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Health Risks of Space Exploration: Targeted and Nontargeted Oxidative Injury by High-Charge and High-Energy Particles

Min Li,¹ Géraldine Gonon,¹ Manuela Buonanno,¹ Narongchai Autsavapornporn,¹
Sonia M. de Toledo,¹ Debkumar Pain,² and Edouard I. Azzam^{1,2}

Abstract

Significance: During deep space travel, astronauts are often exposed to high atomic number (Z) and high-energy (E) (high charge and high energy [HZE]) particles. On interaction with cells, these particles cause severe oxidative injury and result in unique biological responses. When cell populations are exposed to low fluences of HZE particles, a significant fraction of the cells are not traversed by a primary radiation track, and yet, oxidative stress induced in the targeted cells may spread to nearby bystander cells. The long-term effects are more complex because the oxidative effects persist in progeny of the targeted and affected bystander cells, which promote genomic instability and may increase the risk of age-related cancer and degenerative diseases. **Recent Advances:** Greater understanding of the spatial and temporal features of reactive oxygen species bursts along the tracks of HZE particles, and the availability of facilities that can simulate exposure to space radiations have supported the characterization of oxidative stress from targeted and nontargeted effects. **Critical Issues:** The significance of secondary radiations generated from the interaction of the primary HZE particles with biological material and the mitigating effects of antioxidants on various cellular injuries are central to understanding nontargeted effects and alleviating tissue injury. **Future Directions:** Elucidation of the mechanisms underlying the cellular responses to HZE particles, particularly under reduced gravity and situations of exposure to additional radiations, such as protons, should be useful in reducing the uncertainty associated with current models for predicting long-term health risks of space radiation. These studies are also relevant to hadron therapy of cancer. *Antioxid. Redox Signal.* 20, 1501–1523.

Introduction

ASTRONAUTS IN DEEP SPACE encounter many sources of cellular oxidative stress, including exposure to ionizing radiation. The radiation fields in the cosmic environment differ in their composition from those of the background radiations that humans encounter on earth. The terrestrial natural background radiation is mostly due to radon gas and its decay products. Gamma rays generated from the decay of radioisotopes in soil and rocks and the natural radioisotopes in the human body also contribute to background radiation (27). By contrast, the ionizing radiation environment in deep space is dynamic and primarily consists of protons, helium, and high

atomic number (Z) and high-energy (E) (high charge and high energy [HZE]) ions (203). Compared to α particles (~ 2 –10 MeV) emitted from terrestrial radionuclides, the energy spectrum of particulate space radiations is broad and spans several hundred mega electron volts per nucleon. HZE particles constitute only a small component of galactic cosmic rays, but because of their high biological effectiveness, they produce a significant fraction of the effective dose received during missions in space. As HZE nuclei are highly charged, they are densely ionizing and therefore possess strong oxidizing power (61, 249, 273). On impact with biological material, they cause clustered oxidative damage in DNA and other molecules, which may extend along a long column of cells in tissues due to their high penetration (84).

Departments of ¹Radiology and ²Pharmacology and Physiology, Cancer Center, Rutgers University-New Jersey Medical School, Newark, New Jersey.

During long-duration missions in space, exposure to ionizing radiation would easily exceed the guidelines for space exposure (63, 250). As a result, the National Aeronautics and Space Administration (NASA) is greatly concerned about long-term health risks to astronauts (1). The oxidative damage of nucleic acids, proteins, and lipids is directly linked to aging, cardiovascular diseases, neurodegenerative disorders, and cancer among other pathologies (90, 128, 140, 252, 262, 307). Therefore, understanding the various steps involved in HZE particle-induced cellular responses that lead to short- and long-term oxidative stress is important for evaluating the risk of health hazards during prolonged space travel or after return to earth.

Recently, renewed efforts investigating *in vitro* and *in vivo* responses to HZE particle irradiation have significantly advanced our understanding of the induced biochemical changes and the underlying mechanisms, particularly following cellular exposures to low mean absorbed doses (33). The availability of ground-based facilities capable of generating broad- and micro-beams of HZE particles has encouraged these efforts (97, 250). Nevertheless, our knowledge of the biological effects of HZE particles remains limited when compared to that of electromagnetic radiations or other types of particulate radiations (e.g., 2–5 MeV α particles). It should be emphasized that the biochemical and health effects of HZE particles cannot be extrapolated from data with X and γ rays (1, 84, 155, 305). Thus, the studies of HZE particle effects are critical; they may also contribute to the treatment of solid tumors that are particularly resistant to photon radiation (7, 86).

In this article, the focus is on short- and long-term oxidative stresses induced *in vitro* in mammalian cells and *in vivo* in rodents, by low and high fluences of HZE particles. The propagation of oxidative stress from cells targeted with HZE particles to non-targeted cells in vicinity (i.e., bystander effects), and the amplification of such stress among the targeted cells (i.e., cohort effects) will be highlighted. Emerging *in vivo* observations of nontargeted effects involving oxidative injury following partial body irradiation of rodents with HZE particles are also reviewed. The possible consequences of microgravity on oxidative stress and modulation of nontargeted effects are briefly discussed.

New Paradigm

Cells in cultures exposed to ionizing radiation were found to respond to the induced oxidative stress even when they were not directly targeted (199). Such findings were unexpected and led to a paradigm shift in understanding radiation effects (166). It is now widely accepted that radiation traversal through the nucleus of a cell is not a necessary prerequisite for the production of genetic damage or other important biological responses. Cells in the vicinity of directly irradiated cells may respond to the radiation exposure through redox-modulated intercellular communication pathways that propagate the oxidative stress initially originated in the irradiated cells (14, 132, 197, 228). Remarkably, perturbations in oxidative metabolism (e.g., disruption of the balance between oxidant production and antioxidant defense) occur not only in irradiated and bystander cells but also in their progeny as described in other articles in this Forum. A wide range of oxidative damages may occur in the progeny of both irradiated (52, 53, 145, 164, 195) and bystander cells (44, 45, 138,

226). The persistence of such stressful effects in progeny cells has profound implications for long-term health risks (85). It may trigger enhanced genomic instability (140), leading to increased probability of developing cancer with time, or a malignancy recurrence in individuals previously treated with radiotherapy for childhood cancers (78, 206).

In the context of exposure to low-dose radiation, non-targeted stressful effects are likely to develop in cells exposed to densely rather than sparsely ionizing radiations (45, 83). For sparsely ionizing radiation (e.g., γ rays delivered at low-dose rate), protective (16, 151, 215, 232, 304) rather than harmful (133) responses are induced. Therefore, understanding the physical and physicochemical characteristics of HZE particle irradiation, relative to other radiation types, is essential to studies of induced oxidative changes that promote harmful effects.

Characteristics of Densely Ionizing Radiations and the Induction of Oxidative Effects

Direct and indirect effects of ionizing radiation

In mammalian cells, significant chemical modifications take place during or shortly (within 10^{-15} to 10^{-6} s) after the exposure (20, 185, 211). These modifications occur through the direct interaction of the radiation with elements of the exposed cells or indirectly through the generation of oxidizing species from water radiolysis (Fig. 1A). Water is the major ($\sim 80\%$) constituent of cells and is readily decomposed by ionizing radiation, generating various products such as e^-_{aq} (hydrated electron), $\cdot OH$, $H\cdot$, $H_2\cdot$, and H_2O_2 (160, 186, 221, 224). In the presence of oxygen, e^-_{aq} and $H\cdot$ species are rapidly converted to the superoxide/perhydroxyl ($O_2^{\cdot-}/HO_2\cdot$) radicals (31). In summary, in an aerobic cellular environment at physiological pH, the major reactive species present at homogeneity ($\sim 10^{-6}$ s) are $O_2^{\cdot-}$, $\cdot OH$, and H_2O_2 .

The distinguishing features of ionization patterns of various radiations mainly arise from differences in charge-to-mass ratio of the particles (Fig. 1B). In case of HZE particles, the concentration of radiolytic species is very dense in and around the particle track (102, 198) (Fig. 1), causing extensive covalent modifications in affected macromolecules. These modifications lead to changes in signaling events that may affect proteins/genes involved in oxidative metabolism, including activity modulation of oxidases, nitric oxide synthases, and antioxidants (191, 297). An attractive hypothesis is that the nature and concentration of the reactive species along an HZE particle track contribute to recruitment and compartmentalization of signaling molecules (e.g., second messengers). The linear energy transfer (LET) effects of the radiation are therefore an important link between water radiolysis and ensuing biochemical changes (66, 143, 160).

Linear energy transfer

The LET is defined as the amount of energy (E) deposited by an irradiating particle per unit track length (l) ($LET = dE/dl$) (311). It is an average quantity expressed in $keV/\mu m$. Whereas high-energy X rays, electrons, and ^{137}Cs and ^{60}Co γ rays are low-LET radiations, α and HZE particles are typical high-LET radiations (102). The demarcation value between low- and high-LET radiations is $\sim 10 keV/\mu m$ (121). The complexity of radiation damage increases with the LET (34,

FIG. 1. Effects of high atomic number (Z) and high-energy (E) (HZE) ion irradiation in mammalian cells. (A) Traversal of a cell by a densely ionizing HZE particle results in direct and indirect cellular effects on macromolecules. Absorption of ionizing radiation by living cells directly disrupts atomic structures, producing chemical and biological changes and indirectly through radiolysis of cellular water and generation of reactive chemical species by stimulation of oxidases and nitric oxide synthases. Ionizing radiation may also disrupt mitochondrial functions significantly contributing to short- and long-term effects leading to persistent alterations in lipids, proteins, nDNA, and mtDNA. **(B)** The complexity of DNA damage induced by ionizing radiation is highly dependent on the biophysical characteristics of the radiation, in particular its LET properties. Note that tracks of ionizing particle, macromolecules, and organelles are not drawn to scale. HZE, high charge and high energy; LET, linear energy transfer; mtDNA, mitochondrial DNA; nDNA, nuclear DNA. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/ars

240, 274, 285), and the LET effects are critical in determining the relative potency of different types of radiation in causing biological changes (104, 106, 160, 216). For example, following exposure to HZE particles, the yield of locally multiply damaged sites in DNA is greatly increased (106, 284), thereby leading to high biological effectiveness. HZE particles can create multilocus deletions, including the loss of essential genes, leading to the disruption of important physiological functions (155).

The distribution of energy deposition in tissues varies with tissue depth. For low-LET electromagnetic radiation (e.g., X rays), the energy deposition decreases exponentially as a function of penetration-depth in target material. By contrast, charged particles (e.g., HZE particles) have a well-defined range in matter. Specifically, energy deposition by HZE particles is characterized by a relatively low entrance dose in the

target material and a pronounced sharp maximum near the end of their range (the Bragg peak) (148). Beyond the Bragg peak, the energy may be close to zero (Fig. 2). Extremely high-LET values in tissues can be reached at the Bragg peak (274), and this characteristic energy deposition profile of charged particles is exploited in cancer radiotherapy. By positioning the patient so that the location of the tumor coincides with the Bragg peak of the impacting particles, most of the energy is deposited in the tumor, whereas the surrounding tissue is exposed to a significantly reduced amount of energy. Thus, cell killing is specifically enhanced in the tumor region and not in the normal tissue surrounding the tumor (86). Nevertheless, the long-term consequences on nearby cells (those that received a small dose and the unirradiated) have not been investigated, a topic that would enhance our understanding of the effect of dose on propagation of nontargeted effects (77).

FIG. 2. Typical energy loss profiles for photons and an HZE particle (^{12}C) as a function of the distance traveled in tissue. The exponential tail in the photons curve indicates that they deposit their energy through the entire target. In contrast, heavy ions release the highest amount of energy (the Bragg peak) by the end of their range after which little or no dose deposition is expected. For simplicity, tissue is represented by water [adapted from Bert *et al.* (29) with permission].

Several studies have shown that the biological effectiveness of radiation is not solely due to LET (47). HZE ions with the same LET but different charge and velocity may have different biological effectiveness. The track structure or geometry of the physical energy deposition in the biological target greatly affects the cellular response to heavy ions (241) [discussed in refs. (84, 231)]. The track structure reflects the size and precise location of the radiation-induced bursts of reactive oxygen species (ROS) (Fig. 3A). Track structure studies are theoretical in nature; they use computational approaches to understand the spectrum of damages to macromolecules and subsequent signaling effects by radiations of different quality. The radiation quality is defined as the distribution of dose in LET, specifying the fraction of dose in each LET interval (240). The track structure is dynamic and is affected not only by spatial distribution of ionizations (and excitations) but also by tem-

poral aspects of the spatial distribution of the radiolytic species (105, 198, 209, 224). By investigating these microscopic features of ionizing radiation, track structure studies have enhanced our knowledge of numerous radiation-induced phenomena (41, 67, 209). They helped in understanding the signaling events that lead to adaptive protection by low-dose/low-LET radiation *versus* spread of stressful bystander effects by low fluence/high-LET radiation (74, 143, 286). Both high- and low-LET radiations are generated upon interaction of HZE particles with biological matter, and one would expect that their combined effects govern the response of exposed cell populations.

HZE particles and generation of secondary radiations

Secondary radiations arise from interactions of HZE particles with electrons in target atoms and from the fragmentation of the incident HZE particle and target nuclei (68, 81) (Fig. 3A). These secondary radiations are produced along the primary particle track and include photons, protons, neutrons, α particles, other heavy ions with lower velocity, and energetic electrons (δ rays: secondary electrons with enough energy to form tracks of their own). The ranges of δ rays produced by different sources greatly vary. Those produced by HZE particle-traversals can extend up to several cell diameters (62, 190) (Fig. 3B). Due to their long range, these HZE particle-generated δ rays can potentially irradiate and produce biochemical changes in cells that are adjacent to cells directly targeted by the primary particle track. In this respect, the evaluation of the cellular absorbed dose due to δ rays is essential when apparent nontargeted effects are assessed following the exposure of confluent cell cultures to very low fluences of HZE particles. This dose can be as high as 30–40% of the total dose deposited by an HZE particle (62, 101, 209). However, α particles emitted from common radioactive sources (*e.g.*, ^{238}Pu or ^{241}Am) used for cellular experiments do not pose such problems. Interpretations of the apparent nontargeted effects are relatively straightforward as these α particles do not produce secondary radiations that cross-irradiate neighboring cells (126).

For microdosimetric purposes, the trajectory of a high-LET particle is proposed to consist of a cylindrical “core” with dense oxidizing species and a concentric region known as “penumbra” formed by secondary electrons (low-LET δ rays) surrounding the core (54). The yield and precise location of radiolytic species generated by primary and secondary

FIG. 3. Structure of an HZE particle track (primary and secondary ionizations) in relation to an exposed population of cells. (A) Schematic representation of ionizations and excitations generated in biological material (*e.g.*, water) by an incident HZE particle and its fragmentation products. (B) δ rays resulting from the interaction of primary HZE particles with the target materials travel a significant distance and may deposit a biologically significant dose in cells in vicinity.

radiations have been the subject of comprehensive studies (54, 65, 186, 207, 208, 216, 221, 224). The cross talks between signaling events induced by the dense and sparse ionizations are likely to have significant effects on the biological response. In cell populations exposed to low fluences of HZE ions, protective mechanisms (e.g., enhanced DNA repair, increased antioxidant potential) induced by low-LET secondary radiations may mitigate stressful effects propagated from adjacent cells traversed by primary particles (87). Conversely, the nonirradiated cells in the exposed population may transmit rescuing signals to the irradiated cells (56). In this context, experiments evaluating various biological endpoints following the exposure of cell cultures to a specific dose of HZE particles delivered either acutely or at very low-dose rate would be highly informative of the cross talks that are occurring.

Oxygen Effects

The effects of oxygen on biological responses to ionizing radiation became apparent with the discovery of X rays in 1895 by Wilhelm Roentgen. In 1912, Swartz noted that a skin reaction produced on his forearm by a radium applicator was reduced if the applicator was pressed into the skin. He attributed the effect to an interruption in blood flow. In 1923, Petry made the correlation between radiosensitivity and the presence of oxygen through his study of radiation on vegetable seeds (121). Thirty years later, in 1953, Gray *et al.* concluded that the tissue oxygen concentration at the time of irradiation is a factor in radiotherapy outcome (109).

In the presence of O_2 , the free radicals produced during irradiation give rise to other ROS, including hydrogen peroxide (H_2O_2) and organic hydroperoxides (ROOH) (123). These hydroperoxide species together with redox active metal ions (such as iron and copper), commonly found in biological matrices, further contribute to radiation-induced oxidative damages *via* Fenton type reactions (30, 59). In 1949, Thoday and Read performed studies on the frequency of chromosome aberrations in irradiated *Vicia faba* root tips, and they noted that oxygen had a much greater influence on the X ray effects than on the α particle radiation effects (271). Subsequent studies by Barendsen *et al.* (21) have shown that the dissolved oxygen in the milieu in which human cells are grown affects their radiation response in an LET-dependent manner. For radiations with LET greater than $10 \text{ keV}/\mu\text{m}$, the effect of oxygen in the growth environment was found to decrease sharply. The oxygen enhancement ratio (OER) is defined as the ratio of doses administered under hypoxic to aerated conditions needed to achieve the same biologic effect, and the OER value was determined to be unity for radiations with $LET \geq 100 \text{ keV}/\mu\text{m}$ (121).

Baverstock and Burns highlighted the complexity of oxygen effects in cells exposed to high-LET radiation. They suggested that the decrease in OER for cell killing by high-LET radiations is due to O_2 production along the tracks of impacting HZE particles (23, 24). Interestingly, a study by Meesungnoen and Jay-Gerin (187) have shown that the track O_2 concentrations as a function of time in the interval of 10^{-12} – 10^{-6} s correlate well with O_2 concentrations estimated to

FIG. 4. Effect of LET on the temporal variation of oxygen yield and concentration. (A) Time dependences of the O_2 yields (in molecules/100 eV) calculated from Monte-Carlo simulations of the radiolysis of pure deaerated liquid water at pH 7, 25°C , in the interval 10^{-12} – 10^{-6} s, for impacting 24 MeV $^{12}C^{6+}$ (LET $\sim 490 \text{ keV}/\mu\text{m}$, solid line) and 4.8 MeV $^4He^{2+}$ (LET $\sim 94 \text{ keV}/\mu\text{m}$, dashed line) ions, including multiple ionization of water molecules. The dotted line corresponds to the calculated $G(O_2)$ values for the low-LET limiting case of 300 MeV protons (LET $\sim 0.3 \text{ keV}/\mu\text{m}$). **(B)** Time dependence of the calculated corresponding track concentrations of O_2 (in mM) for the different irradiating ions. Typical O_2 concentrations in normal human cells ($\sim 30 \mu M$) are shown by the arrow on the right of the figure [adapted from Meesungnoen and Jay-Gerin (187) with permission].

produce the observed reduction in OER with increasing LET (Fig. 4). Using Monte-Carlo simulations of pure deaerated water radiolysis by 24 MeV $^{12}\text{C}^{6+}$ ions (LET $\sim 490 \text{ keV}/\mu\text{m}$), the initial yield of O_2 along the track was greatly higher than following water radiolysis by 4.8 MeV ^4He ions (LET $\sim 94 \text{ keV}/\mu\text{m}$) or 300 MeV protons (LET $\sim 0.3 \text{ keV}/\mu\text{m}$). The magnitude of the LET effect was particularly significant at early times (10^{-12} s) after irradiation (Fig. 4A). Calculation of the track concentrations of O_2 along the track of a 24 MeV ^{12}C ions (Fig. 4B) showed that it might be more than three orders of magnitude higher than the O_2 levels present in normally oxygenated and hypoxic tumor regions and in normal human cells (187). Such results explain, at least in part, the greater efficiency of high-LET radiations for cell inactivation compared to equal dose of low-LET radiations.

Apart from cellular O_2 tension, other variables of the space environment may also modulate the responses to oxidative stress induced by HZE particles. These include pre-exposure to diagnostic radiation, chemicals, space dust, confinement, and other parameters. These stresses may induce events that alter the cellular redox environment, thereby affecting the response to HZE particles. Recently, the contribution of oxidative stress generated by the non-targeted pathway to the overall responses of cell populations exposed to low fluences of HZE particles has received much attention (64, 133).

Space Radiation-Induced Nontargeted Effects

Ionizing radiation-induced nontargeted effects are broadly defined as the occurrence of important biological changes in nonirradiated cells/tissues due to the exposure of other cells/tissues to ionizing radiation (165) (Fig. 5). Nontargeted effects have been detected in cell cultures where only a small fraction of the cell population is targeted by high-LET α particles (Fig. 5). These effects are known as bystander effects (15, 131, 165, 194, 196, 228). Genetic alterations, including sister chromatid exchanges (199), mutations (200, 310), chromosomal aberrations (225) and micronucleus formation (13), changes in gene expression (12, 136), lethality (260), and neoplastic transfor-

mation (44, 247), are detected in bystander cells of various lineages after the exposure of other cells to α particles. Bystander effects have been also examined after exposure to low-LET radiations, and readers are referred to recent reviews on these effects elsewhere (35, 124, 196, 228).

When cell populations are exposed *in vitro* to low mean absorbed doses from HZE particles, a significant fraction of the cells will not be traversed by a high-LET radiation track (40) (Figs. 3 and 5). Studies of bystander effects in cell cultures exposed to low fluences of HZE particles are only emerging, and conflicting data have been reported. In early experiments with a microbeam, stressful effects were shown to be propagated from HZE particle-irradiated cells to contiguous bystander cells (125, 127, 256). In addition, when HZE particle-irradiated cells were cocultured with bystander cells in a manner in which they shared only the growth medium, stressful responses were also induced in the bystander cells and were similar in nature to those generated in the targeted cells (95, 278, 298–300) (Fig. 5). With relevance to cancer risk, oxidative stress and DNA damage persisted in distant progeny of bystander cells that had been in contiguous coculture with HZE particle-irradiated cells (45, 226). However, transferring the growth medium from irradiated cultures to recipient bystander cells (116, 260), or the targeting of a small number of cells in a population with HZE particles delivered by a microbeam (94), did not induce bystander effects measured by a variety of endpoints in several cell types. Numerous factors may underlie the absence of observable effects in these cases, including timing of endpoint measurement, dilution of the inducing factor, and the metabolic state/redox environment of the recipient cells.

The lack of clear knowledge about nontargeted responses (94, 127, 299) has been singled out by the U.S. National Academies (1) as one of the important factors limiting the prediction of radiation health risks associated with space exploration. This is because during deep space travel, only a small fraction of cells in the human body experiences extremely dense ionizations created along the tracks of the traversing particles in a given day (62). Moreover, the radiation traversals that create these densely ionizing tracks are likely

FIG. 5. HZE-particle irradiation induces targeted and nontargeted (bystander) effects. Communication of stress-inducing molecules from cells exposed to an HZE particle (cells in red) and its fragmentation products (cells in orange or green) propagate stressful effects that lead to induction of oxidative stress in bystander cells (yellow). The progeny of the targeted and bystander cells may also experience oxidative stress. Cells in white are nonaffected bystander cells.

to be separated in tissue location and time (133). Interestingly, a dose-response model that takes into account bystander effects provided an improved fit compared to the targeted effect model in analyses of Harderian gland tumors induced in mice exposed to heavy ions (64). These data probably represent the most comprehensive study for tumor induction by heavy ions. In other studies, Weil *et al.* (289) have suggested circulating inflammatory cytokine and nonirradiated parenchymal cell responses to oxidative stress as contributing factors to the unexpected high relative biological effectiveness of energetic iron ions in inducing hepatocellular carcinoma.

Various *in vivo* and *in vitro* experimental strategies are being adopted to investigate nontargeted effects of HZE particles found in space (Fig. 6). They involve partial body irradiation of rodents, monolayer cell cultures exposed to very low fluences of energetic ions, and cocultures of HZE particle-irradiated cells with nonirradiated cells. For the *in vivo* strategy and in monolayer cell culture studies, the effects of secondary radiation have to be considered when interpreting nontargeted oxidative effects of space radiation. Cells presumed to be bystander might be hit by secondary HZE particle or by δ rays (Fig. 3). Monte-Carlo simulations using programs such as FLUKA and MCNPX multiparticle transport codes are greatly enhancing our understanding of the contribution of secondary radiations in HZE particle-induced bystander effects (101, 162). These studies allow the estimation of the fraction of cells that are not targeted by fragmentation products and also the fraction of cells that are hit by these products together with the doses received. Importantly, they permit the quantification of the radial distribution of the secondary particles as was recently shown (101).

Compared to studies with irradiated monolayer cultures, the use of coculture strategy eliminates complications in interpreting bystander responses attributed to secondary radiations. In the coculture strategy, secondary radiations due to fragmentation of the primary HZE particle are absent as the cocultured cells originate from separate growth vessels (44, 300). Furthermore, this strategy readily permits the investigation of the phenotypic effects (*e.g.*, altered oxidative metabolism) of cells donating or receiving the bystander signal.

On the other hand, the effects of rapidly propagating factors from irradiated cells may be missed because adequate time is required after irradiation to establish the coculture. Importantly, such experiments should be stringently controlled for variations in oxygen tension, pH, temperature, and dilution effects during handling of cells, as these parameters affect cellular physiology, the propagated molecules and the modulated signaling pathways.

Due to our limited knowledge of biological changes in cells targeted by primary HZE particles and in those in vicinity, scientific councils (202, 261) have postponed defining the exposure limits for a Mars mission until more data on the effects of exposure to heavy ions are obtained. Further studies of nontargeted effects involving variations in dose rate and exposure to mixed beams of low- and high-LET radiations (*e.g.*, low fluence protons and HZE particles) are essential toward setting these limits. However, such investigations are currently limited. In the context of *in vivo* studies, partial body irradiation of rodents with HZE particles that target organs with varying degree of radiation sensitivity would be highly informative as the extent of the propagated oxidative stress may greatly depend on the inherent sensitivity of the targeted organ. With respect to risk projections, long-term follow-up studies of oxidative changes associated with genetic and epigenetic alterations in targeted and nontargeted tissues are essential. Studies of chronic inflammation would be informative of oxidative stress as inflammatory responses exacerbate the generation of ROS and reactive nitrogen species (RNS) (91, 156, 168, 237). Characterizing these collective responses and the underlying mechanisms may help reduce the long-standing uncertainty in predicting health risks associated with space missions.

Oxidation/Reduction Reactions and the Biological Effects of Space Radiation

ROS and damage to macromolecules

At homeostatic conditions, ROS maintain normal cellular functions: they regulate the expression of genes (4, 135, 189, 227), modulate ion channel activities (167), and mimic/affect

FIG. 6. *In vivo* and *in vitro* strategies to study nontargeted effects of ionizing radiation and the underlying mechanisms (yellow cells represent potentially affected bystander cells; white cells are nonaffected bystanders). To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/ars

intermediates (e.g., second messengers) in signal transduction (253). However, exposure to high levels of ROS is associated with perturbation of cell proliferation and development of disease (82). For example, in one set of experiments, mice were exposed to isotoxic doses of energetic iron ions (1000 MeV/nucleon) or ^{137}CS γ rays delivered to the whole body. A year after exposure, results showed higher levels of intracellular ROS and mitochondrial superoxide in intestinal epithelial cells of animals exposed to iron particles than to γ rays (69). As highlighted above, the intracellular accumulation of oxidants modifies essential macromolecules such as DNA (122), and the induced genomic changes may be amplified and transmitted to daughter cells (90, 193).

With increasing LET of radiation, an increase in the yield of molecular products (e.g., H_2O_2) is accompanied by a corresponding relative decrease in the yield of e^-_{aq} and certain radicals such as $\cdot\text{OH}$. By contrast, $\text{O}_2^{\cdot-}$ (or $\text{HO}_2^{\cdot}$) is the most abundant radical species produced by high-LET radiation (159, 186). Organic radicals ($\text{R}^{\cdot}$) are also formed following irradiation, most often by H-abstraction reactions. These carbon-based radicals react rapidly with O_2 to give peroxy radicals ($\text{RO}_2^{\cdot}$), which are stronger oxidizing agents than their parent radicals (3). Evidently, the yields of these products and their concentrations have important consequences for the extent and nature of induced damage to cellular DNA (50, 103, 154, 266). Experiments with rat hippocampal neural precursor cells have clearly demonstrated effective ROS generation by high-LET iron ions particularly at mean ab-

sorbed doses of 1 Gy or lower compared with low-LET protons and X rays (Fig. 7) (163). When cellular antioxidant potential is decreased, the interaction of ROS with DNA results in breaks, base damage, destruction of sugars, cross-links, and telomere dysfunction among other alterations (49, 211, 245). In particular, HZE particles produce closely spaced oxidative lesions (oxidatively induced clustered DNA lesions) (266) and cause double-strand breaks, a serious type of DNA lesion that leads to cell death or long-term stressful effects in surviving cells (61, 156). These structural changes result, if unrepaired, in mutations, chromosomal translocations, and other aberrations (119, 214).

Notably, recently developed fluorescence *in situ* hybridization-painting methodologies have revealed that HZE particles induce a high fraction of complex type exchanges, and possibly unique chromosome rearrangements relative to γ rays (120). Assessment of cytogenetic damage in blood lymphocytes of 16 astronauts before and after space missions (of 3 months or more) revealed that the frequency of chromosome exchanges measured within a month of return from space was higher than their preflight yield (99). Although some astronauts showed a temporal decline in chromosome damage with time after flight, others did not. In some astronauts, the yield of chromosome damage actually increased with time (99). Genetic susceptibility and perturbations in oxidative metabolism may explain the increased chromosomal damage in these astronauts (147).

Like in the case of DNA, protein modifications may occur through direct attack by ROS or through reaction with lipid peroxidation byproducts. Such modifications alter protein function and can result in protein degradation. For example, protein carbonylation is an irreversible oxidative process that leads to unrepairable protein modifications (210). It is often associated with the disruption of signaling events and cellular senescence (90). Histones are the essential components of eukaryotic chromatin, and carbonylation of histones may have severe consequences for the maintenance of genomic integrity (294), such as altered chromatin compactness with disrupted DNA repair. Likewise, HZE particle-induced oxidative damage to electron transport chain (ETC) proteins can enhance oxidative stress. Specifically, dysfunctional succinate dehydrogenase subunit C was shown to sensitize cells to low-dose/low-LET radiation through mechanisms partly mediated by increased levels of $\text{O}_2^{\cdot-}$ and H_2O_2 (218). The expression of these effects was shown to contribute to genomic instability after exposure to γ rays (71). Hence, follow-up studies of HZE particle-induced effects on the assembly of macromolecular protein complexes such as the respiratory complexes and mitochondrial translocases would be instructive of defects that may be induced soon or long time after the exposure to HZE particles.

Whereas reports of oxidative damage by HZE particles in genomic DNA, proteins, and lipids are increasing, this is not the case for mitochondrial DNA (mtDNA). The close proximity of mtDNA to sites of ROS production through ETC renders it particularly susceptible to damage, which led Harman to propose four decades ago that damage to mtDNA contributes to the aging process (129). Various pathologies have been attributed to mutations in mtDNA; their severity depends on the nature of the mutation and on the proportion of mutant and wild-type mtDNA present in a particular cell type (137, 281, 282). Tissues that have a high requirement for ATP are most affected by mutations in mtDNA (80);

FIG. 7. Dose response for oxidative stress after ^{56}Fe ion irradiation. Hippocampal neural precursor cells subjected to ^{56}Fe ion-irradiation (1000 MeV/nucleon) were analyzed by flow cytometry for oxidative stress using the ROS sensitive dye CM-H₂DCFDA [5-(and-6)-chloromethyl-2',7'-dichlorodihydro-fluorescein diacetate] 6 h after irradiation. The cells were incubated at 37°C for 1 h in 10 μM of the dye just before harvesting and analysis. At mean absorbed doses ≤ 2 Gy, ROS levels were significantly greater than those generated following the exposure to 250 kVp X rays or 250 MeV protons. The data were normalized against unirradiated controls set to unity. ROS levels induced after ^{56}Fe irradiation were significantly ($p < 0.05$) higher than controls. The standard errors of the mean of three independent experiments are shown [adapted from Limoli *et al.* (163) with permission]. ROS, reactive oxygen species.

they exhibit a variety of metabolic disorders as well as age-associated disease (25, 169).

Short- and long-term HZE particle-induced ROS/RNS could result in damage to mtDNA coding for mitochondrial ETC subunits as well as the biochemical machinery necessary for their proper expression and assembly (28). Interestingly, cells lacking mtDNA are deficient in mitochondrial electron transport ($\rho^{(0)}$ cells), and they do not experience radiation-induced ROS/RNS production (161). $\rho^{(0)}$ cells are more resistant to radiation-induced cell killing than $\rho^{(+)}$ cells (149) and are inefficient at propagating radiation-induced bystander effects (57, 270, 309).

Recent evidence has indicated that base excision repair (115) and mismatch repair (178) may be induced in mitochondria during oxidative insult. Excess oxidative stress appears to target the mtDNA polymerase γ activity required for the replication and repair of mtDNA (110), thereby reducing the overall repair capacity (235, 280, 303). Therefore, subsequent to HZE particle-irradiation, mtDNA might be preferentially damaged or lost due to oxidative stress with an ensuing decrease in respiratory chain activity and decrease of mitochondrial function. Mutations in mtDNA causing disruptions in the proper assembly and/or function of mitochondrial ETCs could lead to an increase in accessibility of reduced components of the ETCs to O_2 , which may result in an increase in prooxidant formation (262). The metabolic oxidative stress may persist and continues to cause *de novo* oxidative damage to critical macromolecules (*e.g.*, nuclear DNA) long after the radiation exposure (36, 98).

The "common deletion" in the mitochondrial genome is induced following cellular exposure to ^{137}Cs γ rays (229). This deletion involves the loss of 4977 base pairs coding for proteins that include subunits of the mitochondrial ATPase, NADH dehydrogenase complex I, and cytochrome c oxidase. Once formed, the common deletion becomes stable (55). Hence, determining whether progeny of targeted and non-targeted cells carry this deletion following the exposure to HZE particles would be informative. The common deletion may lead to inefficient mitochondrial metabolism and thus increased ROS production (32).

Translocation of DNA from mitochondria to the nucleus has been demonstrated in *Saccharomyces cerevisiae* (272), and mtDNA sequences are found in at least 27 sites in human chromosomes (234). It is therefore tempting to speculate that migration of mtDNA fragments to the nucleus following exposure to ionizing radiation may lead to their insertion in nuclear genome, particularly during repair of DNA double-strand breaks (233). The insertion of mtDNA in nuclear genome may result in induction of oncogenes and permanent changes in gene expression (98), which may affect physiological functions and increase health hazards (58, 257). Interestingly, experiments in yeast have shown that the migration rate of mtDNA to the nucleus increases during chronological aging (58). The exacerbation of this phenomenon in older astronauts by HZE particles during space missions would have important implications to the choice of astronaut's age for long duration missions.

Propagation of HZE Particle-Induced Oxidative Damage to Nontargeted Cells

In recent studies with cells exposed to very low fluences of HZE particles (101), significant increases in overall protein

carbonylation were detected in extracts of normal human cell cultures harvested 24 h after the exposure to doses as low as 0.2 cGy of 1000 MeV/nucleon iron ions (LET $\sim 151 \text{ keV}/\mu\text{m}$ in water) (Fig. 8A). At such a low mean absorbed dose, only $\sim 1\%$ of the cells are traversed by a primary particle track. The accumulation of 4-hydroxynonenal (4-HNE) adducts in proteins from the same cultures indicates that increased lipid peroxidation was involved (Fig. 8B) (101). Together, these data strongly suggest the propagation of oxidative stress to cells not targeted by primary iron ions. Interestingly, 4-HNE reactive aldehydes originate from peroxidation of lipids in the membrane that contain key proteins involved in stress-induced intercellular communication, such as connexins, cyclooxygenase-2, and NAD(P)H oxidase (6). Modification of these proteins in targeted cells may modulate transport properties of the plasma membrane leading to propagation of signaling molecules that induce oxidative stress in nearby cells not targeted by a primary HZE particle. In this context, our studies have shown that the magnitude of the effects due to connexin channels in enhancing or reducing the propagation of radiation-induced oxidative stress is a

In confluent cell cultures Oxidative Stress Dose Response to ^{56}Fe ions

FIG. 8. Oxidative stress in confluent normal human fibroblasts harvested 24 h after exposure to low mean absorbed doses of 1000 MeV/nucleon ^{56}Fe ion. Immunoblot analyses of (A) protein carbonylation and (B) lipid peroxidation (measured by 4-HNE protein adduct accumulation). For protein carbonylation, the relative intensity (*i.e.*, fold-change) in oxidation of the overall spectrum of proteins (~ 30 – 130 kDa) in irradiated cells was compared to that in control cells. For 4-HNE protein adduct accumulation, the relative intensity refers to the level of the band with arrow relative to control. Staining with Ponceau S Red was used as loading control [adapted from Gonon *et al.* (101) with permission]. 4-HNE, 4-hydroxynonenal.

function of radiation quality (LET effect). The abundance of reactive species along the track of an HZE particle may be the factor that affects permeability of connexin channels (9–11). These results are consistent with earlier studies showing that the cellular redox environment together with gap junction communication are the major mediators of the propagation of stressful responses from α particle-irradiated cells to bystander cells in the vicinity (13, 17, 201, 308).

The notion that bystander cells participate in the responses of cell populations exposed to low fluences of HZE particles was further validated with studies in progeny of bystander cells as follows (45). Bystander cells were cocultured with cells exposed to low (10 cGy) or high (200 cGy) mean absorbed doses of 1000 MeV/nucleon ^{56}Fe ions. Excess levels of spontaneous DNA damage were observed in the progeny of these bystander cells, and these effects correlated well with extensive protein carbonylation (45) (Fig. 9A). Remarkably, these effects did not occur in the progeny of bystander cells that were cocultured with cells exposed to low (25 cGy) or moderate (200 cGy) absorbed doses of 1000 MeV protons (45). Our recent data show that these nontargeted effects of HZE particles occur *in vivo* and are persistent (142). Significant increases in protein carbonylation were also detected in mitochondria isolated from the nontargeted liver of rats that were exposed, 22 months earlier, to a mean absorbed dose of 50 cGy of 500 MeV/nucleon ^{48}Ti ions targeted to the head only (Fig. 9B). Furthermore, the effects were associated with defects in mitochondrial protein import and altered the expression of mitochondrial protein translocases. Defects in mitochondrial protein import resulting from an initial exposure to HZE particles may amplify the oxidative stress and lead to late health effects, including degenerative diseases and metabolic disorders (70, 139, 242, 268). These data raise the possibility that mitochondrial markers could be used as a

sensitive indicator of both targeted and nontargeted effects of radiation (174, 220). Cellular exposure to ionizing radiation may cause damages to key components of the protein translocation machinery, and this in turn may result in global defects in protein targeting/import. Alternatively, a reduction in import efficiency may also represent an adaptive response to minimize ROS-mediated metabolic changes. In fact, proteomic analyses of mitochondria from nonirradiated tissues of rodents exposed to HZE particles (142) revealed both stressful effects [e.g., decrease in proteins involved in fatty acid elongation, a process required for energy storage and synthesis of lipids (283)], and protective responses [e.g., upregulation of Nrf2 signaling implicated in important antioxidant functions (157, 158)]. Extending these studies to examine changes in mitochondrial structure/functions after sequential exposure to low- and high-LET radiations would be of great interest. Adaptive responses encompassing DNA repair and anti-oxidation reactions may be triggered following exposures to low doses of low-LET radiations (e.g., γ rays, protons), particularly when delivered at low-dose rate (73, 152, 293, 304). The protective mechanisms may defend against the damaging effects of a subsequent exposure to HZE particles (18). Interestingly, genes responsible for both generating and scavenging ROS were upregulated in liver of mice that were flown on a 13-day space shuttle mission (STS-118) (19).

Space radiation, generation of nitric oxide, and inflammatory responses

Ionizing radiation can stimulate inducible nitric oxide synthase activity in targeted cells (179, 191, 287), thereby generating large amounts of nitric oxide ($\cdot\text{NO}$). Reaction of $\cdot\text{NO}$ with $\text{O}_2^{\cdot-}$ forms the peroxynitrite anion (ONOO^-), which is highly reactive and capable of attacking a wide range

FIG. 9. *In vitro* and *in vivo* long-term effects of HZE particle irradiation on protein oxidation. (A) Immunoblot showing protein carbonylation in the progeny of bystander human cells (20 population doublings following isolation) that were cultured for 5 h with 1000 MeV/nucleon ^{56}Fe ion-irradiated cells. (B) Immunoblots showing protein carbonylation in nontargeted liver of Sprague-Dawley rats whose head only was exposed, 22 months earlier, to 500 MeV/nucleon ^{48}Ti ions. Note that the radiation doses described in the figures refer to the absorbed dose by the targeted cells or organ.

of cellular targets, including lipids, thiols, proteins, and DNA bases (287). When generated during inflammatory responses, ONOO⁻ and other RNS can react with nucleic acids, lipids, and proteins in neighboring bystander cells, which may lead to cell death or genomic instability (182). Importantly, space flight-induced effects on the immune system (204) and the ensuing inflammation and perturbations in oxidative metabolism (91, 156, 237) increase the risk of cancer (237, 295). When macrophages and neutrophils are recruited to sites of inflammation, they generate ROS and RNS, which can cause a large spectrum of oxidative damage in neighboring cells (48, 75), hence contributing to out-of-field or abscopal effects (142, 192, 212, 269). Interestingly, low doses/low fluences of protons and HZE particles have been shown to trigger numerical and phenotypic changes in immune/inflammatory cells (112–114, 259) and changes in cytokine levels (236, 246) in mice. The secreted cytokines (107) may then perturb physiological functions in normal surrounding cells (248). Further studies on the effect of low doses/low fluences of space radiation on inflammatory/immune cell trafficking, DNA damage, polarization, and signaling in radiosensitive (*e.g.*, bone marrow, lung) and radioresistant (*e.g.*, brain) organs should fill important gaps in our knowledge of *in vivo* space radiation targeted and nontargeted effects.

At normal concentration of •NO, nitrosation is essential for basal cellular functions. For example, protein S-nitrosation (addition of the NO moiety onto cysteine thiols) is known to regulate diverse cellular processes, such as cell survival, cell death, signal transduction, and DNA repair (92, 173, 219, 296). Virtually all enzymes contain cysteine residues that can be subjected to nitrosation, and many enzymes are involved in mediating the response to ionizing radiation, including caspases, thioredoxin, HIF-1 α , MnSOD, NF- κ B, p53, and Bcl-2 (93). Thus, studies of transnitrosation and denitrosation of proteins with roles in antioxidation, DNA repair, and cell survival as a function of time after exposure to space radiation would be informative in elucidating the mechanisms underlying the development of genomic instability or induction of adaptive responses to the induced stress (179). They may identify biochemical events that underlie protective effects induced by low-LET space radiation, which defend against oxidative stress induced by densely ionizing HZE particles (87).

HZE particles and metabolic enzymes

Iron-sulfur (Fe-S) clusters are modular cofactors of proteins that participate in many important cellular processes, including enzymatic catalysis, electron transport, and regulation of gene expression (243). Mammalian cells contain two aconitases that contain [4Fe-4S] clusters: the mitochondrial enzyme (ACO2) involved in the TCA cycle, and a cytosolic enzyme referred to as the iron regulatory protein-1 (IRP1).

The TCA cycle in the mitochondrial matrix is a central pathway of oxidative metabolism. This pathway is critical for the oxidation of acetyl-CoA and for the production of reducing equivalents that are used by the respiratory complexes to produce ATP. Aconitase (ACO2) is one of the eight enzymes that participate in the TCA cycle. It reversibly catalyzes the conversion of citrate to isocitrate. The cubane [4Fe-4S]²⁺ cluster in the active site of aconitase is essential for this catalytic activity, but it also makes the enzyme highly susceptible to oxidative stress (141). The cytosolic IRP1 in its [4Fe-4S]

cluster form functions as an aconitase. When the Fe-S cluster is absent, for example, as a result of iron depletion or the presence of ROS, IRP1 binds to mRNAs that contain a specific stem-loop sequence known as the iron-responsive element. When IRP1 binds to the 5' untranslated region (UTR) of ferritin mRNA, the translation of ferritin—an iron storage protein—is repressed. When IRP1 binds to the 3' UTR of transferrin receptor mRNA, which produces an iron uptake protein, the transcript is protected from degradation. Consequently, IRP1 binding to mRNAs reduces iron sequestration while increasing cellular iron uptake. A recent study shows that loss of IRP1 activity has serious physiological consequences, such as polycythemia and pulmonary hypertension in mice (100).

In progeny of bystander cells that were in coculture with cells pre-exposed to a mean dose of 200 cGy of 1000 MeV/nucleon ⁵⁶Fe ions, the ACO2 activity was reduced by 65% ($p < 0.0001$) (45). However, ACO2 protein level remained unchanged, suggesting oxidative damage to the [4Fe-4S]²⁺ cluster in the active site of the enzyme. Parallel experiments were conducted with the progeny of bystander cells that had been in coculture with cells pre-exposed to 10 or 200 cGy mean doses from protons. Interestingly, increased ACO2 activity was observed in the progeny of bystander cells that were cocultured with low dose-irradiated cells, and there was no change in the case of high doses. In both cases, ACO2 protein levels remained practically unaffected. These results further support the idea that oxidative effects can be propagated from HZE particle-irradiated cells to neighboring bystander cells. The situation was more complex for the distant progeny of bystander cells that were in coculture with cells exposed to densely ionizing radiations. In this case, aconitase activity was decreased likely due to persistent oxidative stress (44, 45). Such a decrease though might lead to reduced NADH supply for electron transport, thereby limiting free radical production (43) with concomitant attenuation of oxidative stress.

Measurement of aconitase activity may be used as another sensitive marker of oxidative stress in irradiated cells and tissues. Interestingly, it was suggested that the oxidized form of aconitase (ACO2) might be selectively degraded by the Lon protease in mitochondria (38, 267). However, the studies in progeny of bystander cells indicated that the ACO2 activity was decreased without detectable decrease in ACO2 protein level. Thus, expanding the studies of mitochondrial aconitase activity/protein levels and degradation of the aconitase protein by Lon protease may provide critical understanding of radiation-induced biochemical events in mitochondria. In addition, studies of IRP-1 would be informative of the effects of HZE particles on iron metabolism. Changes in IRP-1 levels/activity would affect oxygen transport, many enzymatic reactions and thereby radiation sensitivity (130).

Mitigation of HZE particle-induced oxidative stress

It is commonly accepted that the cellular effects of HZE particles on macromolecules are mainly due to direct rather than indirect effects involving water radiolysis products (121). Hence, protective effects of antioxidants against the initial damage are not expected. However, studies have indicated a protective effect of antioxidants when administered before radiation exposure. Whether the protective agents act against oxidizing species generated during

irradiation or thereafter is not clear. Notably, incubation of human cells with selenomethionine (SeM) 18 h before irradiation completely prevented HZE particle-induced increase in oxidative stress and attenuated cell killing, suggesting a countermeasure role of SeM against space radiation-induced adverse effects (150) (Fig. 10). Interestingly, it was suggested that SeM prevents adverse biological effects by enhancing the DNA repair machinery in irradiated cells (150), although this effect on DNA repair may be secondary to selenium-dependent effects on glutathione peroxidase and thior-

edoxin metabolism (258). In other studies, a diet containing vitamin A given to rats 1 week before accelerated ^{56}Fe ion-irradiation significantly inhibited the expression of inflammation-related genes and reduced induced neoplasms by almost half (46). Likewise, a diet containing antioxidant blueberry or strawberry extract [rich in polyphenolic compounds (144)] given to rats 8 weeks before exposure to 1.5 or 2 Gy doses of ^{56}Fe ions was found to enhance their ability to perform behavioral tasks (230). Similar beneficial effects of antioxidants have also been shown by *in vitro* studies of neuronal cells. Irradiation of hippocampal neural precursor cells with a mean absorbed dose of 1 Gy of energetic ^{56}Fe ions elicited two to fivefold higher levels of ROS compared to nonirradiated controls (163). Supplementing the growth media with the antioxidant lipoic acid (LA), before or after irradiation, reduced ROS levels below background levels (163). Antioxidants delivered before irradiation can therefore mitigate the adverse effects of heavy ions. The efficacy of LA in protecting against HZE particle-induced oxidative stress was confirmed *in vivo*: radiation-induced impairment in spatial memory retention was mitigated in middle-aged mice fed with a diet rich in LA 3 weeks before exposure to a mean absorbed dose of 3 Gy of ^{56}Fe ions targeted to the head and throughout the period of cognitive testing (275). Notably, postirradiation treatment of cultured cells with ascorbate (added 30 min after radiation) reduced the induction of CD59⁻ mutants in human-hamster hybrid A_L cells exposed to high-LET ^{12}C ions (279). Identifying novel therapeutic strategies against oxidative stress is currently under intense investigation. The antioxidant potential of hydrogen therapy (251), magnesium ions (244), polyamines (276), melatonin (172), and anti-inflammatory triterpenoid compounds (89) is being determined. The efficacy of these compounds will likely be apparent in long-term studies, as increasing data indicate that oxidative stress is persistent following HZE particle exposure and is associated with decreases in antioxidant defense (117).

Central to antioxidant defense are superoxide dismutases (SODs), glutathione, glutathione peroxidases, and catalase (51, 170). In progeny of bystander cells cocultured with energetic ^{56}Fe ion-irradiated cells, oxidative stress was associated with decreases in activities of manganese and copper-zinc SODs, and catalase and glutathione peroxidases (45). With relevance to radiation protection and radiotherapy, similar effects in progeny cells were not induced when bystander cells were cocultured with low-LET radiation exposed cells (*e.g.*, protons, γ rays) (45). Low doses of low-LET radiation (^{137}Cs γ rays) delivered at low-dose rate upregulated antioxidant defense as judged by increased glutathione levels together with the upregulation of γ -glutamylcysteine synthetase expression (39, 73, 152).

The use of antioxidants in the treatment of radiation injury and other diseases associated with oxidative stress (*e.g.*, Crohn's disease, rheumatoid arthritis, osteoarthritis) continues to be advocated (88, 222, 223). For example, SOD has been shown to protect other enzymes, mitochondria, membranes, microsomes, and DNA (222); its anti-inflammatory properties were discovered well before the protein was identified as an enzyme (184). Indeed, the protective effects of antioxidants support the role of ROS in mediating the stressful effects of HZE radiation (290). However, the dose, timing, and mode of delivery of antioxidants should be carefully evaluated. For example, the superoxide radical serves as both an initiator and

FIG. 10. The radioprotective effects of selenomethionine. (A) Effect of SeM on ROS generation in ^{56}Fe ion-irradiated MCF10 breast epithelial cells as measured by the dichlorofluorescein dye assay performed *in situ* using a fluorescence plate reader (briefly, the cells were incubated in 50 μM dye solution for 30 min before irradiation). The experiments were carried out using at least six replicates for each treatment group at each dose level and the results are presented as means $\pm$ SD. (B) Effect of SeM (5 μM) on cytotoxicity measured by a standard clonogenic assay in MCF10 cells irradiated with iron ions. Two separate experiments were performed, with four flasks per point in each experiment. Each data point represents the average (mean $\pm$ SD) surviving fraction values from the four flasks in one experiment. The results in (A) and (B) show significant radioprotective effects of SeM supplementation [adapted from Kennedy *et al.* (150) with permission]. SeM, selenomethionine.

a terminator of the free radical-mediated chain reaction resulting in lipid peroxidation (183). Thus, depending on dose and situation, SOD may alleviate or exacerbate the toxic effects of radiation injury. In addition, the effects of chronic exposure to HZE particles on nutrient absorption, including radioprotectors must be considered. Earlier studies indicated that nutrient absorption in mice was significantly reduced following the exposure to γ rays (217, 239). Decreases in intestinal sugar absorption were associated with a rapid decrease in SOD and catalase abundance, followed by dramatic upregulation within 4 h and then downregulation a few days later (238).

Gravitational Changes in Space and Oxidative Stress

Another prime concern to astronauts' health is reduced gravity (79). Microgravity leads to bone loss (153). Like ionizing radiation, it alters physiological functions (60, 265) and contributes to oxidative stress (153). Interestingly, analyses of urinary excretion before, during, and after long-duration space flight (4–9 months) on the Russian space station MIR and short-duration missions on the shuttle revealed opposite results in-flight and post-flight. Whereas decreased oxidative damage was observed in-flight, increased damage was observed post-flight (265). These results were linked to decreased production of oxygen radicals from the ETC due to the reduced energy intake in-flight. The enhanced post-flight excretion of oxidative damage products (*e.g.*, isoprostane, 8-iso-prostaglandin F_{2 α} , and 8-oxo-7,8-dihydro-2 deoxyguanosine) was attributed to a combination of increased metabolic activity and decreased antioxidant defense in-flight. In humans and rodents, oxidative stress manifested by increased lipid peroxidation products and decreased antioxidant enzyme activity (176, 177) was detectable for several days after space flight (264). Other studies have shown increased oxidation of body fat (264) and significant changes in gene expression (255) during flight. Notably, irradiation with heavy ions may accelerate or worsen the loss of skeletal integrity triggered by musculoskeletal disuse (5). It was suggested that oxidative stress is likely to synergize these effects (302). Recently, with the increase in the number of facilities where HZE particles are generated, there has been a great interest in studying the effects of simultaneous or sequential exposures to HZE particles and reduced gravity (291).

Increased lipid peroxidation and decreased blood antioxidant levels have also been reported to occur in human erythrocyte membranes under reduced gravity (175–177). Importantly, long-term space missions appear to increase triglyceride and high-density lipoprotein/low-density lipoprotein (HDL/LDL) cholesterol ratio (175). In humans, abnormal LDL and HDL plasma levels are major risk factors for developing cardiovascular diseases (8, 42, 188). Prominently, ionizing radiation has also been shown to have an impact on lipid peroxidation (2, 37, 118, 263). Therefore, reduced gravitational conditions combined with ionizing radiation and other space hazards may increase the risk of developing cardiovascular diseases, including atherosclerosis (288). Further studies to elucidate the molecular mechanisms underlying changes in lipoprotein metabolism in cells maintained under conditions that mimic the space environment will be highly significant for risk assessment (301).

Astronauts are also exposed to hypergravitational conditions during the launch of the spacecraft and upon reentry to

the earth environment (146, 306). Hypergravity was shown to increase malanodialdehyde (an indicator of lipid peroxidation) in rat kidney and heart tissues (306). In human glioblastoma cells, exposure to hypergravity modified signal transduction induced by ionizing radiation through the p53 pathway (213). Altered gravity also modulated prostaglandin H synthase and lipoxygenase pathway in human erythroleukemia cells (171). In rabbits subjected to hypergravity, enhanced resorption activity was observed in activated osteoclasts (205). Technological advances in the development of specialized centrifuges to study hypergravity effects are opening new avenues to study the biological response at the cellular and molecular levels (277).

Whereas the combined effects of radiation and gravity on biological changes are being examined in populations where all the cells are affected, the effects of combined stresses on the spread of oxidative effects to nontargeted cells has not been examined. Whereas the directly irradiated cells may not ultimately survive the induced oxidative stress, the communication of harmful effects to nontargeted cells and their enhancement by altered gravitational conditions may have an important role in late effects of space radiation.

Perspective

Overwhelming evidence indicates that oxidative stress contributes to elevated levels of DNA damage, abnormal growth, and disruption in metabolic pathways, which can lead to cancer and other diseases. Whereas our knowledge of redox-modulated processes in human cells exposed to low-LET radiations is fairly advanced, the *in vitro* and *in vivo* effects of space radiation on these processes and the underlying signaling events, particularly in the context of exposure to low fluences of energetic particles with varying LET, are still unclear (204). This is further complicated by the fact that astronauts are usually exposed to heterogeneous ionizing radiations. A prior exposure to a low dose of low-LET radiation may induce protective processes, thereby attenuating the damaging effects of a subsequent exposure to high-LET ionizing radiation at least in the short term. This is important because the low *flux* of high-LET HZE radiations in space relative to comparatively higher *flux* of low-LET protons makes it highly probable that for any given cell in the body, proton events will precede any HZE event. Characterizing these responses at partial oxygen tensions (Po₂) relevant to space activities will be crucial to developing more accurate biologic- and physics-based models for predicting health risks to astronauts.

Several factors contribute to oxidative stress during space expeditions. In addition to changes in gravitational conditions, ionizing radiation and oxidative metabolism, astronauts are also exposed to hypobaric hyperoxic conditions as they perform extra-vehicular activities (EVA; 100% oxygen for 8–10 h) (180, 181). The pre-EVA protocol for U.S. astronauts typically includes a 2.5 h prebreathe of 95–100% oxygen (181) to reduce the risk of decompression sickness. After the 2.5 h prebreathe, astronauts are exposed to hypobaric 100% oxygen for 8–10 h during EVA (180). Numerous studies indicate that hyperoxia induces physiological injury (22, 72). Therefore, studies of the effects that may result in an increased cellular Po₂ are essential to our understanding of redox-modulated processes. Decreased Po₂ in cells results in reduced cytotoxicity, mutagenicity, and DNA breaks (76, 96, 111, 134).

Lower than ambient Po_2 alters the growth rate of cells (26), induces expression of specific genes (108, 227, 254, 292), and modulates ion channel activities (167). Increased Po_2 is likely to result in greater oxidative damage. Specifically, the effects of Po_2 on propagation of HZE particle-induced nontargeted effects require more attention and needs to be carefully evaluated. By affecting radiation-induced lesions that signal expression of the bystander response, cellular Po_2 may have important consequences on the magnitude and nature of the propagated responses.

Currently, NASA's prediction of the health risks of space ionizing radiation relies predominantly on epidemiological studies of the A-bomb survivors and data from medically or occupationally exposed populations. These individuals were not exposed to protons or HZE particles that are prevalent in space. Accordingly, risk estimates for astronauts are extrapolated from loosely related epidemiological data by using radiation quality and dose-rate effectiveness factors. To refine these predictions, mechanistic studies in animal and cell culture models are essential for estimating the corresponding risks for humans (1). Through an integrated understanding of redox-modulated events in cell populations and tissues exposed to HZE particles and other space radiations, a rational path toward preventing the occurrence or delaying the progression of cancer and degenerative diseases during or after space missions may be developed. In addition, studies of targeted and nontargeted effects are relevant to hadron therapy. The propagation of cytotoxic effects among HZE particle-irradiated and bystander tumor cells have the potential to enhance the efficacy of radiotherapy and help achieve tumor eradication. Conversely, the propagation of oxidative stress from irradiated tumor cells to neighboring normal cells may contribute to the development of second cancers outside the treatment field.

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Abbreviations Used

4-HNE = 4-hydroxynonenal
 ETC = electron transport chain
 EVA = extra-vehicular activity
 H₂O₂ = hydrogen peroxide
 HDL = high-density lipoprotein
 HZE = high charge and high energy
 IRP1 = iron regulatory protein-1
 LA = lipoic acid
 LDL = low-density lipoprotein
 LET = linear energy transfer
 mtDNA = mitochondrial DNA
 NASA = National Aeronautics and Space Administration
 nDNA = nuclear DNA
 •NO = nitric oxide
 OER = oxygen enhancement ratio
 ONOO⁻ = peroxynitrite anion
 Po₂ = partial oxygen tension
 RNS = reactive nitrogen species
 ROS = reactive oxygen species
 SeM = selenomethionine
 SOD = superoxide dismutase
 UTR = untranslated region

Address correspondence to:

Dr. Edouard I. Azzam

Department of Radiology

Cancer Center

Rutgers University-New Jersey Medical School

205 South Orange Avenue

Room-F1212

Newark, NJ 07103

E-mail: edouard.azzam@rutgers.edu