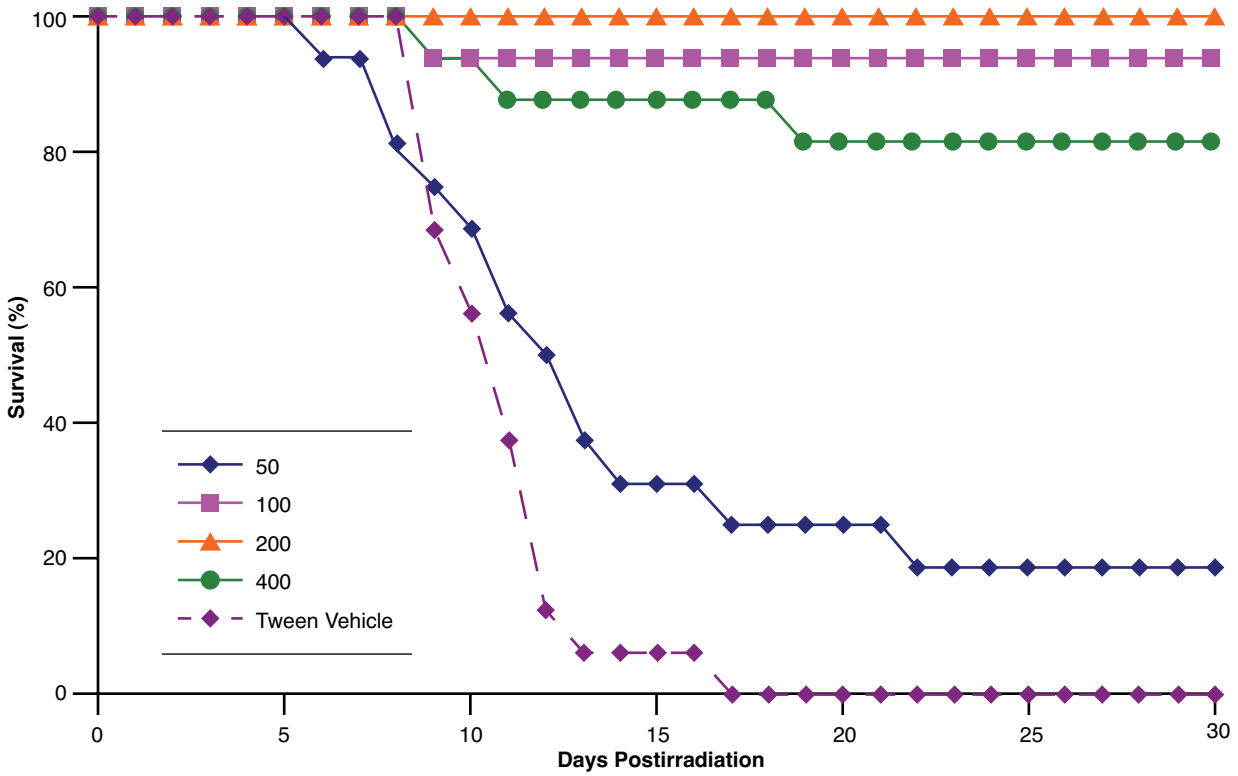

Figure 11-4. Gamma-tocotrienol as a radiation countermeasure at 11 Gy. Thirty-day survival of mice (n = 16 per group) treated 24 hours before receiving 11 Gy of cobalt-60 gamma radiation, with a single subcutaneous injection of a vehicle (5% polysorbate 80) or γ-tocotrienol at doses of 50 to 400 mg/kg body weight. Mice that received a γ-tocotrienol dose of 100, 200, or 400 mg/kg exhibited a significant increase from the vehicle control group.

increase radioprotection when combined with it.[91] Selenium, copper, and zinc were shown to be marginally radioprotective, but they enhance the radioprotection by amifostine.[92]

The parenteral administration of superoxide dismutase increased survival in mice exposed to ionizing radiation.[93] Intravenous injection of this enzyme in mice at a dose of 200 mg/kg given 1 hour before irradiation with X-rays resulted in a DRF of 1.38. A single injection of only 35 mg/kg given 1 hour before irradiation with X-rays also increased survival (DRF: 1.12). The highest DRF reported for this enzyme is 1.56, achieved in mice given two intravenous injections: once at a dose of 200 mg/kg given 1 hour before irradiation with X-rays, and the other at a dose of 35 mg/kg given 1 hour after irradiation.[93] Although further studies on protection by parenteral superoxide dismutase (SOD) were reported, mimetics of SOD showed promise of radioprotection. Eukarion-189, a salen-manganese complex, and superoxide dismutase/catalase mimetic enhanced 30-day survival, with a DRF of 1.15.[94]

Recently, flavonoids were found to be potential nontoxic radioprotectants. Genistein, a nontoxic isoflavone from soybeans, protected mice when given as a single subcutaneous injection at a dose of 200 mg/kg 24 hours before lethal irradiation.[95] The 30-day survival in the genistein-treated group was 97%, as compared to 31% of the vehicle-treated mice and 0% of untreated mice. One of the reasons for the protection by genistein may be due to the extended quiescence followed by reduced senescence of bone-marrow repopulating LSK^+ ($Lin^+Sca1^+Kit^+$) cells.[96]

The ocimum flavonoids orientin and vicenin protected mice from radiation-induced intestinal and bone-marrow syndromes with DRFs of 1.30 and 1.37, respectively.[97] Both of these flavonoids protected mice from prenatal radiation-induced genomic instability and reduced delayed chromosomal aberrations and tumorigenesis in adult mice.[98]

Eicosanoids

The eicosanoids are a large group of potent inflammatory mediators derived from the 20-carbon fatty-acid precursor, arachidonic acid. The compounds in this family that were examined for their abilities to

increase the survival of irradiated animals include 16,16-dimethyl prostaglandin E, ($DiPGE_2$, a synthetic analogue of the naturally occurring prostaglandin GE_2), leukotriene C (LTC), and platelet-activating factor (PAF). $DiPGE_2$, at a toxic dose that induced diarrhea 5 to 15 minutes before irradiation, elicited a DRF of 1.72, but some protection could still be achieved when the compound was given 1 hour before irradiation.[99] Misoprostol, a stable analogue of prostaglandins, increased the survival of intestinal clonogenic cells by 600%. Diarrhea and other side effects of misoprostol were significantly decreased by mixing misoprostol with iloprost (a prostanoid), which simultaneously decreased the radiation protection efficacy.[100] LTC_4 was shown to be effective in increasing the survival of hematopoietic stem cells in mice exposed to cobalt-60 gamma radiation.[101] Despite the high DRFs obtained with these compounds, serious irreversible toxicity associated with prostanoids prevented further exploration for human use.

Biological Response Modifiers, Immunomodulators, and Cytokines

The original immunomodulators were generally crude, whole-cell, microbial preparations (such as Bacillus Calmette-Guérin [BCG] and *Corynebacterium parvum)* used because they could nonspecifically stimulate host immune responses. Later, the active components of these cells (such as endotoxin and zymosan) were identified and isolated from their cell walls. Further work led to the purification, identification, and synthesis of the specific portions of the cell fragments that were responsible for stimulating immune responses (such as endotoxin and glucan from zymosan). Stimulation of cells by immunomodulators results in the release of cytokines, which act as specific stimulators of host immune responses. Recent advances include the development of biologically defined molecules and recombinantly produced cytokines (such as interleukin 1 [IL 1] and granulocyte-macrophage colony-stimulating factor [GM-CSF]), which are relatively nontoxic but allow specific manipulation of various components of the immune and hematological systems.

Bacterial endotoxin was probably the first biological response modifier shown to be a radioprotectant.[102] The window of protection for endotoxin is very narrow due to its high toxicity. A less toxic product from endotoxin obtained by acid hydrolysis was found to have almost the same radioprotective efficacy. This product, 3D-monophosphoryl lipid A (3D-MPL), at a dose of 0.2 to 0.5 mg/kg body weight, given intraperitoneally 16 to 20 hours before radiation, protects mice from radiation-induced lethality, with a DRF of 1.2.

Glucans, which are β-1,3-linked polysaccharides, in soluble and particulate forms showed differential radioprotective efficacy, with the particulate form being more radioprotective. Particulate glucan showed a DRF of 1.22 at a dose of 75 mg/kg, while soluble glucan provided a DRF of only 1.02 at a dose of 250 mg/kg. There are several other biological response modifiers that showed varying degrees of radioprotection.[103] Polysaccharides MNZ, GLP/Bo4, GLP/Bo5 (from *Saccharomyces cerevisiae*) and MNR (from *Rhodotorula rubra*) also provided high DRFs, but these high values may be due to impurities.

Trehalose dimycolate, also known as cord factor, is a glycolipid consisting of 6,6′-diesters of the sugar D-trehalose. It is isolated from the cell walls of *Mycobacteria*, *Nocardia*, and *Corynebacteria*, and is an active component of Freund's complete adjuvant. Like glucan, trehalose dimycolate is a potent immunostimulant that is capable of increasing host defense mechanisms against a variety of organisms and of increasing survival after irradiation.[104,105]

Cytokines are another class of immunomodulators with radioprotective efficacy. Neta et al[106,107] showed IL 1 protected irradiated mice when given either 20 hours before or 2 hours after irradiation. Radioprotection with a DRF in the range of 1.15 to 1.25 was maximized when IL 1 was given 20 hours before radiation at doses of 4 or 8 μg/kg body weight. Acidic fibroblast growth factor (FGF) 1 was radioprotective, with a DRF of 1.16 when given before irradiation.[108] FGF1 and FGF2 induced radiation resistance of crypt cells.[109] A chimeric form of FGF1 and FGF2 augmented activity useful for epithelial proliferation and radioprotection.[110] Tumor necrosis factor α (TNF-α) was also shown to be radioprotective in mice. It has been suggested that TNF-α does not protect tumor cells from radiation, but protects only normal cells. On the other hand, it is also reported that specific inhibition of TNF-α receptors by genetic knock-out protected lungs from radiation.[111] Ammonium trichloro (dioxyethylene-0-0′) tellurate (AS101), a synthetic immunomodulator, was shown to protect mice from hematopoietic injury.[112]

Whitnall et al investigated the mechanisms of action of androst-5-ene-3beta,17beta-diol (5-androstenediol [5-AED]) because of its ability to reduce mortality (Figure 11-5), thrombocytopenia, and neutropenia in irradiated mice and nonhuman primates. 5-AED displays extremely low toxicity and androgenicity.[113–115]

In-vitro studies of human hematopoietic progenitor cells showed they are a direct target of 5-AED.[115] Incubation with 5-AED reduced apoptosis and promoted survival of these cells when exposed to gamma radiation, and this effect was dependent on activation of NFκB and resultant induction of G-CSF, consistent

with the demonstration of G-CSF induction in mice treated with 5-AED.[116,117]

Two other cytokines may be potentially useful agents: GM-CSF and interleukin 3 (IL 3). Several growth factors that are specific for different hematological cell populations have been discovered and can be produced by recombinant DNA methods. One of these, a specific human recombinant GM-CSF (rhGM-CSF), accelerates marrow repair or engraftment and may contribute to increased nonspecific resistance. It functions by increasing the number of circulating granulocytes and platelets in normal animals and accelerating the recovery of these cells after irradiation. This factor was used in treating some victims of the radiation exposure accident in Goiânia, Brazil. The effectiveness of GM-CSF in ameliorating radiation-induced cytopenia can be seen from data obtained in the minimal-shielding experiment.[62] In that experiment, the survival of partially shielded monkeys that were given supportive therapy was enhanced. Unshielded animals rapidly became neutropenic and died within 15 days. In the shielded animals that survived beyond 30 days, peripheral granulocytes began to recover slowly between days 20 and 40. In contrast, shielded animals treated with GM-CSF showed evidence of granulocyte recovery well before day 20, and granulocyte levels quickly reached supranormal levels. Therefore, it appears this factor is a useful adjunct to radiation-injury therapy. However, its effectiveness as a regeneration agent in radioprotective regimens is much lower than that for IL I and TNF. Other evidence suggests that GM-CSF may act synergistically when combined with other cytokines.[118]

IL 3 has not yet been evaluated for its ability to in-

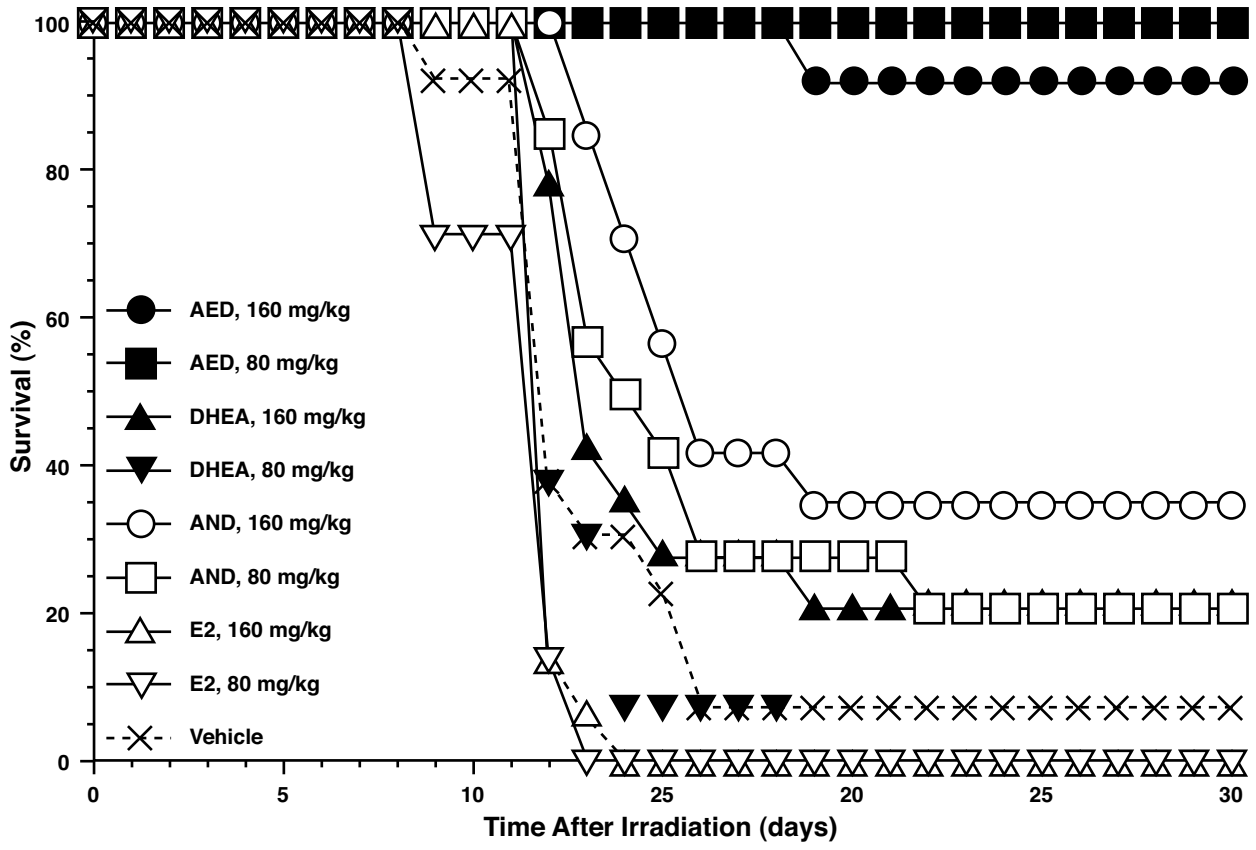

Figure 11-5. Survival time courses of female B6D2F1 mice after subcutaneous injection with 5-androstenediol, dehydroepiandrosterone, 4-androstenedione, or estradiol 24 hours before whole-body gamma-irradiation (11 Gy, 0.6 Gy/min). Survival after 5-androstenediol ($P < 0.001$) or 4-androstenedione (80 mg/kg: $P < 0.05$; 160 mg/kg: $P < 0.01$) was significantly greater than with the vehicle.
AED: 5-androstenediol
AND: 4-androstenedione
DHEA: dehydroepiandrosterone
E2: estradiol

crease survival after irradiation. Unlike the described action of the cytokines (whose major target cells are primarily the more mature functional cells in the system), IL 3 is reported to act specifically in stimulating the growth of pluripotent progenitor cells.[119]

Kiang et al found that the geldanamycin derivative 17-(dimethylaminoethylamino)-17-demethoxygeldanamycin (17-DMAG) improved mouse survival from cobalt-60 gamma irradiation at a lethal dose.[120] 17-DMAG inhibited the radiation-induced activation of the inducible nitric oxide synthase pathway, thereby blocking apoptosis[121] and autophagy.[122] This drug also inhibited the radiation-induced activation of p53–Bax signal transduction[121] and the radiation-induced increases in cytokines (Kiang, unpublished data, 2010).

Combination Agents

Rationale

Agents that act as protectors, mitigators, or therapies contribute in different ways to counter radiation injury by protection, repair, and regeneration. Each of them also has its limitations. Neither chemical nor enzymatic means of protection minimize direct damage. In addition, it is almost impossible for any protective or repair agent to either completely eliminate all of the reactive intermediates formed or repair all of the damaged molecules. Regardless of the efficiency of scavengers and repair agents and their concentration within the cell at the time of irradiation, some molecular damage and cell death still occurs. The effectiveness of agents that function in the regeneration strategy is limited because the agents require a pool of surviving functional cells on which to work. That pool of hematopoietic stem cells and highly radiosensitive progenitor cells becomes depleted even at sublethal radiation doses.

It is reasonable to expect that optional survival would be provided by an agent or combination of agents that would operate using two or more of these strategies. Such a formulation would maximize the effectiveness and minimize its limitations. Protective agents prevent the production of reactive species resulting from the radiolysis of water. Mitigators attenuate the injury. Therapeutic agents repair the damage to critical target molecules and allow regeneration of critical cells. A combination of these agents increases the surviving fraction of stem cells, progenitor cells, and mature cells of the hematopoietic system after irradiation. By allowing stem cells to survive at higher radiation doses, the net effect is to increase the threshold radiation dose that limits the effectiveness of regenerative agents. Taken together or at intervals with protective agents and mitigators, these agents further enhance the organism's survival by maximizing the proliferation and function of the extra stem cells provided.

It would be difficult to produce one drug that would be able to ameliorate radiation injury by performing protection, repair, and regeneration. Two or more agents might be used either together or at intervals, but this is not ideal; a single dose is the simplest dosing regimen that is desirable for military personnel under battle conditions or for first responders in emergency situations. Therefore, the goal is a single treatment consisting of a combination of two or more agents with the capabilities of protection, repair, and regeneration.

Combination Agents

The concept of using a combination of agents that function by different mechanisms to achieve protection was developed and studied in the 1950s and 1960s.[7,92] In many of the combinations examined, synergistic effects were seen. These results are particularly significant because increased protection with the combinations was often achieved using substantially lower doses of individual drugs than those required for protection when each agent was given separately. For example, one study examined various combinations of five different radioprotective agents: cysteine, β-mercaptoethylamine (MEA), aminoethylisothiouronium bromide-hydrobromide (AET), glutathione, and serotonin.[123] MEA, AET, or serotonin used alone provided similar protection, with a DRF of 1.7; cysteine was less effective, with a DRF of 1.12; and glutathione was marginally protective, with a DRF of 1.05. The most effective regimen was a combination of all five agents, which produced a DRF of 2.8. In this combination, the MEA dose was one half, and the AET dose was two thirds that used when the drugs were given individually.

Additive and synergistic effects were demonstrated with various combinations of aminothiols, antioxidant vitamins and minerals, immunomodulators, prostanoids, and cytokines. It is likely that a first-generation agent will be a combination of subtoxic doses of two or more of these agents (Table 11-3).

Mitigation of Performance Decrement

Because a single, self-administrable agent is sought as a radiation countermeasure, it might also be necessary to include moderators of performance decrements such as nausea, vomiting, diarrhea, or hypotension in any regimen that is developed. While measures to enhance resistance to the lethal effects of radiation have been extensively studied, the application of pharmacological interventions to mitigate performance and behavioral deficiencies has not been addressed sufficiently, even though

TABLE 11-3

RADIOPROTECTIVE EFFICACY OF SELECTED COMBINED AGENTS

Agents		Dose (mg/kg)		Dose Reduction Factor*		
A	*B*	*A*	*B*	*A*	*B*	*A+B*
IL 1[1]	TNF	150†	5‡	1.19	1.12	1.38
Glucan-P[2]	Amifostine	75	200	1.22	1.33	1.51
Selenium[3]	Amifostine	1.6	400	1.1	2.2	2.5
$DiPGE_2$§	Amifostine	0.4	200	1.4	1.9	2.2

*Dose reduction factor = radiation $LD_{50/30}$ dose for drug/radiation $LD_{50/30}$ dose for excipient
†μg/mouse
‡ng/mouse
§Unpublished data
$DiPGE_2$: 16,16-dimethyl prostaglandin E_2
IL 1: interleukin 1
$LD_{50/30}$: the dose of radiation expected to cause death to 50% of an exposed population within 30 days
TNF: tumor necrosis factor
Data sources: (1) Neta R, Oppenheim JJ, Douches SD. Interdependence of IL-I, TNF, and CSFs in radioprotection. *J Immunol*. 1988;140:108–111. (2) Patchen ML, MacVittie TJ, Weiss JF. Combined modality radioprotection: the use of glucan and selenium with WR-2721. *Int J Radiat Oncol Biol Phys*. 1990;18:1069–75. (3) Weiss JF, Hoover RL, Kumar KS. Selenium pretreatment enhances the radioprotective effect and reduces the lethal toxicity of WR-2721. *Free Rad Res Communs*. 1987;3:33–38.

these are immediate military concerns. Although it is possible for radioprotective agents to prevent some performance decrements, drugs that increase survival generally have not enhanced performance. In fact, except for a few notable exceptions, they usually exacerbate radiation-induced performance decrements.[73,74] Groups of drugs are being developed that will, perhaps, stabilize performance by modulating cellular permeability, altering regional blood flow, and interrupting the release or action of various mediators. Drugs are being identified that can modulate postirradiation nausea, vomiting, diarrhea, and other performance decrements.

Radiation Countermeasures and Supportive Therapy

Radiation countermeasures will be most effective in personnel exposed to radiation doses within the ranges required to produce the hematopoietic subsyndrome (approximately 2.0–8.0 Gy) and mild gastrointestinal subsyndrome (approximately 8.0–10.0 Gy), and in whom no associated injuries are present. In the event of more severe radiation injury, or if radiation injury is combined with traumatic or burn injuries (a likely occurrence on the battlefield or after a radiation leak or explosion accident), radioprotective measures alone will be insufficient and additional supportive therapy will be required. Although the effectiveness of radiation countermeasures may be reduced in the face of

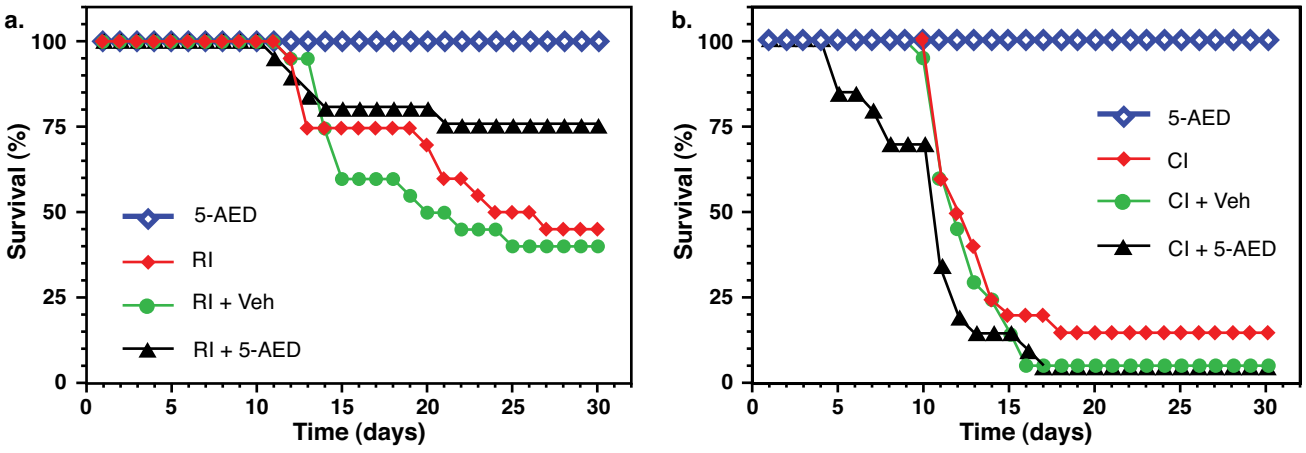

Figure 11-6. Comparative effects of 5-androstenediol on survival in mice receiving either radiation alone **(*a*)** or radiation followed by wound trauma **(*b*)**. B6F2D1/J female mice received cobalt-60 gamma radiation at 9.75 Gy alone or followed by a 15% body-surface-area wound. Then the mice were subcutaneously injected with a vehicle (polyethylene glycol 400) or 30 mg/kg 5-androstenediol at 2 hours, 24 hours, and 48 hours after radiation alone or combined injury.
5-AED: 5-androstenediol
CI: combined injury
RI: radiation injury
Veh: vehicle

more severe radiation injury or combined injury, it should be noted that their use at the time of irradiation will likely increase the effectiveness of supportive therapies provided days later.

Traumatic injury can reduce the ability of pharmacological agents to increase survival from a lethal radiation dose (Figure 11-6). Ledney et al[124] reported that mice treated with 5-AED dissolved in PEG-400 (polyethylene glycol 400) within 2 hours after exposure to 9.75 Gy of cobalt-60 gamma radiation showed 76% survival, whereas mice treated with just PEG-400 showed 40% survival. However, this protection was not seen in mice receiving 9.75 Gy followed by a 15% total-surface-area wound. In the irradiated and wounded mice, death began to occur about 1 week earlier than in the irradiated-only mice, and all mice died at the same rate regardless of treatment with 5-AED.[124] A similar observation was also found with trehalose dimycolate treatment.[104]

This difference in protective response between irradiated-only and combined-injury mice may be due to a more profound activation of the inducible nitric oxide synthase pathway, increases in serum cytokine concentrations and bacterial infection, reduction of cell adhesion and extracellular matrix, and increases in toll-like receptor signaling, resulting in physiological perturbations[125] so as to induce apoptosis[121] and autophagy.[122] Finally, multiple organ dysfunction and failure occur and mortality is manifested. Various interventions to enhance resistance to radiation and wounds may be used in combination to prevent infection in severely injured subjects. To avoid infection, the natural and artificial defenses must be in balance so that the host resistance is sufficient to control the number of microorganisms. Therefore, as normal defenses are compromised due to suppression by radiation, artificial interventions are required to maintain resistance above the threshold for infection (Figure 11-7).

The potential synergy between therapeutic agents, such as antibiotics, and substances that may be used as radioprotectants is indicated by data on the use of glucan and the antibiotic pefloxacin in the management of postirradiation mortality. In that experiment, only 25% of mice given 7.9 Gy of whole-body cobalt-60 gamma radiation survived. Treatment with either glucan alone at 1 hour or with pefloxacin alone for 24 days after irradiation resulted in 48% and 7% survival,

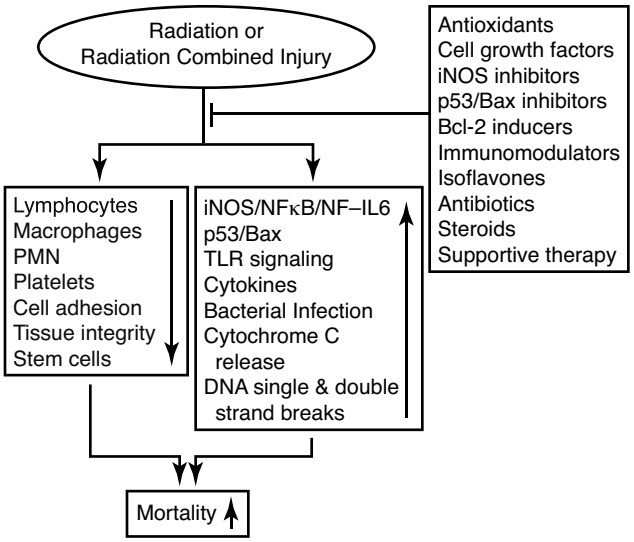

Figure 11-7. Radiation or radiation combined with wound attenuate the normal defenses. Various interventions against radiation injury or radiation injury combined with wound injury may be used in combination to improve the chance of survival in severely injured subjects.
Bax: B-cell-lymphoma-2–associated X protein
Bcl-2: B-cell lymphoma 2
DNA: deoxyribonucleic acid
iNOS: inducible nitric oxide synthase
NFκB: nuclear factor κ-light-chain-enhancer of activated B
NF–IL6: nuclear factor–interleukin-6
p53: protein 53
PMN: polymorphonuclear leukocytes
TLR: toll-like receptor

respectively. However, if the two treatments were combined, survival was 85%.[126] An increase in DRF was demonstrated when glucan was combined with selenium and amifostine.[127] Combining α-tocopherol with WR-3689 (a methylated form of amifostine) reduced the toxic dose of WR-3689 without compromising the DRF.[128] Other combination modality strategies were reviewed by Weiss et al.[92] Recently, a mixture of dietary antioxidants was shown to protect hematopoietic cells and improve survival after total-body irradiation.[129] Curcumin, when combined with copper (II) in a ratio of 1:1, showed higher radioprotection as compared to curcumin alone.[130] Combining salts of copper, selenium, and zinc increased radioprotection by amifostine or 5-aminosalicylic acid.[92,131]

DEVELOPMENT OF A RADIOPROTECTIVE REGIMEN

A variety of factors must be considered when evaluating and developing candidate radiation countermeasure drugs for military use, and a compromise must be reached between the ideal and the achievable. To screen radiation countermeasure agents in animals at the Armed Forces Radiobiology

Research Institute (AFRRI), an optimal drug dose for screening is determined. Drug doses are selected in a stepwise, up-or-down fashion to assess toxicity over 14 days. A drug dose that does not result in any adverse effects is established and known as the "no-observed-adverse-effect" level. The drug dose to be used for initial radioprotection experiments is one fourth the no-observed-adverse-effect level. Then the optimal timing of drug administration, the optimal drug dose, and the optimal administration route can be determined. It should be noted that ease of administration, simplicity of dose schedule, minimal side effects, and a wide safety margin are particularly important because it may be necessary to administer a radioprotective drug repeatedly for several days.

Pharmacological Side Effects

Side effects (ie, toxicity) are a major obstacle in fielding agents to prevent, mitigate, or treat radiation injury. No chronic toxicity is acceptable. Acute toxicity (such as nausea, vomiting, and hypotension) are common, especially with the sulfur compounds. For a fieldable drug, any acute side effects will have to be reduced in severity so that military performance is not impaired. If that is not possible, these effects should at least be controllable by other conveniently applied therapies.

Additionally, these agents must not significantly increase the user's vulnerability to chemical or biological agents or antidotes, exacerbate other battlefield injuries, negatively affect behavior, or interfere significantly with wound healing. The agent should have a wide safety margin (ie, therapeutic index) to compensate for the "if one is good, then two must be better" philosophy.

New Directions

Past nuclear accidents at Chernobyl, Three Mile Island, Goiânia, and Tokaimura, and recent global developments in the possession of weapons-grade nuclear fissionable materials by several nations are indications that a radiological/nuclear incident is only a matter of time. Therefore, there is an urgent need to develop a safe and effective radiation countermeasure. Such a need prompted intense efforts by the National Institute for Allergy and Infectious Diseases and the Department of Defense Threat Reduction Agency to devote considerable resources to developing radiation mitigators and prophylactic agents. These efforts are already yielding sporadic successes. Among the drugs that were screened and exploited under the direction of these two agencies or AFRRI, a few are showing moderate successes. 5-AED,[111] a toll-like receptor 5 agonist,[132] genistein,[133] SOM230,[134] and Ex-RAD[52] have been observed in completed small-animal studies. Some have already been afforded investigational new drug status by the US Food and Drug Administration and are in phase I clinical trials with humans.

Simultaneously, newer approaches are being explored. One approach being developed involves incorporating the human MnSOD gene into a minicircle plasmid and testing its radioprotective potential.[135] The MnSOD-containing plasmid was radioprotective in vitro and in vivo. One problem encountered in the current radiation countermeasure discovery programs is a lack of efficacy of oral drugs. Application of nanotechnology may make drugs that are currently delivered by injection available orally. In this technology, the drug is encapsulated in a nanoparticle, allowing it to pass through the stomach and be delivered into the bloodstream. Nanoencapsulation has been shown to increase the cellular delivery of drugs as much as 3- to 10-fold.[136]

At AFRRI, a permanent intramural screening program has been instituted to test potential radiation countermeasures that may be developed independently at the institute or referred from various sources. At this writing, four radiation countermeasure candidates have been granted investigational new drug status by the US Food and Drug Administration. All four are AFRRI products: two initiated independently at AFRRI, and two the results of collaborations with biotechnology firms.

Systems Biology Approach

Applying bioinformatics tools, it should be possible to search the database of chemicals maintained by the National Center for Biotechnology Information and identify chemicals that may have chemical structures similar to well-established radiation countermeasures. The compounds can be screened for their abilities to protect cell lines from radiation as measured by clonogenic survival. Selected drugs from this initial screening would be subjected to mechanistic studies in these cells by high-content screening to establish if the clonogenic survival is accompanied by the restoration of pathway-specific genes affected by radiation. Those chemicals surviving these rigorous initial tests will be subjected to in-vivo screening in rodents and further development. Since this approach would miss effective countermeasures that depend on cell interactions or mechanisms not present in the cultures, a parallel program of initial screening in vivo should be maintained.

SUMMARY

The development of radioprotective agents has been dominated by the study of sulphydryl compounds, particularly the aminothiols. These compounds function by a variety of mechanisms, almost all of which increase survival in the irradiated organism by minimizing the radiation-induced damage to critical biological molecules. These compounds suffer from one major drawback: high levels of protection are accompanied by unacceptable side effects. Therefore, it has been necessary to search for less toxic compounds for radiation injury alone and for combined injury.

Among the candidates being evaluated are naturally occurring dietary components such as selenium, vitamin A, vitamin E, genistein, and drugs of low toxicity that are being used clinically, such as MPG. The drawback to these agents is that the protection achieved is relatively low. However, some vitamin E isoforms and genistein display more protection than reported previously for dietary components. These compounds merit further exploration.

The net effect of protective compounds is an increase in the number of stem and progenitor cells that survive the initial radiation insult. To exploit this early benefit, agents that stimulate the proliferation and differentiation of those cells would help optimize cell repopulation of organ systems that were depleted by radiation-induced cell death. The use of regeneration agents, such as immunomodulators and cytokines, alone has been shown to enhance survival after irradiation. When these agents are administered along with a protective agent, additive or synergistic effects are seen. Most importantly, these effects are often achieved using subtoxic doses of the individual agents.

Combining those agents that use a protection or repair strategy with those that promote regeneration offers the advantages of circumventing side effects, enhancing the effectiveness of relatively nontoxic agents that provide only mild protection when given alone, and maximizing the therapeutic benefit provided by each agent. The use of pharmacological agents to increase survival after irradiation will be most effective for personnel exposed to low or intermediate doses of radiation who have minimal associated traumatic or burn injuries. Indeed, in a mass casualty situation, those agents may be the only type of medical intervention available. On the other hand, with smaller numbers of casualties, especially those with combined injuries, it is likely that additional supportive therapies will be available. The early application of radiation countermeasures will minimize the need for subsequent interventions and will enhance the effectiveness of the interventions that are provided.

Many factors must be considered in defining the desired properties of a potentially fieldable first-generation agent. Since the development of WR-2721 (amifostine), emphasis has been placed on studying agents that produce DRFs greater than 2. This emphasis may actually have hampered efforts to field a suitable agent. Some agents with lower DRFs can provide significant protection and may be more appropriate for field use. The agent should also have a high therapeutic index because it will most likely be self-administered. Whether or not the agent can be taken orally is ultimately an important consideration.

Based on candidate agents now available, it may soon be possible to recommend a countermeasure regimen that meets the requirements. The recommendation will probably include a combination of at least two of the candidate agents described above. Fielding a first-generation agent that satisfies most of the requirements discussed above is an achievable goal that will satisfy, at least in part, a critical immediate need of the armed forces.

Fielding the first-generation agent is only an initial step. Much work needs to be done to develop an agent that is effective against high-LET radiation. This need will become increasingly urgent as nuclear terrorism threats inrease. Second- and third-generation agents will be developed only through intense studies that are aimed at defining the mechanisms of radiation injury on the molecular and cellular levels and determining how organisms can be stimulated to protect themselves against this injury. The search for more efficacious radiation countermeasures must continue using newer bioinformatics and systems biology approaches.

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