Pharmacological Countermeasures for the Prevention and Treatment of Toxic Radiation Exposure in Space Flight

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PREFACE

Space travel technologies have rapidly advanced over the past forty years. Man has accomplished initial heroic flights into low earth orbit, travel to the Moon, the development of a reusable shuttle system for routine orbital missions, and the development of orbiting space stations that have allowed for long-term space habitation. Next on the agenda are plans for long-term space travel to include a return to the Moon and eventual exploration of Mars. Despite the current state of technologic and medical sophistication, there remain significant barriers to deep space exploration. One of the most formidable of these barriers is developing efficacious and cost effective countermeasures to protect the flight crew from the acute and chronic effects of toxic radiation exposure during space travel.

Despite years of research into the origins and emission levels of galactic cosmic radiation sources, space agencies have yet to offer a cost-effective and efficacious solution to the issue. The focus of this capstone is to briefly review space radiation types, doses, and toxicities, then critically evaluate the current clinical and experimental data on pharmacological prevention and treatment of radiation toxicity. The goal is to generate a document to serve as guidance for space exploration bodies regarding available and future efficacious therapies to help develop a standard protocol for the prevention and treatment of radiation toxicity in space and to guide future research directions in this field of study.

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Ionizing radiation toxicity in space poses a significant occupational risk to astronauts and is an area of critical concern for space traveling agencies. However, despite technological advances in other areas, a viable solution to this problem has not been developed. The focus of this capstone is to briefly review space radiation types, doses, and toxicities and then critically evaluate the current clinical and experimental data on pharmacological prevention and treatment of radiation toxicity. The document is intended to serve as a guide for space exploration bodies regarding available and future efficacious therapies for toxic radiation exposure and to assist with the development of a standard protocol for the prevention and treatment of radiation toxicity in space. In addition, guidance is offered for future research directions in this field of study. Based on this work, radiopreventive pharmacotherapy should be limited to the treatment of high-level solar particle event exposures. The only agent currently acceptable for this purpose is high-dose amifostine, in addition to traditional supportive measure and medications. Other agents and the treatment of low-level radiation are not recommended at this time given the limited risk/benefit ratio of these agents.

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LIST OF ABBREVIATIONS

DRF: Dose reduction factor

GCR: Galactic cosmic radiation

Gy: Gray

ip: Intraperitoneal

IU: International units

iv: Intravenous

LET: Linear energy transfer

LD: Lethal dose

po: Per os (orally administered)

Q: Quality factor

RBE: Relative biologic effectiveness

sc: Subcutaneous

SPE: Solar particle event

Sv: Sievert

GLOSSARY

Dose Reduction Factor: A measure of the reduction in the biologically experienced radiation dose compared to the actual radiation dose delivered, as a result of some intervention. By definition, the DRF equals the $LD_{50/30}$ of the experimental treatment group divided by the $LD_{50/30}$ of the vehicle-treated controls.

Gray: Tissue radiation absorption unit of measure in joules per kilogram.

Nuclear Spallation: Process whereby heavy ion particle nuclei collide with materials in their path of travel and cause them to fracture into multiple smaller high-energy nuclei.

Q Factor: $Q=13.7 \ln(1+z/4660)$. Accounts for variation in charged particle radiation absorption based on particle size/charge

Rad: Tissue radiation absorption unit of measure in ergs per gram.

Relative Biological Effectiveness: Accounts for different biological effects of various radiation sources/types and converts them to a relative gamma-radiation standard.

Rem: Q factor multiplied by the radiation energy in rads.

Sievert: Q factor multiplied by the radiation energy in Grays.

CHAPTER ONE: INTRODUCTION TO SPACE RADIATION

Astronauts are a group of individuals who are subjected to a unique set of occupational risks. These risks include microgravity, the space vacuum, temperature extremes, micrometeorite strikes, isolation, and radiation exposure. The latter of these risks would seem to present the greatest challenge to occupational health risk reduction strategies for this population.

Ionizing radiation is the predominant type of radiation encountered in space. Ionizing radiation refers to high-energy radiation sources that strip electrons from other molecules in their path of travel. The released electrons then interact with other surrounding molecules in a similar manner leading to an event cascade of interactions resulting in a massive free electron release and the formation of the accompanying electron acceptor. When these interactions occur within living tissue, two biologically significant events may take place; the radiation particle may interact directly with cellular molecules and cause a direct disruption of their integrity, or more frequently they operate indirectly by interacting with intracellular and extracellular water, generating secondary electrons and reactive oxygen free radical species that may result in damage to cellular components and processes.[1,2]

There are several terms used to describe the dose of radiation absorbed by tissue. The Gray (Gy), which is equal to one joule per kilogram of tissue, is

the standard international unit for an absorbed radiation dose. Another unit of measure still frequently found in the literature is the rad. One rad represents the absorption of 100 ergs of radiation energy by one gram of tissue. For the purpose of conversion, one rad equals 0.01 Gray. When measuring the absorbed dose of the variably sized high-energy radiation particles found in space, this measurement is further complicated by the great variability in actual absorbed tissue dose per radioactive particle dose in joules per kilogram or ergs per gram encountered. This variability is determined by multiplying the energy level in Grays times (Q) a quality factor that is experimentally determined for each radioactive particle ($Q=13.7 \ln(1+z/4660)$). When this calculation is completed based on the radiation absorption unit in Grays, the new unit of measure is the Sievert (Sv). Because the Q factor has no units, one sievert equals one Gray or 1 joule per kg. When the calculation is based on the rad, the resultant absorption unit of measure is the rem. One rem equals 100 ergs/gram. It should be readily apparent then that one rem equals 0.01 sieverts.[2,3]

The three main sources of ionizing space radiation that have been identified are galactic cosmic radiation, solar energetic particles, and trapped particles (Van Allen belts). Each of these radiation sources has their own risk characteristics that are a function of the type of ionizing radiation produced. Unlike terrestrial radiation, most types of ionizing radiation encountered in space are high-energy (1MeV-3000MeV) particles that include heavy ions, electrons, neutrons, and protons. Each of these ionizing radiation types has

distinct levels of tissue penetration usually described as its linear energy transfer (LET).[4] Furthermore, the biologic effect of the various high linear energy radiation types may have significantly different severity levels despite having the same radiation energy level. This difference is measured as its relative biologic effectiveness (RBE). The RBE is calculated to standardize high-energy radiation to the effects of low-energy gamma radiation. The RBE therefore equals the gamma radiation dose to cause an observed biological effect divided by the dose of the specific high-energy radiation species required to produce the same effect.[5]

Van Allen belt trapped particles are ionizing radiation particles that are trapped in the Earth's intrinsic magnetic field[4]. This radiation source is only an issue in Earth orbit, but can be a huge source of total radiation exposure on orbital missions, depending on the orbital height and inclination.

Solar energetic particles are generated during solar particle events (SPE), usually related to peak solar activity of the eleven-year solar cycle. The high-energy protons generated by these events are very difficult to shield astronauts from and represent a constant threat to mission success and crew health. The largest of these events produce solar particles with energies as high as 3000 MeV and are capable of delivering more than 1000 rem in less than twenty-four hours, over 600 rem is generally lethal to humans[6]. Solar

particle events are sporadic, difficult to predict, and have a directional element that determines their risk to spacecraft inhabitants and electronics.

CHAPTER TWO: BIOLOGICICAL CONSEQUENCES OF RADIATION

Galactic cosmic radiation is an ever-present ionizing radiation source in space, although it is somewhat attenuated in earth orbit by the Van Allen radiation fields. Galactic cosmic radiation (GCR) is a source of high-energy heavy particles, not generally found in other radiation sources. These heavy particles have an extremely high LET, making it difficult if not unfeasible to adequately shield against. Galactic cosmic radiation represents the main continuous radiation threat to interplanetary travel. Effective shielding is limited due to the high-LET of these particles. Shielding material interaction with GCR produces secondary radiation through both molecular ionization and through the process of nuclear spallation. Nuclear spallation is a process whereby heavy ion particle nuclei collide with shielding material causing the heavy nuclei to breakup into a greater number of lighter, usually less energetic, nuclei[4]. Each of these smaller nuclei may continue to undergo spallation, and/or activate secondary radiation sources.

The biologically damaging effects of ionizing radiation exposure occur at the single cell level. Ionizing radiation can interact with intracellular molecules to generate destructive free radicals. The free radical moieties then interact with other intracellular molecules and generate damage to cellular proteins, DNA, RNA, and lipids. Protein and RNA damage generally tend to be

tolerated, as both are expendable and usually short-lived molecules. The effect of cellular lipid ionization varies greatly based on degree of ionization. Extensive lipid free radical production can lead to loss of cell membrane integrity and cellular demise. Free radical damage to nuclear DNA can be catastrophic, but may not show its effects for some time. Extensive DNA damage may lead to early cell death through cellular inability to produce necessary homeostatic proteins or through the process of apoptosis. More subtle DNA damage may effect non-critical chromosomal areas and have no effect, or may lead to oncogenic transformation, or to heritable genetic defects in the case of cells with reproductive potential.[3,7] These processes are usually the result of cellular interaction with multiple small radioactive particles or secondary radiation sources leading to broad and extensive cellular damage. Heavy ions, such as those found in GCR, are large enough to interact directly with nuclear DNA through nuclear spallation, and have the potential to induce these irreparable changes through a single interaction.[8]

There are two separate but related issues to consider when studying radiation exposure, acute and delayed health risks. Most acute radiation health risks are categorized as deterministic or non-stochastic, that is they have a threshold value at which they occur. Deterministic radiation effects are of greatest concern because they are generally certain to occur within a radiation dose interval, incapacitating and/or life threatening, and have the potential to greatly impact mission success. Deterministic effects are generally related to mass cellular death or tissue and organ dysfunction.

Tissues with the highest turnover rate are at most risk. Stochastic effects, on the other hand, tend to be delayed, do not have a threshold dose, and do not always occur.[4] The two most concerning stochastic effects are cancer and heritable genetic mutations.

In consultation with the National Council on Radiation Protection (NCRP) and Measurements (report No. 132, 2000) and Occupational Safety and Health Administration (OSHA) (29 CFR 1918.1096), NASA has determined acceptable annual and lifetime radiation exposure limits for astronauts stratified on age of first exposure and sex. These standards still allow for roughly an overall increased lifetime cancer incidence of three percent. These standards are meant to apply to space operations in low earth orbit. Radiation exposure limits for deep space exploration have not been addressed by OSHA or the NCRP. Although NASA has set career ration exposure limits on its astronaut population, the goal is not to approach these limits if at all possible. Rather, the principal of ALARA (as low as reasonably achievable) is applied first and foremost, with the set career limit values as the upper threshold of acceptable exposure.[9]

CHAPTER THREE: RADIATION COUNTERMEASURES

Current radiation countermeasures focus on spacecraft shielding and creation of heavily shielded safe havens. At issue is the extraordinary high cost of launching these heavy structures into space and their inability to provide adequate shielding from heavy ions at a feasible shield thickness.[10]

Enhancement of biologic radiation resistance, an alternate method to limiting radiation toxicity, has received relatively little attention. This involves enhancing the radiation resistance of the intracellular milieu through pharmacological or physical intervention. To date the majority of studies have looked at free radical scavenger supplementation to counter the effects of both primary and secondary ionizing radiation. Some of these therapies have shown promising results in experimental animal studies. Other studies have focused on up-regulation of cellular DNA repair enzymes to enhance cellular repair in response to radiation injury in ex vivo and in vitro models.[11] Additionally, a phenomenon called hormesis has been described whereby low-level chronic radiation exposure leads to a relative cellular resistance to radiation toxicity. The mechanism appears to be related to cellular adaptation through up-regulation of radiation-resistance genes.[12] Recent work has also found a dramatic reduction in acute mortality in laboratory rats subjected to myeloablative doses of radiation through post-exposure transfusion of mesenchymal stem cells. The treatment appears to restore a necessary

growth milieu allowing for autologous reconstitution of the host hematopoietic system and subsequent animal survival. Most all of these studies have focused on the deterministic effects of radiation toxicity, but mechanistically, it would seem likely that stochastic effects would be diminished as well.[13]

There have been relatively few studies evaluating various pharmacological agents for their ability to prevent or limit radiation toxicity or act as rescue therapy, helping to limit post-radiation exposure morbidity or mortality. The studies that have been performed have often been preliminary and/or performed only at the in vitro or ex vivo level. Other study downfalls include use of unpurified plant extracts that have varying levels of theoretical agents mixed with other compounds that may markedly inhibit, supplement, or may be responsible for the observed data. Very few compounds have actually been studied in a human model, and fewer still have Federal Drug Administration (FDA) approval for clinical use. In fact, only one agent, amifostine, has specific FDA approval for use in radiation toxicity. Some others would be acceptable off-label uses of relatively safe compounds.[14] Other well-studied agents are non-FDA regulated compounds such as vitamins and essential metals that are safe and available for human usage at appropriate doses.

There are several strategies for pharmacological prevention of radiation exposure toxicity. Some agents may act in a prophylactic manner by acting to

prevent or limit radiation-induced cellular damage by making tissue more resistant to radiation exposure. Others may act as a rescue therapy, limiting post-radiation morbidity and mortality by augmenting cellular, tissue, and organ system recovery.[15] The ideal compound(s) will have a long shelf life at room temperature, a high degree of bioavailability, low toxicity at required doses, a low side effect profile, and preferably be efficacious through oral, subcutaneous, or intramuscular administration. A standard measure of pharmacological efficacy is the DRF (dose reduction factor). The DRF is a measure of the reduction in the biologically experienced radiation dose compared to the actual radiation dose delivered, as a result of pharmacotherapy. By definition, the DRF equals the $LD_{50/30}$ of the experimental treatment group divided by the $LD_{50/30}$ of the vehicle-treated controls. The $LD_{50/30}$ is the dose of radiation necessary to kill 50% of exposed animals within a thirty-day period. This value can be extrapolated to $LD_{XX/YY}$ studies where X equals the percent lethality of the radiation exposure and Y equals the time period in which death must occur. Further, the DRF has also been used to compare biologically measurable insults to cells and tissues based on the percent manifesting the insult and the defined time frame in which it occurs. With this background, a discussion of the pharmacological agents that have shown a reasonable degree of efficacy in either experimental studies or clinical trials will follow.

CHAPTER FOUR: PHARMACOLOGIC RADIOTHERAPEUTICS

Amifostine

Amifostine was the first major drug breakthrough for the treatment of radiation toxicity. Amifostine was developed in the 1950s by the United States army as part of its antiradiation drug development program. The goal was to develop compounds that could treat soldiers who had been exposed to nuclear detonations in the battlefield. Of the four thousand compounds selected for further study at the Walter Reed Army Institute of Research, WR-2721, also known as amifostine, was the only agent to have significant therapeutic benefit and an acceptable toxicity level. Amifostine (S-2-(3-aminopropylamino)-ethylphosphorothioic acid, Ethyol) is a nucleophilic sulfur containing prodrug that is metabolized to its free thiol metabolite WR-1065. WR-1065 is a strong antioxidant acting to scavenge free radicals, assisting DNA radical repair through hydrogen transfer, and reducing cellular oxygen availability via thiol oxidation reactions. It also protects sulfhydryl enzymes as a consequence of protein-aminothiol disulfide formation and stabilizes DNA through DNA-disulfide bridging, which in turn inhibits DNA replication and allows for increased time for DNA repair mechanisms to operate. This agent shows some cell selectivity, allowing for radioprotection of normal cells, while enhancing cancer cell susceptibility to radiation-mediated injury.[14,16]

Amifostine has been demonstrated to be an effective clinical radioprotectant, but its use has been somewhat limited due to its toxicity. In humans, amifostine related toxicity includes hypotension, flushing, sneezing, nausea, vomiting and chills.[14] Its battlefield utilization has been all but abandoned secondary to the performance decrement associated with these reactions. Profound hypotension is the most concerning toxic reaction associated with amifostine, and is its main dose-limiting effect. If amifostine is combined with antiemetics and dexamethasone, it can be tolerated at doses as high as 910 mg m^{-2}.[16]

Amifostine and its metabolites have been analyzed for radioprotective activity at in cell suspension models, in experimental animal studies (monkeys and rodents), and in human clinical trials. All of these experimental protocols have demonstrated the therapeutic efficacy of amifostine treatment. Amifostine therapy has a definite tissue-dependent efficacy. It does not cross the blood-brain barrier, and therefore it has previously been shown to have very little if any radioprotective effects on tissues of the central nervous system. Amifostine's efficacy in other tissues vary from DRFs just above one for parenchymal cells of the lung, kidney, and bladder, to DRFs of about three for hematopoietic cells and salivary gland tissues. When given to lethally and supralethally irradiated mice, it significantly increased survival rates (DRF=1.8-2.4).[17-19]

Although abandoned for acute toxic radiation exposure in the battlefield by the army, amifostine has been adapted for clinical use by the civilian sector. It underwent clinical trials as a radioprotective agent for use in patients undergoing high-dose fractionated radiation for the treatment of cancer. Its greatest therapeutic value was noted for prevention of xerostoma and stomatitis during radiation treatment for head and neck cancers. Based on these studies, the Food and Drug Administration approved amifostine for clinical use in 1996.[16,20]

Despite amifostine's role as a therapeutic radioprotectant for acute lethal radiation exposures in experimental animal models, there has not been a case report of its use for this purpose in humans. Its use for this purpose in humans must be extrapolated from the animal studies and its therapeutic benefit in cancer radiotherapy. Amifostine is not an ideal therapeutic agent for toxic radiation exposure given its toxicity and limited efficacy, but it is the most effective drug that has been tested and is therefore the current gold standard radiopreventive agent against which all others are compared.

Glucans

Glucan, a beta 1-3 polysaccharide derived from the inner cell wall of the yeast *Saccharomyces cerevisiae* has been shown to have promise as a pharmacological radioprotectant. Although its exact mechanism of action as

a radioprotectant has not been elucidated, glucan is known to act as an immunomodulator. It has been demonstrated to be a potent stimulant of both the innate and adaptive immune systems in laboratory animals. Recent studies in irradiated animals have established the efficacy of glucan as a radioprotective agent and have helped define a probable mechanism for this effect. Two glucan preparations have been tested under these conditions; glucan P, a particulate glucan preparation and glucan F, a soluble form of the agent. Both glucan preparations have shown efficacy as radioprotectants with DRFs ranging from 1.21 to 1.17. [21-23] Glucans are relatively low toxicity agents, which are metabolized by macrophages to glucose. Soluble glucan is generally preferred to the particulate form due to the possible occurrence of microemboli with glucan P.

Glucan functions as a prophylactic radioprotectant with therapeutic optimization seen when administered twenty hours prior to radiation exposure. A recent experimental study was conducted where mice were given either glucan (75mg/kg iv) or saline (iv) twenty hours (n=20/group) prior to an 8.5 Gy radiation exposure dosed at 0.4 Gy/ minute. The results of this study evaluated out to 30 days showed 100% survival of the glucan-pretreated animals and no survivors in the saline control group.[21]

Another in vivo based study looked at the ability of glucan to prevent bacteremia. Bacteremia and subsequent sepsis from bacterial translocation

from the gastrointestinal tract is the leading cause of mortality after clinically significant radiation exposures. In this study, mice were again subjected to 8.5 Gys of radiation following pretreatment with either glucan (75mg/kg iv) or saline (iv). Mice from each group were then euthanized and liver tissue was removed and cultured for bacterial growth. At fifteen days post radiation exposure, greater than 75% of control animal hepatic tissue cultures showed bacterial growth whereas less than 10% of the glucan treated tissue cultures had bacterial colony formation. Given that this time interval was too short for increased hematopoiesis to be of benefit, the authors speculated that the observed decrease in hepatic bacterial infection in the glucan group was due to activation of the more radioresistant macrophage cell population. To test this hypothesis peritoneal macrophages were isolated from glucan-treated and control mice subjected to 8.5 Gy radiation exposures.[21] Under these conditions macrophages from the glucan treated group had a significantly higher level of activation and phagocytic activity compared to the control group based on 5' nucleotidase activity and carbon clearance halftimes, respectively.

A related study was initiated to evaluate the effect of glucan on multipotent hematopoietic stem cell survival. Here mice were treated with glucan P (75mg /kg iv), glucan F (250mg/kg iv), or saline (iv). Twenty hours after treatment bone marrow and spleen cells underwent cell survival studies at various in vitro doses of radiation, which demonstrated a significant survival advantage in the glucan P (DRF= 1.21) and F (DRF = 1.17) treated groups compared to controls (DRF = 1.0). Further analysis of these cells showed a

significant increase in cell viability and activation based on tritiated-thymidine cell suicide analysis. Furthermore, bone marrow cells from glucan treated mice subjected to 8.5 Gy radiation exposure produced significantly higher levels of GM-CSF and showed initially higher levels of cells in the more radioresistant S-phase of the cell cycle compared to saline controls.

These data lend credibility to the argument that the potent immunomodulator effect of glucans is at least partially responsible for its observed efficacy as a radioprotective agent. From a mechanistic standpoint it is logical to hypothesize that up-regulation of radioresistant macrophages by glucan contributes to early post-radiation survival through an enhanced innate immune function. Furthermore, the greater long-term survival of glucan treated animals may be secondary to enhanced regenerative hematopoietic stem cell survival conferred through glucan-mediated cycling into the more radioresistant S-phase of the cell cycle. Given the interactive and interdependent nature of the innate and adaptive immune systems, it may be that this is not mediated directly by glucan, but by the cytokine milieu created by glucan-mediated macrophage up-regulation.

Administration of glucan has not only been shown to enhance survival of lethally irradiated mice when given alone, but has a significant synergistic effect when combined with aminothiols. In fact, when glucan is given in addition to the FDA approved aminothiol WR-2721, it not only synergistically

enhanced the survival of lethally irradiated animals, but it did so at a nontoxic and usually minimally efficacious dose of the highly toxic aminothiol. When mice were pretreated with low dose WR-2721 (200 mg kg^{-1} ip) 30 minutes prior to radiation exposure followed by glucan F (250 mg kg^{-1} iv) one hour post-exposure the $LD_{50/30}$ for each group was 8.0 Gy for saline controls, 8.25 for glucan F, 9.5 for WR-2721, and 12 Gy for glucan F + WR-2721. Similar, but less pronounced results were obtained when combined under the same experimental conditions with the aminothiol WR-3689.[21,24]

Antagonistic results were obtained when glucan therapy was combined with the nonsteroidal anti-inflammatory agent, indomethacin. In this protocol, mice received subcutaneous indomethacin (5 mg kg^{-1}) twenty-four hours prior to receiving glucan P (75 mg kg^{-1} iv) or intravenous saline. The animals then received a radiation dose of 8.5 Gy twenty hours after treatment. This study reported a 100% mortality for saline treated and indomethacin/saline treated controls, Whereas 100% of Glucan-P treated animals survived to the thirty-day experimental end point. When glucan treatment was preceded by indomethacin in these animals thirty-day survival fell to less than 60%, indicating a possible role for prostaglandin up-regulation in glucan-mediated radioprotection. As will be discussed later in this paper, indomethacin has been shown to act as a radioprotective agent in other studies.[21] This observed antagonistic effect underscores the need for tested treatment combinations, as some combinations of radioprotectants may actually decrease the therapeutic efficacy.

Glucan has been demonstrated to have significant efficacy as a prophylactic radioprotectant and synergistic adjunctive therapy when combined with aminothiols in laboratory animal studies. It shows promise as an agent in both preventive and rescue radiation toxicity protocols, but further studies including human clinical trials need to be accomplished to verify efficacy and establish drug safety prior to its incorporation into clinical treatment regimens.

Vitamins

Several essential vitamins have been shown to have significant antioxidant properties and have been tested as possible radioprotective agents. The most promising of these are Vitamins A, C, and E. Each of these agents acts as free radical scavengers and has demonstrated some reduction in radiation-induced DNA and cellular damage in vitro. In some cases, preliminary in vivo studies have corroborated these results. Other studies have shown enhanced survival in animals that received lethal or supralethal radiation exposures.[25]

Vitamin E

Of the vitamin antioxidants, vitamin E is the most studied radioprotectant. Vitamin E is an essential, lipid soluble vitamin that exists in the mammalian body in eight forms, each with its own biologic activity. Alpha-tocopherol is a potent antioxidant and the most biologically active form of vitamin E in the human body.[26-27] Numerous studies have demonstrated the ability of Vitamin E to function as both a prophylactic as well as rescue radioprotective agent. It has been found to be both an independent radioprotectant as well as a synergistic agent when combined with other radioprotective compounds.[28-30]

The radioprotective effects of Vitamin E have been shown at a cellular level as well as in whole animal studies. When cells where treated with 500 micromolar Vitamin E in vitro, then exposed to gamma radiation up to 6 Gy (0.7 Gy per minute), there was complete cessation of the normally observed dose-dependent single stranded breaks in transfected plasmid DNA.[31] Additional studies looking at the radioprotective efficacy of alpha-TMG, a water soluble Vitamin E derivative, on chromosomal damage were performed. In these studies mice were given relatively nontoxic doses of alpha-TMG (600mg kg^{-1}), then received up to 12 Gy of gamma radiation.[30] Ex vivo analysis of bone marrow cells from these animals revealed a significant reduction in aberrant metaphases and number of micronucleated erythrocytes; DRFs 1.22 and 1.48, respectively. The same group went on to demonstrate that these

effects were observed whether alpha-TMG was given prior to or after radiation exposure.

Administration of Vitamin E has also been demonstrated to enhance survival of supralethally-irradiated animals. Kumar et al tested the efficacy of vitamin E (400 IU kg^{-1} sc) administered twenty-four hours prior to a 10.5 Gy (0.6 Gy/min.) radiation exposure on thirty-day survival enhancement. They reported a 79% thirty-day survival in vitamin E treated animals compared to only 4% of vehicle only controls subjected to a lower radiation dose (9.5 Gy (0.6 Gy/min)). When these conditions were repeated at 9.5 Gy for both groups, the vitamin E treated group yielded an 88% thirty-day survival. No survival was seen in the vehicle control group. No efficacy was found under similar experimental conditions when vitamin E was administered orally. The authors speculated that first pass metabolism of vitamin E to quinines by the liver may be the reason for lack of efficacy when given orally, but this issue remains to be elucidated.[28] In addition to enhanced post-radiation survival, a double-blind placebo controlled clinical study showed treatment with vitamin E (1000 IU/day) in combination with pentoxifylline (800 mg/day) induced a marked reduction in superficial radiation induced fibrosis. Here 60% of women on combined therapy had reduced disease compared to 43% of controls. Neither drug demonstrated efficacy when used alone.[32]

Vitamin E has a demonstrable effect on radiation-induced changes in both humans and laboratory animals. At therapeutic doses, Vitamin E has established anti-cancer characteristics and does not produce any notable behavioral decrement. Although it has a relatively low DRF (1.1-1.2) compared to other agents, its status as an essential vitamin for the maintenance of normal human physiology, pharmacologic stability and availability, and relatively low toxicity make it an ideal candidate as an applicable radiopreventive agent. Given these characteristics, and the possible efficacy of vitamin E as a preventative and rescue agent, vitamin E should be a priority compound for further studies as part of a combination radioprotective drug regimen.

Vitamin A and C

Vitamins A and C are also established antioxidants, but with a less well defined role in radioprevention than Vitamin E. Neither vitamin A nor C has been established to have a significant impact on post-radiation survival enhancement, but studies have been limited. There are several preliminary studies that have shown a possible role for both of these compounds in reducing radiation induced DNA damage, improving post-radiation wound healing, and inhibiting endothelial damage, but the data are often mixed and inconclusive. It is clear that these compounds are important players in the antioxidant system and as such contribute to the functional milieu of enzymes, cofactors, and redox agents that lead to optimal free radical dissipation in vivo.[25, 33]

Essential Trace metals

Trace metals are an essential part of human nutrition and play an important role in the maintenance of normal homeostasis and stress responses. These metalloelements are requisite components of the metalloenzymes that are crucial to antioxidant physiology. Metalloenzyme complexes not only operate to quench free radical species, but play a critical role in cellular recovery to injury, including DNA and RNA repair and wound healing.[34] Research has focused on the role of trace metals in the prevention of radiation toxicity and has identified copper, iron, manganese, zinc, and selenium as important radioprotective agents. Metalloelements exist in vivo primarily as chelates, not as their ionic counterparts, which exist at levels too low to measure with current clinical instrumentation. Metalloelement chelates are less toxic than the ionic species, more bioavailable, and therefore a more appropriate form for use as pharmacologic agents.[34] As with most of the other pharmacologic radioprotective agents discussed, the data to support the use of these agents is based on cell culture and laboratory animal data.

Selenium

Selenium is an essential trace metal in the human body that has been found to be necessary for normal immune system and thyroid function. Additionally, it plays an important role in the antioxidant system as a

necessary cofactor for glutathione peroxidase and related proteins.[35] These antioxidant enzymes are important players in the performance of free radical scavenging. Selenium's essential role in normal immune function and in the system of free radical scavenging led to its evaluation as a possible radioprotective agent.[36-38] Two forms of selenium have been tested as radioprotective agents in laboratory animals; the organic form selenomethionine (LD_{10}= 16 mg kg^{-1}), and the inorganic form, sodium selenite (LD_{10}=3.2 mg kg^{-1}). These studies found lower toxicity and greater efficacy with selenomethionine. Studies have had mixed results regarding the efficacy of selenium compounds in irradiated animals. These differences seem to be dependent on route of administration, with enteral supplementation demonstrating little efficacy compared to intraperitoneal injections (DRF=1.2).[39]

Selenium seems to act as both a radiation prophylactic as well as rescue agent. When selenomethionine (4 mg kg^{-1} ip) was given to mice twenty-four hours before (T-24h), one hour before (T-1h), or fifteen minutes after (T+15m) a 9 Gy radiation exposure dosed at 0.2 Gy per minute, significant efficacy was obtained. Under these conditions post-radiation survival was 93%, 71%, 79%, and 23% for the T-24h, T-1h, T+15m, and saline control groups, respectively. Similar survival enhancement was observed at higher (10 Gy) radiation doses with 36% of the T-24h group surviving compared to none of the control animals.[39]

Selenium has also demonstrated a synergistic effect when given in combination therapy with aminothiols. Sodium selenite (1.6 mg kg^{-1} ip) was administered twenty-four hours prior to WR-2721 (400 mg kg^{-1} ip (LD_{10}= 800 mg/kg)) given thirty minutes prior to various radiation doses. In this study, the thirty-day survival was significantly enhanced by the addition of selenium to the aminothiol regimen. In fact, 83% of animals radiated to 20 Gy and 31% radiated to 24 Gy (1.0 Gy per minute) survived with combined therapy versus 17% and 0% of similarly irradiated animals that received WR-2721 alone. The results of this study demonstrated an increase in the DRF to 2.6 when selenium was combined with WR-2721 compared to 2.2 with WR-2721 alone. This same group examined the effects of different selenium doses combined with WR-2721. They found that a selenium dose of 0.8mg kg^{-1} enhanced WR-2721 radioprotection and decreased lethal drug toxicity. However, there was an observed increase in behavioral drug toxicity.[39]

In preliminary studies, selenium has shown a route dependent efficacy as a weak radioprotectant. It has, however, demonstrated the capacity to significantly enhance the efficacy and decrease the lethal toxicity of the FDA approved aminothiol WR-2721. These early studies indicate a possible role for selenium in both prophylactic and rescue therapy in toxic radiation exposure. Further studies with this compound will be critical in elucidating the efficacy and role for selenium as part of a sanctioned treatment protocol.

Iron

Iron is another trace metal that is essential for the maintenance of human physiologic processes. Over two-thirds of the iron in the human body is found in hemoglobin and myoglobin which are necessary for oxygen storage and delivery to tissues. In a well-nourished adult, approximately 15% of iron is stored in an inactive form to be mobilized as future needs arise. Iron supplementation is toxic in excessive doses.[35] CNS depression, coma, and death are the most concerning consequences of acute iron toxicity. In addition, chronic iron toxicity such as is present in patients with hemachromatosis has been linked with the development of coronary artery disease and some forms of cancer. Fortunately, normal physiologic mechanisms in the adult limit the absorption of most excess ingested iron through duodenal regulation. Children however, remain very vulnerable to iron ingestion toxicity.[35]

Aside from its role in oxygen transport, iron plays a requisite role in wound healing, tissue repair, and gene regulation. Iron chelates also act as relatively strong superoxide dismutase and catalase mimetics. It is iron's enzyme mimetic activity as well as its role in DNA and tissue repair that is thought to account for most of its radioprotective activity. In addition, it is thought that depletion of enzymatic activity through radiolytic loss of iron from iron-dependent enzymes further contributes to cellular inability to recover from ionizing radiation insults.[34]

Most research accessing the role for iron in post-radiation toxicity has been limited to in vivo and ex vivo laboratory animal studies. Pre-radiation exposure prophylaxis and post-radiation exposure rescue protocols have been performed on laboratory animals using the iron chelates 3-5 diisopropylsalicylate (FE (III) (3-5 $DIPS)_3$) and FE (III) hematoporphyrin chloride (FEHP).[34] These analyses were limited to animal survival, as well as hematopoietic cell activity and viability studies.

Fe (III) hematoporphyrin chloride was evaluated for its ability to effect hematopoietic cell recovery and activation after toxic radiation exposure to DBA/2 mice. When an intraperitoneal injection of FEHP was given at various time-points post-radiation exposure, it induced a significant increase in the total number of splenocytes as well as granulocyte-macrophage colony forming units. Given this finding, the radioprotective properties of FEHP were hypothesized to be due in part to its role as a stimulating immunomodulator.[40] Animal survival studies with this agent remain to be completed.

When the iron Chelate FE (III) (3-5 $DIPS)_3$ was studied as a prophylactic radioprotective agent at approximately one-fourth its acutely toxicity dose it increased survival by 84% in $LD_{30/50}$ irradiated male mice. Surprisingly, pretreatment of these animals with even lower doses of FE (III) (3-5 $DIPS)_3$ increased survival up to 300 percent above vehicle-treated control

animals. Post-radiation rescue studies with similar doses of FE (III) (3-5 $DIPS)_3$ failed to increase survival compared to vehicle-treated controls when given to mice after $LD_{100/30}$ irradiation.[41]

These data show some promise for iron as a radioprotectant. The studies, however, are very limited, and the role for iron in the treatment of toxic radiation exposure remains unclear.

Copper

Copper is one of the best-studied trace metal radioprotectants. Copper is also essential for maintenance of human life, mostly due to its role as an enzymatic cofactor. Copper plays a significant role in important physiologic processes such as neurotransmitter formation, energy production, collagen cross-linking, and free radical reduction. In addition, copper is involved in the absorption, storage and metabolism of iron. The majority of dietary copper is absorbed in the small intestine and to a lesser extent in the stomach. Most copper in the human body is stored in the liver, from where it is mobilized on demand and transported in the blood by ceruloplasmin to the target tissues. Copper toxicity seems to have its greatest effect on the central nervous system (CNS) and liver. Acutely lethal doses cause central nervous system depression leading to a loss of cardiopulmonary regulation. Lower doses of copper produce a reversible sedative-hypnotic effect that may be beneficial in

radiation protection. Chronic copper toxicity, on the other hand, manifests primarily as hepatic dysfunction.[34,35]

Many studies have been performed to evaluate the radioprotective effect of copper and to attempt to determine a possible mechanism of action to account for its activity. The majority of these studies are limited to experimental animal research or in vitro analysis. What follows is a discussion of some of the key studies.

Cu $(II)_2$ (3-5 diisopropylsalicilate)$_4$ [Cu $(II)_2$ (3-5 DIPS)$_4$] is a copper chelate with demonstrated superoxide dismutase mimetic properties. Cu $(II)_2$ (3-5 diisopropylsalicilate)$_4$ was first noted to produce a 58% survival in mice receiving whole-body $LD_{100/30}$ irradiation when it was given at a dose of 80 micromoles kg^{-1} sc twenty-four hours prior to radiation exposure. This finding was statistically significant when compared to vehicle-treated control animals that had a 100% mortality rate.[42]

These initial studies were followed by others that demonstrated an equivalent or better efficacy when Cu $(II)_2$ (3-5 DIPS)$_4$ was given at a much lower dose (20 micromoles kg^{-1} sc) only three hours prior to an $LD_{50/30}$ radiation exposure. In this study, treated animals had a 97% survival rate compared to the approximately 50% survival rate observed in the vehicle-treated controls. Cu $(II)_2$ (3-5 diisopropylsalicilate)$_4$ was then tested for its

ability to act as a rescue agent. The chelate was given three hours post $LD_{50/30}$ irradiation dosed at 2.5 or 5.0 micromoles kg^{-1} sc and increase survival from 48% in vehicle-treated controls to 88% or 93% in the Cu $(II)_2$ $(3\text{-}5\ DIPS)_4$ treatment groups, respectively.[43]

Another experimental protocol was performed to evaluate the efficacy of orally administered copper chelates in the treatment of radiation-induced toxicity. In this study, male mice were given Cu $(II)_2$ $(3\text{-}5\ DIPS)_4$ 25 or 50 micromoles kg^{-1} po at twenty-four or four hours prior to a $LD_{50/30}$ radiation exposure. Here, treated animals had respective survival rates of 75% or 95%, compared to the expected survival rate of 50% in vehicle-treated control animals.[44,45]

Copper chelates were also studied to evaluate their effects on post-irradiation immunodeficiency and tissue injury. While pre and post-radiation administration of $CU(II)_2$ $(3\text{-}5\ DIPS)_4$ were unable to inhibit radiation-induced loss of hematopoietic cells and their precursors, both were able to significantly enhance the repopulation of splenic and bone marrow lymphocytic tissues in mice. These animals were also shown to have increased levels of multipotent stem cell colony forming units, compared to controls. In addition, copper chelate-treated animals demonstrated an earlier return of T-cell dependent antibody responses and T-cell and B-cell mitogenic activity. Similarly, a

reduction in post-irradiation tissue damage and improved tissue repair were observed in the $CU(II)_2$ (3-5 $DIPS)_4$ treated animals.[46-50]

Copper has also been demonstrated to promote recovery of radiation-induced DNA damage, and therefore help prevent oncogenic transformation. This effect is thought to be mediated by copper's anticlastogenic quality. This causes inhibition of DNA replication through suppression of S-phase cell growth, thus allowing for increased time for DNA repair processes. Copper is also known to have a high binding affinity for nucleic acids.[51] This characteristic may actually act to stabilize the DNA double helical conformation through interstrand and intrastrand bridging.

Although most studies have demonstrated the efficacy of copper as a radioprotective agent, there are a few authors who have presented contrary data. Despite the excellent survival advantage of mice given low dose $CU(II)_2$ $(3\text{-}5\ DIPS)_4$ prior to a lethal (10Gy) radiation exposure, when higher doses of copper (133-901 micromoles kg^{-1} sc) were used, no improved survival was observed. These experiments were repeated and validated in later studies. Similarly, low dose copper chelates given after $LD_{50/30}$ irradiation markedly improved the survival of treated animals. However, when it was given at 80 micromoles kg^{-1} sc (a normally efficacious prophylactic dose) after radiation exposure, all animals treated with this higher rescue dose died before the vehicle-treated control animals. It was postulated that these larger doses

inhibited de novo synthesis of other important metalloelement-dependent enzymes by promoting incorporation of the incorrect metalloelement through altered concentration gradients.[52] This would, in effect, produced less effective or nonfunctional enzymes.

Copper may have other beneficial therapeutic properties for the treatment of radiation toxicity. Co-administration of copper has been shown to reduce the acute lethal toxicity of the FDA approved radioprotectant amifostine (WR-2721). Animals dosed with 3,922 micromoles kg^{-1} ip WR-2721 alone had a 97% mortality rate. However, when 5 micromoles kg^{-1} ip $CU(II)SO_4$ was given three hours prior to the same dose of WR-2721, the mortality rate decreased to only 10%. If the copper sulfate was given twenty-four hours prior to the WR-2721, the mortality rate was 30%, while coadministration of the agents led to a 90% mortality rate. The later observation was attributed to the formation of CU(II)-sulfide and $CU(II)_3(PO_4)_2$ when the drugs were mixed for co-administration.[53]

Copper has generally been shown to have significant radioprotective effects in experimental animal models. Although the exact mechanism of action has not been elucidated, copper does possess enzyme mimetic, anticlastogenic, antiinflammatory, antimutagenic, and anticarcinogenic activities. Furthermore, nontoxic doses of copper chelates appear to be useful as both a prophylactic and rescue therapy agent for toxic radiation exposures.

In addition, it produces a synergistic effect when combined with WR-2721. Given these results, copper should continue to be evaluated as a possible clinical agent for the treatment of radiation exposure toxicity.

Manganese

Manganese, also one of the trace minerals essential to human life, has shown some promise as a radioprotective agent. Manganese is necessary for the normal function of numerous body systems, including neurological, endocrine, musculoskeletal, and reproductive systems. The majority of manganese in the human body is found concentrated in the liver, pancreas, bone, and brain.[35] As with the other essential metalloelements discussed, it has a significant role as a cofactor in the functioning of various enzymes. Of particular interest to radiation protection strategies is its role in prevention of free radical tissue injury via the formation of manganese superoxide dismutase and tissue repair enzymes. The clinical effects of manganese deficiency and toxicity have been described in the medical literature. Of relevance to its possible use as a radioprotectant is its toxicity profile, which includes schizophrenia and Parkinsonism.[34]

The initial promising studies with manganese as a radioprotectant were completed with the inorganic salt $MnCl_2$. These studies showed a significant survival advantage and earlier return to immunocompetence in irradiated animals treated with manganese, compared to vehicle-treated controls.[54]

These early experiments led to a more in depth evaluation with the more bioavailable chelated form of manganese.

The manganese chelate Mn-(3-5 diisopropylsalicilate)$_2$ [Mn-(3-5 DIPS)$_2$] was given to mice at 165 micromoles kg$^-$ sc three hours prior to an $LD_{50/30}$ (8 GY) radiation exposure. Treated animals in these experiments had a thirty-day survival of 96-100%, in contrast to the 50% survival rate expected at this level of radiation. In another prophylaxis study, mice were treated with Mn-(3-5 DIPS)$_2$ dosed at 120, 240, or 480 micromoles kg^{-1} sc prior to an $LD_{100/30}$ radiation exposure. Here, researchers observed a survival rate of 16% and 27% in animals dosed with 240 micromoles kg^{-1} or 480 micromoles kg^{-1} Mn-(3-5 DIPS)$_2$, respectively. Animals in the 120 micromoles kg^{-1} Mn-(3-5 DIPS)$_2$ or the vehicle-control groups had no survivors.[55]

The efficacy of manganese chelates as a radioprotective rescue agent has also been demonstrated in numerous experiments. Henderson et al treated mice exposed to $LD_{50/30}$ irradiation with Mn-(3-5 DIPS)$_2$ (30,60, or 120 micromoles kg^{-1} sc) three hours after exposure. These authors reported a 130% increase in survival with a rescue dose of 120 micromoles kg^{-1}, when compared to vehicle-treated controls. A separate study by the same researchers treated mice with Mn-(3-5 DIPS)$_2$ (30, 60, 90, 120, or 150 micromoles kg^{-1} sc) after subjecting them to $LD_{50/30}$ irradiation. This study confirmed an optimal rescue therapy dose of 120 micromoles kg^{-1} Mn-(3-5

DIPS)$_2$. Animals treated with 120 micromoles kg^{-1} had an increased survival rate of 229% above control animals, whereas, mice treated with 90 or 150 micromoles kg^{-1} Mn-(3-5 DIPS)$_2$ exhibited a 200% and 214% increased survival rate, respectively.[56] Another rescue therapy study completed at higher radiation doses confirmed these earlier findings. This time mice were administered 60, 120 or 240 micromoles kg^{-1} sc Mn-(3-5 DIPS)$_2$ after an $LD_{100/30}$ (10 Gy) radiation dose. This study also found the optimal therapeutic rescue dose of Mn-(3-5 DIPS)$_2$ tested to be 120 micromoles kg^{-1} with a 223% increased survival rate in these animals. This was found to be statistically significant compared to the 12% survival observed in vehicle-treated control animals.[57]

A time-variable rescue therapy trial with manganese chelate demonstrated the critical importance of therapeutic optimization based on time of Mn-(3-5 DIPS)$_2$ administration relative to radiation exposure. When Mn-(3-5 DIPS)$_2$ was given to mice, dosed at 90, 120, or 480 micromoles kg^{-1} sc, one or three hours after an $LD_{50/30}$ (8 Gy) radiation exposure, a significant difference in therapeutic efficacy based on time of treatment was observed. Animals treated one hour after irradiation had an increased survival rate of 7%, 21%, or 29%, whereas animals treated three hours post-radiation exposure had a 20%, 30%, or 130% increased survival rate for the doses tested, respectively.[58]

Other studies sought to optimize manganese treatment by evaluating by evaluating the efficacy of Mn-(3-5 DIPS)$_2$ given both before and after irradiation. Animals in one study were given 150 micromoles kg^{-1} sc Mn-(3-5 DIPS)$_2$ three hours before and then 30, 60 or 90 micromoles kg^{-1} sc two, four, and six days after an $LD_{100/30}$ radiation exposure. The experimental treatment groups had respective survival rates of 28, 12, and 16 percent. There were no survivors in vehicle-treated controls.[57]

Both preradiation prophylaxis and postradiation rescue therapy studies have also been performed with oral administration of manganese chelate. Although these studies failed to match the improved survival rates to the levels observed for animals that received the same doses of Mn-(3-5 DIPS)$_2$ given subcutaneously, they still demonstrated significantly improved survival rates when compared to controls.[57] Whether this difference in route of Mn-(3-5 DIPS)$_2$ administration is due to absorption time, oral bioavailability, or other factors is not clear. It is possible that optimization of oral dosing and time of administration may yield efficacies equal to those seen with subcutaneous administration of the compound. Future studies should help to elucidate this issue.

Data from the research on manganese demonstrate consistent evidence that manganese has radioprotective qualities when given before and/or after radiation exposure in laboratory animals. Although its mechanism of action

has not been clearly identified, its role in metalloenzyme complex formation and its superoxide dismutase mimetic properties are likely to be, at least partially, responsible for its efficacy. Further studies need to be completed to help elucidate its mechanism of action and to further delineate its potential role as a clinically useful radioprotective drug.

Zinc

Zinc is an essential metal that has a significant role in the processes of immune function, wound healing, DNA and RNA regulation and repair, tissue growth, and developmental regulation.[34,35] Most of these activities are regulated, at some level, by zinc-dependent enzymes. Zinc is found in almost every cell of the human body and is a requisite component of about one hundred different regulatory enzymes.[35]

As with the other essential metals previously described, the chelated form of zinc has a higher bioavailability and safer toxicity profile than inorganic zinc salts. The LD_{50} for the chelated zinc compounds is between 164 and 176 micromoles kg^{-1}. This acutely toxic dose shows variability based on compound makeup and route of administration. Intraperitoneal administration demonstrates the highest toxicity and is associated with significant granuloma formation.[34]

Although it has not been as well studied as some of the other essential metals, preliminary trials have demonstrated a possible role for zinc as a therapeutic agent for radiation toxicity. In a prophylactic treatment trial, mice were given $Zn(II)_2$ $(acetate)_4$, 39 or 153 micromoles kg^{-1} sc, twenty-four hours before an $LD_{50/30}$ (6.7 Gy) radiation exposure. The zinc treated animals had a 62% or 81% survival rate, respectively, whereas only 25% of control mice survived.[59] Time of prophylactic administration turned out to have a significant impact on efficacy. When the inorganic zinc salt, $Zn(II)Cl_2$, was given at 37 micromoles kg^{-1} sc one or three days prior to a 6.3 Gy radiation dose, 100% of treated animals survived. When the same dose was given one or three hours before irradiation, only a 60% survival was seen in the treated animals and 40% survival in the control animals.[60] A larger study was performed to study optimization of both dose and time of administration of zinc chelate treatment prior to various levels of radiation exposure. In this study mice were given 31, 61, 92, or 122 micromoles kg^{-1} ip $Zn(II)_2$ $(acetate)_4$, ten, thirty, or one hundred and twenty minutes prior to a 9, 10, 11, 12, or 13 Gy radiation exposure. This study noted maximal zinc efficacy at 92 micromoles kg^{-1}. When this dose was given ten, thirty, or one hundred and twenty minutes pre-exposure to a 9 Gy irradiation, a survival rate of 88%, 80%, or 90% was observed in treated animals, respectively. Survival rates in comparably treated animals dropped to 3%, 57%, or 35% when the radiation exposure was increased to the supralethal dose of 12 Gy.[61]

Detailed studies using zinc as a radiation toxicity rescue therapy are lacking. One small study gave 37 micromoles kg^{-1} sc $Zn(II)_2Cl_2$ after a 7.7 Gy radiation exposure with a resultant survival rate of only 5% in the treated group.[34] As observed with the other essential metals discussed, this relatively low prophylactic dose may cause excessive toxicity when given in the post-radiation period. Much lower doses may yield a therapeutic benefit, but further studies must be performed in order to resolve this issue.

Several theories have been put forth to explain zinc's radiopreventive properties. One of is that the observed zinc–induced metallothianine up-regulation in the liver may have a significant effect. Metallothianine has significant reduction qualities, acting to quench reactive $O2^-$ and hydroxyl free radical species.[34] Unlike the other essential metal chelates, zinc chelates do not exhibit an superoxide dismutase mimetic activity to partially account for its therapeutic efficacy. Also, given Zinc-dependent enzymatic roles in DNA regulation and repair, it is thought that radiation protection is also, in part, due to the zinc-dependent processes of nuclear transcription, translation, and endonuclease activity.[62,63]

Experimental studies have also shown zinc to inhibit the normally observed post-radiation decrease in hematocrit, thrombocytes, erythrocytes, and leukocytes. Additionally, zinc is required for normal T-cell activation,

therefore, it may act mechanistically to inhibit post-irradiation immunoincompetence or to enhance hematopoietic recovery.[34]

2-MERCAPTOPROPIANYLGLYCINE

Amifostine has become the gold standard radioprotective drug. It is very effective at protecting multiple body systems from radiation toxicity including the hematopoietic and gastrointestinal compartments. Other agents have been shown to potentiate its efficacy and/or allow for a comparable efficacy at a lower less toxic dose of WR-2721. One agent with this characteristic is 2-mercaptopropianylglycine (MPG). MPG is a thiol agent derived from the sulfhydryl acylation of glycine. This agent is normally used in the treatment of liver diseases and myopia. MPG has also been demonstrated to be an oxygen free radical scavenger, and has a very low toxicity profile.[64] Preliminary studies with MPG have shown that mice given 20 mg kg^{-1} ip thirty-minutes before a supralethal 15 Gy irradiation had a delay in post-radiation gastrointestinal syndrome, but that ten-day survival rates remained unchanged from control animals at zero percent. When WR-2721 was given to comparably irradiated animals, the ten-day survival rate was 70%. When MPG, 20 mg kg^{-1} ip, was given thirty-minutes prior to irradiation, in addition to WR-2721 the survival rate increased to 95%. Additionally, where as all of the control and single drug therapy treatment groups expressed various levels of gastrointestinal syndrome, none of the animals given both MPG and WR-2721

had gastrointestinal symptoms. When these animals were followed out to thirty-days after radiation exposure, the WR-2721 treated group had only a 23% survival rate, whereas the group receiving both drugs had a 65% survival rate. All of the vehicle-treated control animals expired before day ten.[64]

Given the observed decrease in gastrointestinal symptoms in MPG treated animals, and the complete lack of these symptoms in animals treated with both MPG and WR-2721, researchers studied the jejunal mucosa of these animals for histological changes. They found that vehicle-treated controls had less than 17% of the number of jejunal crypts as did sham-irradiated control mice, and there was a 54% decrease in viable crypt cells. MPG-treated mice had about 34% of sham-irradiated control crypts with only a 29% reduction in viable crypt cells. The number of crypts present in the jejunum of animals treated with both agents was increased to 65% of sham-irradiated controls and had about 74% crypt cell viability.[64] These data demonstrated a clear tissue preservation effect that seemed to be independent of immune system preservation.

MPG has a low toxicity profile and is clinically available for use in human subjects. This, along with its ability to potentiate WR-2721, and MPG's known antioxidative properties, make it a possible candidate for a combined therapeutic approach for the treatment of toxic radiation exposures. Further studies to determine the usefulness of MPG are certainly warranted.

CALCIUM CHANNEL BLOCKERS

Calcium channel blockers are a group of drugs that operate to inhibit calcium influx into cardiac and vascular tissue via a mechanism of receptor specific blockade. Many of these agents are FDA-approved clinically available drugs for the treatment of a variety of medical conditions including hypertension, cardiac diastolic dysfunction, and cardiac arrhythmias. These drugs are frequently prescribed in the United States and have proven clinical efficacy and safety for the treatment of these disorders. Given that cellular calcium influx plays an instrumental role in cell death after free radical oxidative injury, it was hypothesized that calcium channel blockers may hold some therapeutic benefit for the treatment of radiation exposure toxicity. Several of these agents have been evaluated.[34] Here, the discussion will be limited to results reported for two of the most heavily prescribed calcium channel blockers, diltiazem and nifedipine.

Diltiazem

Diltiazem is a member of the benzothiazepineone class of calcium channel blockers. It is frequently prescribed for the treatment of atrial fibrillation and hypertension. Although the data are limited, some promising preliminary results have been obtained using diltiazem as a radioprotective agent. Here mice given diltiazem (61, 122, or 244 micromoles kg^{-1} sc) fifteen minutes prior to an $LD_{100/30}$ radiation exposure showed a 17%, 58%, or 93%

survival rate, respectively. Vehicle-treated control animals in this trial demonstrated only a 2% survival rate.[65] In another study, when mice were given 244 micromoles kg^{-1} sc diltiazem ten minutes before a 10 Gy irradiation, there was a 100% survival rate in the diltiazem-treated group. The survival rate fell to 80% when the same dose of diltiazem was given thirty-minutes before the same dose of radiation. Only 12% of vehicle-treated control animals survived in this set of experiments. When a rescue therapy protocol was performed, mice were given 244 micromoles kg^{-1} sc diltiazem ten minutes after an $LD_{100/30}$ radiation dose. Here, experimentally treated animals had a 100% survival rate, whereas only 2% of control animals survived to thirty days.[65]

Nifedipine

Nifedipine is a member of the diester dihydropyridine class of calcium channel blockers and is used primarily as an antihypertensive agent. It too has been shown to have radioprotective qualities. When nifedipine was given at a dose of 4 micromoles kg^{-1} ip thirty-minutes before an $LD_{50/30}$ (8.5 Gy) irradiation, the thirty-day post-irradiation survival rate was 100% compared to only 61% in control animals.[65] When the dose of nifedipine was increased to 9 micromoles kg^{-} ip and given three hours before an $LD_{100/30}$ (9.8 Gy) radiation dose, the survival rate was 83%. What is also of interest is that the vehicle-treated control animals had a much higher than expected survival rate. The ethanol vehicle was given to mice at a 96% or 18% formulation and yielded post-$LD_{100/30}$ survival rates of 61% and 18%, respectively. These data still

demonstrated the superiority of nifedipine treatment, but gave rise to the notion that ethanol may have a beneficial radioprotective effect. Researchers then treated mice with 9 micromoles kg^{-1} ip nifedipine, ethanol vehicle, or distilled water prior to an 8.1 Gy radiation exposure with thirty-day survival rates of 67%, 30%, and 0%, respectively. Next, a nifedipine dose variability study was performed. Mice were given nifedipine (2.3, 4.5, or 9 micromoles kg^{-1} ip) thirty minutes prior to an $LD_{100/30}$ (10.5 Gy) irradiation. In this study, a dose-dependent response was seen with survival rates of 0%, 40%, or 79%, respectively. This compares to the 6% survival rate observed in ethanol-treated control mice.[65]

Further preliminary studies found that when diltiazem was given in combination with Zn(II) $(aspartate)_2$ a significant survival advantage was seen after an $LD_{100/30}$ (10.5 Gy) irradiation, compared to either drug administered separately. Additionally, when diltiazem and nifedipine were co-administered, a synergistic radioprotective effect was observed.[66]

The mechanism of action of calcium channel blockers as radioprotectants is not known. It has been proposed that calcium channel blockers act by inhibiting calcium influx into cells after radiation injury. Additionally, like many of the other radioprotective agents discussed in this paper, calcium channel blockers have receptor-independent free radical scavenging characteristics. These agents are FDA approved and have an

excellent safety record proven by heavy clinical usage. Further investigation needs to be performed with these drugs in order to delineate their use and suitability as part of a multi-regimen therapeutic modality for toxic radiation exposure.

PLANT EXTRACTS

Several herbal extracts borrowed from the Indian Ayurvedic system of medicine have shown radioprotective effects in preliminary experimental trials. Although these agents are not yet fully accepted by western allopathic medicine, their medicinal value for some medical conditions is becoming apparent. The inherent problem with these agents is the lack of standardized dosing and the unknown combination of chemical species within the extracts. However, many current prescription medications and some breakthrough anticancer medications are purified species derived from plants. The value of alternative and herbal medicines is gaining some credibility in the scientific worlds. The United States National Institutes of Health recently opened up a subsection to fund alternative medical studies. The literature holds several radioprotection studies using herbal extract therapy, however some of these projects have limited relevance or suffer from poor experimental design. This discussion of herbal extracts for radioprotection will be limited to two compounds, Triphala and mint extract. Both of these agents have undergone

well-designed experimental studies and have been shown to have some efficacy as radioprotective agents.

Triphala

Triphala, an Ayurvedic regenerative drug, is a compound made from the fruits of three plants; *Terminalia chebula*, *Terminalia bellerica*, and *Phyllenthus emblica*, mixed in equal proportions. An extract of Triphala in double deionized water was tested by Jegetia *et al* for its ability to increase survival in irradiated laboratory mice. First, they accomplished a drug toxicity profile in order to determine safe dosing parameters. Mice received intraperitoneal injections of Triphala at doses ranging from 200-1000 mg kg^{-1}. They noted an LD_{50} of 280 mg kg^{-1} and no lethal toxicity at doses at or below 240 mg kg^{-1}.[67]

Survival studies were then performed with intraperitoneal doses of Triphala (5, 6.5, 10, 12.5, 20, 25, 40, 50, or 80 mg kg^{-1}) on mice exposed to $LD_{100/30}$ (10 Gy) irradiation. This study showed the 10 mg kg^{-1} dose to be most efficacious with a thirty-day survival rate of about 60%, compared to only 4% in vehicle-treated controls. There was an observed dose-dependent increase in efficacy up to 10 mg kg^{-1}. Doses greater than 10 mg kg^{-1} demonstrated a dose-dependent decline in survival as doses increased. The decreased survival at doses greater than 10 mg kg^{-1} was postulated to be secondary to an increasing toxicity effect on radiation compromised animals.[67]

These data are intriguing, but much further work needs to be completed. As with all herbal extracts, there is an inherent complication of variable drug concentrations. There is also an unknown compound makeup variability, as the extracts are a composite of hundreds to thousands of chemical species naturally found in the plants used to produce the agent. It is possible that several of these chemicals have radioprotective properties. These chemical agents may have variable efficacy based on the ratios of the therapeutic chemical species present. It is also possible that the whole extract may contain chemical(s) that act to inhibit the effect of a therapeutic one, or may be responsible for drug toxicity with little or no therapeutic benefit.

Mint Extract

Another herbal agent, mint extract (*Mentha arvensia*), has also received attention as a possible radioprotectant. This compound has previously been shown to be antimutagenic[68] and to contain numerous chemicals that possess antioxidative and free radical scavenging properties.

When mint extract was given intraperitoneally at various doses ranging from 2.5-80 mg kg^{-1} for five consecutive days prior to an $LD_{100/30}$ (10 Gy) radiation exposure, 10 mg kg^{-1} was found to have the greatest therapeutic benefit with a 25% survival rate. There was a dose-dependent increase in survival up to 10 mg kg^{-1} and a dose-dependent decrease in survival at doses

above 10 mg kg^{-1}. All animals that received 80 mg kg^{-1} died, as did all vehicle-treated controls.[69]

Next, mice received mint extract, 10 mg kg^{-1} ip, for five consecutive days, then, were irradiated at 6, 7, 8, 9, or 10 Gy. All mint extract-treated animals subjected to 6 or 7 Gy irradiation survived compared to only 80% of vehicle-treated control animals that received 7 Gy irradiation. The survival rate for mint extract-treated mice receiving 8, 9, or 10 Gy radiation exposures was 75%, 58%, or 25%, respectively, whereas it was 42%, 17%, or 10% for vehicle-treated control animals. Based on the 7Gy radiation data, mint extract was calculated to have a DRF of 1.2.[69]

Like Triphala, the data for mint extract as a radioprotectant shows some promise. Although not as well studied as some of the other radioprotective agents discussed, the data lend support for the further study of these and other herbal compounds. Many of these extracts are available for purchase in the United States and are not regulated by the Food and Drug Administration. We must be cautious in interpreting results with these agents. Given the discussed compound variabilities and unknowns associated with these extracts, their clinical application is not recommended at this time.

MELATONIN

Melatonin (N-acetyl-5 methoxytryptamine) is the acetylated methylether derivative of serotonin. It is widely available over the counter in the United States and in a pharmacologically pure form in other countries for the treatment of insomnia and circadian cycle phase shifting. It is a relatively low toxicity compound that can form covalent bonds with essential metals.[70]

In initial studies, melatonin was found to be an effective radioprotectant. In one study, mice were given melatonin in various analog forms (propionyl, butanoyl, hexanoyl, octanoyl, decanoyl, or hexadeconoyl) at 1.1 mmol kg^{-1} ip, 0.78 mmol kg^{-1} ip Wr-2721, or vehicle, thirty-minutes before an $LD_{100/30}$ (9.5 Gy) irradiation. This experimental protocol demonstrated thirty-day survival rates of 43%, 32%, 75%, 95%, 65%, and 48% for the noted melatonin analogs, respectively. Mice treated with WR-2721 had a 50% survival rate, while there were no survivors in the vehicle-treated control group. The best results were obtained by the melatonin-octanoyl derivative with a 95% thirty-day survival rate. The survival rate with the melatonin analogs were noted to vary based on the lipid solubility profile of the analog tested.[71] In another study, the same dose and route of melatonin administration given one-hour before irradiation still proved therapeutic, but was not nearly as efficacious as was demonstrated when given thirty-minutes prior to irradiation. In addition, when the dose of melatonin was halved one hour before irradiation, there was no difference in efficacy compared to vehicle-treated control mice.[70] The usefulness of melatonin as a clinical radioprotective agent remains to be

determined. However, given its relatively low toxicity and availability in a pharmacologically pure form for clinical human use, research into the efficacy of this drug should be given some level of priority.

OTHER AGENTS

Other agents have received attention in the literature, however the data demonstrating their efficacy as effective radioprotective agents are absent or the studies lacked significant survival data. These agents include 5-Androstenediol, indomethacin, prostaglandins, curcumin, and substituted anilines.[15,34,72,73] Newer agents that may proven highly effective, but have not yet been well analyzed are the nitroxide agents and the amifostine analog, phosphorothioate acid, a more orally bioavailable and less toxic relative of amifostine. In addition, various intravenous antibiotics and combination bowel sterilizing regimens, whose administration may have an effect on post-irradiation survival, were not discussed. Instead, focus was limited to agents that are postulated to increase survival through enhancement of cellular radioresistance and recovery mechanisms.

CHAPTER FIVE: SUMMARY AND RECOMMENDATIONS

The issue of radiation toxicity is an area of critical concern for space traveling agencies. A viable solution to this critical problem needs to be developed before long-duration space travel can be safely undertaken. The risks to astronauts include not only incapacitating and/or life threatening radiation exposures from solar particle events, but also chronic and delayed effects such as cancer, cataracts, and neurological compromise. In addition, astronauts of reproductive age risk passing on heritable genetic deficits to their progeny as a result of radiation-induced mutations to gamete DNA. The possibility of teratogenic insults also exists in the event of pregnancy in space. At this time prevention is aimed at reduced human exposure times based on the concept of ALARA, astronaut flight restrictions based on maximum career exposure limits, and spacecraft shielding technologies. Medical therapy for radioprevention thus far has focused only on supportive care. This form of medical management includes intravenous hydration, antiemetics, anxiolytics, antibiotics, and drugs used to enhance reconstitution and activation of the hematopoietic system.

The pharmacological agents presented in this paper represent an armamentarium of drugs that have shown a significant dose reduction factor either alone or in combination with other agents. An intelligently designed multimodality protocol to include preventive and supportive pharmacological

agents along with the use of optimal spacecraft shielding would be the best prophylaxis against radiation toxicity. From a pharmacological standpoint, the ideal therapy will offer the greatest DRF and lowest toxicity, while having a long shelf life at room temperature, a high bioavailability, and easy route of administration. The current state of research into such agents remains amazingly sparse, especially given the need for these agents in today's nuclear battlefields, cancer treatment regimens, as well as the government and civilian push for increased space exploration.

Based on the presented data, it seems reasonable to make the following recommendations for radioprevention in long-duration space flight. First, low-level ionizing radiation, such as found in GCR, should not be treated with experimental radioprotectants given the lack of evidence to support their efficacy and safety in humans at their extrapolated treatment doses. In addition, the treatment efficacy of GCR high-energy particle exposure with these reagents is only theoretical, based on extrapolation of gamma-radiation studies. At this time, prevention of radiation toxicity from low-level background radiation should remain focused on shielding strategies, ALARA, and career radiation limits. Once research and new drug development data reach an acceptable clinical state, pharmacological radiopreventive therapy for low-level GCR can be revisited.

Further elucidation of the biological effects of the high energy particles found in GCR is needed. This can and should be accomplished using experimental animals and cell culture suspensions in the laboratory setting. Here quantitative exposures to high-energy particles from advanced particle generators can be performed. The limiting factors in this setting are the availability of advanced particle generators, their high cost of operation and relatively low yield. A more feasible alternative is to study the biological effects of high-energy particles on laboratory animals in space. Although the protective effects of the Van Allen belts significantly reduce the density of high-energy particles in low earth orbit, the relative effects of exposure could still be determined in this setting. The use of short-lived animals such as mice would facilitate evaluating the chronic and lifetime effects of GCR exposure. This can be accomplished on the research facilities of the International Space Station. The data generated from these experiments should help clarify the actual RBE of GCR particles and help guide possible therapeutic interventions. Furthermore, once the biological effects of GCR are delineated on animals in this setting, it can serve as a model to evaluate the therapeutic efficacy of experimental radioprotective agents against galactic cosmic radiation exposure.

The treatment of high-level radiation exposures that lead to the acute life-threatening and debilitating deterministic effects, as occur with solar flaring, also need to be further addressed. Pharmacological prophylaxis or rescue therapy should be instituted when large solar particle events are

vectored toward spacecraft inhabitants. Given the catastrophic effects of large solar particle events, the risk of toxicity from these chemoprotective agents is warranted. I recommend high dose amifostine for these exposures, as this FDA approved agent has the highest efficacy (survival DRF or 1.6 to 2.4) of the studied compounds. Its toxicity profile, while significant, is acceptable given the morbidity and mortality associated with no therapy. The use of other agents in combination with amifostine cannot currently be recommended. These drugs, while promising, have not been studied in humans as radiotherapeutic agents. Their effects on amifostine, whether inhibitory, additive, or synergistic, are not known. Given current dosing recommendations, amifostine should be dosed at 910 mg m^{-2} thirty minutes prior to exposure, if possible. The use of amifostine should be combined with the currently accepted supportive measures to include pain control agents, iv hydration, antibiotics, antiemetics, and anxiolytics.

Further work must be accomplished in the area of combination therapy. Data suggests that a combination drug therapy will yield the highest radiation dose reduction factor while allowing for a lower efficacious drug dose and thus reduced drug toxicity. Based on the current literature, a promising multi-modality therapy for experimental investigation would include the use low dose amifostine, glucan, vitamin E, 2-mercaptopropianylglycine, and one or more of the essential metals. This would allow for a reduction in the therapeutic amifostine dose as well as a reduction in its toxicity. In addition, this regimen focuses on multiple biological pathways to attain its effects. Amifostine and

vitamin E act to scavenge radiation generated free radicals, while amifostine also acts to stabilize double stranded DNA helices and facilitate DNA repair processes. 2-mercaptopropianylglycine, a low toxicity FDA approved compound, potentiates the efficacy of amifostine and significantly reduces the therapeutic dose and thus lowers its toxicity. Glucan, a naturally occurring polysaccharide immunomodulator with little toxicity, enhances the immune response to bacterial infections in irradiated animals. Finally, the essential metals, especially zinc, manganese, copper, and selenium are essential for the function of metalloenzyme complexes and possess enzyme mimetic, anticlastogenic, antiinflammatory, antimutagenic, anticarcinogenic, immunological, and/or tissue repair activities. The doses and relative times of administration of these agents need to be experimentally determined to optimize their therapeutic efficacy. Once land based studies have been optimized, experimental trials on laboratory animals can be advanced to space based habitats, such as those found on the International Space Station.

With the current push for deep space exploration and the entry of commercial enterprises into the space environment, the issue of radiation toxicity must be addressed. Current research strategies should focus not only on clinical translational research of the discussed radiotherapeutic agents, but expand into other areas. This includes intelligent drug design to formulate agents that will allow for S-phase arrest, up regulation of DNA repair enzymes, increases in superoxide dismutase and other important antioxidant enzymes,

free radical scavenging, and immune system enhancement. Treatment modalities should include classical pharmaceutical compounds, cellular therapeutics, gene therapy, and perhaps herbal agents and permissive radiation hormesis. In addition to standard supportive antibiotics, the concept of prophylactic gastrointestinal decontamination should be investigated. Prophylactic GI decontamination with benign oral antibiotics, while still undergoing clinical trials, has been shown to significantly reduce the incidence of gram-negative infections in critically ill intensive care patients. The effect of this treatment is thought to result from decontamination of intestinal organisms that lead to bacterial translocation and sepsis, the same events that occur in the altered intestinal epithelium after high-dose radiation exposure.

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