ABSTRACT

The unique feature of the space radiation environment is the dominance of high-energy charged particles (HZE or high LET radiation) emitted by the Sun and galactic sources, or trapped in the Van Allen radiation belts. These charged particles present a significant hazard to space flight crews, and accelerator-based experiments are underway to quantify the health risks due to unavoidable radiation exposure. There are three principal properties of charged particles that distinguish them from conventional radiation, *i.e.* gamma rays and x-rays. First, they have a defined range in matter rather than an exponential absorption profile. Second, they undergo nuclear reactions to produce secondary particles. Third, and most important, they deposit their energy along well-defined linear paths or tracks rather than diffuse fields. The structured energy deposition pattern interacts on multiple scales with the biological structures of DNA, cells and tissues to produce correlated patterns of damage that evade repair systems. Traditional concepts of dose and its associated normalization parameter, RBE (relative biological effectiveness), break down under experimental scrutiny, and probabilistic models of risk based on the number of particle traversals per cell may be more appropriate. Unique patterns of DNA damage, gene expression, mobilization of repair proteins, activation of cytokines and remodeling of cellular microenvironment are observed following exposure to high LET radiation. At low levels of exposure the communication of bioactive substances from irradiated to unirradiated “bystander” cells can amplify the damage and cause a significant deviation from linearity in dose vs. response relations. Under some circumstances, there is even a multigenerational delay in the expression of radiation-induced genetic damage (genomic instability) which is not strictly dose dependent. These issues and the experimental evidence derived from ground based experiments at particle accelerators are presented along with speculation about how modified inertial conditions might perturb homeostatic responses to radiation to further complicate risk assessment for space flight.

Keywords: bystander effect, charged particle, chromatin, gene expression, radiation, track structure

INTRODUCTION

The biological responses to exposure by charged particle radiation are of critical concern to radiotherapy and to risk assessment. Protons and light ions have important roles in radiotherapy because they have defined ranges, can be controlled electromagnetically and may be more biologically effective at cell inactivation than x-rays. In the area of risk assessment, exposure to alpha particles from radon and occupational exposure to cosmic rays for astronauts and transatlantic commercial aircraft crews are the focus. In both situations, it is the response of normal cells and tissues that is most important. The level of exposure of normal tissues associated with a tumor site sets the limits for radiotherapy prescriptions and the process of conversion from normal to abnormal state(s) (e.g. malignant transformation) determines the level of risk.

FEATURES OF CHARGED PARTICLES

The charged particle environment in space is dominated by protons which are derived from the sun as emissions of solar wind, plus protons that are trapped by the Earth’s magnetic field into annular Van Allen radiation belts, and massive short-term emissions of particles in the form of solar flares or coronal mass ejections, collectively referred to as solar particle events or SPEs. Superimposed on the proton fields are cosmic rays, which are fully ionized atomic nuclei of all stable elements dominated by hydrogen, helium, oxygen and iron (McCormack *et al*., 1987). These high energy (*E*) high atomic number (*Z*) particles are often referred to as HZE (Schimmerling, 2003). Ground based facilities such as the Loma Linda University proton synchrotron and the Brookhaven National Laboratory’s NASA Space Radiation Laboratory (NSRL) can produce beams of charged particles of the appropriate composition and energy to simulate a significant portion of the space radiation environment (Miller, 2003). Microbeam facilities and specialized radioisotope sources complement accelerator facilities for biological studies.

The fundamental feature of charged particle radiation that distinguishes it from x-rays or gamma rays is that its deposition of energy is structured along a linear “track”, whereas high-energy electromagnetic radiation (photons) deposits energy in a diffuse pattern. Ionizing photons deposit their energy in matter by several processes that dislodge electrons to produce ions (photoelectric effect, Compton scattering and pair production). In each interaction, the photon energy is scattered from its original direction of motion resulting in a spreading of a narrow beam of photons. The associated energy loss as a function of depth is exponential and is characterized by a linear attenuation coefficient. By contrast, energy loss by charged particles is dominated by Coulombic interactions and is described by the Bethe-Bloch relationship (Kiefer, 1990). Figure 1 contrasts the attenuation of photons and charged particles by matter. The loss of energy per unit track length is proportional to the charge of the particle squared divided by its velocity squared (*z^2/v^2*). This results in an enhanced rate of energy loss at the end of a particle’s range (v —> 0) and a so-called “Bragg peak” form for the dose vs. depth distribution. The massive nature of the particles restricts their lateral scattering but the secondary electrons they generate (delta-rays) are...
ejected normal to the track and are absorbed as the inverse square of the radial distance. The overall appearance of a track seen in photographic emulsions is illustrated in Figure 2. Tracks are seen to take the appearance of a dense “bottle brush” pattern with a central “core” of silver grains surrounded by a diffuse “penumbra”.

A final important property of charged particles is that they undergo nuclear reactions to produce multiple secondary particles (most often protons, neutrons and alpha particles) that create their own tracks of molecular damage and may extend the range of damage beyond that of the primary particle.

The most common description of radiation exposure uses the concept of dose. Absorbed dose is the total amount of energy absorbed by the target material per unit mass (Todd, 2003). However, dose says nothing about the distribution of the energy with respect to the structural elements of the target mass. Whereas common doses of x-rays (e.g. 100 rad or 1 Gray) can be reasonably expected to produce a uniform pattern of ionization throughout a target (cell, tissue, animal), this is not true for charged particles. In the center of a track, the local dose may be thousands of Gray but a few microns away may be close to zero (Cucinotta et al., 2000). Thus dose is misleading and it is extremely important to understand the relationship of the energy deposition pattern in the context of the structure of the target. Figure 3 contrasts uniform vs. structured energy deposition.

**Interaction of Charged Particles with Chromatin**

The usual target for radiobiology investigations is DNA or chromatin. If one superimposes the pattern of ionizations in a single track with DNA in solution, or with chromatin, it can be seen that multiple ionizations may localize to short sequences of base pairs or to individual nucleosomes. The macroscopic dose associated with such an event at an average exposure of one track traversal per cell would be of the order of 0.1 Gy, whereas the specific energy deposited in a directly hit $5 \times 10^{-10}$ nm nucleosome would be 80,000 Gy (Cucinotta et al., 2000). Importantly, the physical events associated with the passage through a cell of a charged particle are produced within $10^{-15}$ seconds (Kiefer, 1990). A typical radiotherapy x-ray device produces dose rates of the order of 10 Gy per minute and would require hours to create the same local dose of interest. The free radicals created by the ionization process are highly reactive and complete most of their reactions within $10^{-9}$ seconds which allows them to diffuse only about 3 nanometers from their point of origin before reacting with each other or organic molecules (Ward, 1996). For charged particles, the spatial distribution of chemically reactive species reflects the original physical track. For the x-rays the short lifetime of free radicals mitigates against their being present in any given small volume at the same time. The ultimate result is that the time scale of enzymatic DNA repair is incompatible with the instantaneous damage produced by the track but is effective in repairing damage from the x-rays at relevant exposure levels. The yield of double strand breaks (dsb) and the proportion of dsb to single strand breaks (ssb) produced in DNA are correspondingly higher for charged particle radiation than for photons. Furthermore, new classes of damage termed multiply clustered lesions or multiply damaged sites are derived from the concentration of ionizations in small regions of chromatin. These sites will lead to deletions whose size vs. frequency distribution is expected to correlate with the dimensions of the tracks.
A unique aspect of charge particle-induced chromatin damage is that structurally related sites may be simultaneously damaged if they lie along a single track as shown in Figure 4. For example, if the orientation of a track is along the “beads-on-a-string” distribution of nucleosomes in a particular region of chromatin, then multiple inter-nucleosomal segments of DNA unprotected by histones may sustain dsb’s. Single and multiple-bead lengths of DNA may be deleted in the process and have been detected as a unique form of damage (Rydberg, 1996). Multiple sites of damage can be created in single nucleosomes or in short sequences of DNA. For higher order chromatin structure, the passage of a track through 30 nm solenoid fibers lead to characteristic fragment distributions consistent with a zig-zag packing of nucleosomes (Rydberg et al., 1998). At the level of interphase chromatin territories, passage of a track may lead to multiple breaks within a single chromosome and the production of multiple intra-chromosomal rearrangements. Such complex aberrations as well as “shattered” chromosomes are produced by charged particles at high frequency vis-à-vis x-rays. Because the arrangement of territories in interphase nuclei is not random, it is also predicted that certain classes of translocations associated with chromatin lying at the contact surfaces of adjacent territories will be produced at higher frequencies by charged particles. Stated another way the spectrum of mutations, deletions and aberrations is different for charged particle radiation than for photons.

Quantitation of Exposure

To overcome the inadequacies of dose as a sufficient descriptive variable for exposure the notions of fluence and LET have been explored. These ideas are focussed on the number of particles that traverse a sample and a measure of their intensity. Fluence, or more properly planar fluence, is the number of particles that pass through a unit area of planar surface usually expressed as particles/cm². The number of traversals of a target cell or cell nucleus is dependent on the fluence and the area of the target – also known as its geometric cross section. Because individual targets are either “hit” or “unhit” when placed in a field of moving point-like particles the distribution of hits per cell can be described by the Poisson distribution. In this formulation the probability of a cell being traversed by exactly n particles is \( p(n) = \frac{x^n e^{-x}}{n!} \) where \( x \) is the average number of particles per target area. For low doses or fluences, many cells will be unhit while some are traversed by multiple particles. For example, 1 Gray of 600 MeV/n silicon ions produces an average of 9.9 hits per 10 micron diameter cell, and very few cells are struck by less than 3 particles. By contrast, 0.1 Gy results in an average of 1 hit per cell but 37% of cells are unhit, 36% are traversed by one particle, and 26% are struck by 2 or more particles. A low fluence environment is expected for HZE particles in space, but Curtis (Curtis et al., 1998) has estimated that for a 3-year Mars mission, 7–13% of neurons in the central nervous system will be traversed by an iron ion. Similarly, 20 million of the 43 million cells in the hippocampus would be directly hit by ≥ 1 particle of Z ≥ 15.

As a measure of track intensity, the parameter of linear energy transfer or LET has been used (Blakely et al., 1984). LET is the energy transferred to the target per unit length of track and is usually expressed as kiloelectron volts per micron (keV/µm) and scales as \( z^2/v^2 \). A fast proton (\( Z = 1 \), 250 MeV/n) has an LET of about 0.4 keV/µm, while a radon decay alpha particle (\( Z = 2, 1.25 \) MeV/n) has an LET of about 90 keV/µm and a fast iron ion (\( Z = 26, 600 \) MeV/n) has an LET of about 200 keV/µm. Though useful, LET is not a uniquely defined function as different combinations of \( z \) and \( v \) can give the same LET. Dose, fluence and LET are interrelated by the following relation. Dose (cGy or rad) = \( 1.602 \times 10^{-7} \times \text{fluence} \times \text{LET} \) (keV/µm).

A complete description of track structure requires specification of the radial energy distribution and various track structure models have been advanced. In such models the ionization density outside a several nanometer dense track core is determined by the energy distribution of secondary electrons produced by the primary ion and their radial absorption. This leads to an exponential decrease in ionization density with distance from the track center (Goodhead, 1989). As Z increases the height of the ionization density increases while the radial extent scales with velocity (kinetic energy of the primary particle and thereby the energy distribution of secondary electrons). Slow, high charge particles (e.g. alpha particles or iron ions at the end of their range) produce compact intense tracks while fast, low charge particles produce more spread out patterns of ionization (e.g. high energy protons, helium or carbon ions).

One problem frequently encountered is how to compare the effectiveness of one type of radiation to another. It would be convenient to be able to state that radiation type B is N-fold more effective than type A at equal dose, and this is common practice (Cucinotta, 2003). The scalar quantity used is called the relative...
biological effectiveness, or RBE, and is the ratio of the dose of a standard (250 kVp X-rays or $^{60}$Co gamma rays) to the dose of a test radiation that produces the same biological effect (Blakely et al., 1984). In survival studies one might compare doses that produce equal survival levels such as an LD$_{50,30}$ or lethal dose to 50% of the population at 30 days and divide the LD$_{50,30}$ of gamma rays (e.g. 7 Gy for a strain of mice) by the LD$_{50,30}$ of neutron ions (e.g. 3.5 Gy) to determine an RBE of 2. Having established the RBE, one may now define the dose equivalent (H, in Sieverts) as the dose of the test radiation multiplied by the RBE: $H = D \times RBE$. This approach is useful as long as all conditions are the same and, most importantly, if all the qualitative features of the biological response are the same. Typically, RBEs are not constant with dose, even in a single experiment. The RBE at the 90% survival level is often greater than the RBE at the 50% survival level due to differences in repair at low dose vs. high dose. The value of RBE is often not the same among cell types, organisms or even dose rates but does give some guidance as to trends. National and international advisory panels have attempted to establish consensus RBE values that reflect the general properties of different radiation types and these quality factors (Q) are expressed in terms of fluence as 0.01 mutations per cell per 1.25 x $10^7$ particles/cm$^2$. If the presumptive geometric target (nuclear area for the cell type of interest) is a 4 micron diameter circle with an area of 12.6 $\mu$m$^2$, then the probability of mutagenesis per traversal is 0.8/12.6 = 0.064. Put another way, 12.6/0.8 = 15.75 is the average number of traversals required to produce 1 mutation per cell. This formalism can be used to sum over all particle types and energies to give a total probability for the risk (mutation in the example) for any arbitrary particle distribution and avoids the difficulties created by the RBE concept. It also provides the opportunity to compare different particles with each other by normalizing their effects to fluence. To gain insight into the effect of ionization density due to differences in track structure, a cross section vs. LET distribution is sometimes employed.

Unique Non-Genotoxic Effects of Charged Particle Exposure

Having illustrated the formalisms using chromatin damage as an example, it is appropriate now to examine other radiation responses to illustrate the unique actions of charged particles. It is generally agreed that double strand breaks, mutations and chromosome rearrangements are early events leading to malignant transformation. In cell models, in particular C3H10T$^{1/2}$ cells, transformation studies have illustrated a strong dependence on track structure and LET (Yang et al., 1985). In rodents, carcinogenesis studies using rat skin, mouse and rat mammary gland, mouse leukemia and mouse Harderian gland tumors all show striking effectiveness for charged particles in the initiation of cancer and a strong dependence on LET (Blakely, 2000). In many tumor cells there is a continuous elevated level of genomic rearrangement with time characterized by polyploidy and aneuploidy. This genomic instability has been examined in another context with charged particles. It has now been observed with hematopoietic stem cells, skin fibroblasts and many cultured cells that there is a non dose-dependent change induced in cells that results in the late expression of genetic damage which is quality dependent (Lorimore et al., 1998). This takes the form of independent polyclonal mutations and aberrations that arise after > 20 cell divisions (Kadhim et al., 1995) and may persist beyond 60 divisions (Morgan et al., 1996). This form of genomic instability appears to be highly elevated following charged particle exposures but is not...
frequent following photon irradiation. Defects in
telomeres, persistent elevation of oxidative status leading
to a late crisis (Morgan et al., 1996), or multiple dicentric
bridge-breakage and reannealing cycles are some of the
mechanisms under consideration for genomic instability.

It has been known for some time that radiation
exposure can lead to changes in gene expression pattern.
Fornace et al. (1999) have examined tumor cells, myeloid
cell lines and circulating lymphoid cells following gamma
ray exposure. They detected a large number of radiation-
induced genes using microarray analysis and found that
cell cycle control, DNA repair and signal transduction
genes were amongst the set whose transcription profiles
were altered. Woloschak (1997) found differential
expression as a function of radiation type (gamma rays vs.
neutrons) in a small set of genes studied in Syrian hamster
embryo cells. Most recently, Nelson et al. (in press)
conducted full genome microarray studies of the
nematode C. elegans and showed that 599 of 17,871
genes analyzed (3.4%) showed differential expression
3 hours after exposure to 3 Gy of gamma rays, protons or
high energy iron particles. 193 were up-regulated, 406
were down-regulated and 90% of these were affected only
by a single species of radiation. A novel statistical
clustering technique identified the regulatory relationships
between the radiation-modulated genes and showed that
genes affected by each radiation species were associated
with unique regulatory clusters. This suggests that
independent homeostatic mechanisms are activated in
response to radiation exposure as a function of track
structure or ionization density.

Barcellos-Hoff (1998) has shown differential gene
expression at the tissue level by examining the
composition and remodeling of extracellular matrix
(ECM) in murine mammary glands and liver. She found
using immunofluorescence techniques that collagens I,
III, & IV, laminin and other matrix components were
synthesized at different concentrations, different locations
and according to different time courses following
exposure to heavy ions than for gamma rays.
Furthermore, she found that latent transforming growth
factor β (TGF-β) in the ECM was directly converted to its
active form by the charged particles but not by gamma
rays (Barcellos-Hoff, 1998). TGF-β regulates a variety of
tissue functions including the redox status of cells. Cell
membrane lipids also show LET-dependent variations in
production of oxidation products which might in turn alter
the functions of any number of transport or signal
transduction complexes (Ziegler et al., 1998). These
observations show that cells may be uniquely
reprogrammed by high LET radiation and may remodel
their own microenvironment as a consequence.

Another important feature of charged particles is
revealed at low dose or fluence. Nagasawa and Little
(1992), Lorimore et al. (1998) and Mothersill and
Seymour (2001) have described the so-called “bystander
effect” which is illustrated in Figure 5. Bystander effect is
the observation that when an isolated individual cell in a
population is traversed by a particle, both the “hit” cell
and many of its “unhit” neighbors (bystanders) respond to
the radiation exposure. The response may take the form of

Figure 5. Damage created in a cell struck by a single charged particle may lead to the spread of signals or toxic products to many
neighboring cells via intercellular junctions, soluble molecules or remodeling of the extracellular matrix. This “bystander effect”
amplifies the risk from charged particles at low fluence or dose.
gene up-regulation (e.g. p21 and p53 genes), apoptosis, cell cycle arrest, sister chromatid exchange, mutation or genomic instability. The cells need not be in direct contact to exhibit the effect, and transfer of culture medium from an exposed culture to an unexposed set of cells can often transfer the damage-inducing molecule(s). Protection by reactive oxygen species-degrading enzymes such as superoxide dismutase or catalase blocks the bystander effect in some systems while proteases block the effect in others. In certain high-density cultures, connexin 43 type gap junctions mediate the process, which can be blocked by heptanol or by knockout mutations. The existence of the bystander effect complicates risk assessment for low fluences of charged particles by providing an amplification mechanism for damage. In fact, certain models now suggest that much of the risk from high LET radiation exposure at less than 0.1 Gy may be due to the bystander effect (Brenner and Elliston, 2001).

Speculation on Other Potential Interactions

We have illustrated some of the known effects of charged particle radiation on cells and tissues biased towards nuclear targets. Let us now speculate as to whether there are other cellular structures that might be important targets and whether these have the potential to mediate interactions between altered gravity fields and space radiation at the level of cells and tissues. Again, the critical feature of the instantaneous traversal of a cell by a charged particle is the linear track of high ionization density with dimensions of tens to hundreds of nanometers in diameter and many centimeters in length.

Organelles such as mitochondria, lysosomes or transport vesicles are obvious targets and could be damaged leading to cytoplasmic degradation, interruptions of respiration or even induction of apoptosis. Many smaller macromolecular complexes intersected by a track would also be subject to denaturation by the local ionization and action of free radicals. If cellular architecture aligned such complexes along a track, they might be subject to collective inactivation. Such complexes might include membrane rafts, caveolae, focal adhesions, ribonucleoproteins and individual cytoskeletal members. “Hit” lipid rafts might sustain lipid chain reactions that oxidize components, alter their fluidity and disrupt signal transduction complexes integrating extracellular receptors and intracellular kinase cascades (Mylonas and Kouretas, 1999). It is known that nitric oxide synthase and protein kinase C are restricted to such membrane domains in the form of caveolae and play roles in signaling (Janneny, 1998). Messenger RNA is transported along microtubules and microfilaments and deployed to cytoplasmic sites as ribonucleoprotein particles (RNP) where translation occurs at the site of gene product use. Dendrites and axons of neurons deploy numerous mRNA’s in this way (Carson et al., 1997). Denaturation of RNPs would directly lead to altered gene expression patterns and delay recovery due to transport issues.

Disruption of the cytoskeletal elements, including microfilaments, microtubules and intermediate filaments, could affect many transport functions and the large polyanionic surfaces of filaments and tubules that organize other macromolecular complexes. A track traversal colinear with a cytoskeletal element would have the potential to disrupt entire complexes organized along its length. Disruption of focal adhesions could disturb cell adhesion and overall shape from redistribution of stresses and strains via tensegrity mechanisms (Wang et al., 2001). Cell nucleus deformation would alter chromatin domain relationships and their responses to subsequent radiation exposures. Interactions of cytoskeleton through integrins with ECM define a bi-directional signaling pathway that may be track sensitive (Janneny, 1998). Most of these potential consequences have yet to be explored experimentally. The complex dynamic cellular structures whose geometries put them at risk for strong interactions with particle tracks could also mediate responses to altered acceleration levels and thereby integrate gravity and radiation effects on cells and tissues. Several attempts have been made to detect such interactions during space flight using irradiated nematodes, yeast and mammalian cells, but to date no convincing interaction has been demonstrated (Nelson, 1996; Nelson et al. 1999).

The tracks of HZE particles may be tens of centimeters in length and therefore traverse many cells. At the tissue and organ level, therefore, there may be disturbances reflecting functional capabilities that derive from linear, cylindrical or planar geometries that organize groups of cooperating cells such as in an intestinal villus. Tracks intersecting these cell arrays would have the capability of perturbing their joint function. This possibility was originally identified by D. Grahn as a “microlesion” and conceived of by Todd as a group of killed and damaged cells (Todd, 1992). A broader definition of a functional microlesion might now be considered in which coordinated disturbances mediated by remodeled ECM, bystander communication and cell death all contribute. For tissues such as liver or connective tissue that are rather isotropic, the effects should be modest. However, for epithelial sheets and tubes such as in hair follicles (Chase and Post, 1956), glands, or the highly layered and interconnected central nervous system, tracks might lead to substantial lesions. Figure 6 illustrates interactions of tracks at the tissue level.

Finally, at the systemic level, the cytokine environment modulated by the combined stress of space flight, extracellular matrix remodeling during bone and muscle loss altered by gravity, and acceleration-mediated gene expression patterns in the immune system and/or central nervous system may all contribute to a cellular environment in which reactions to radiation exposure are altered. This could tip the balance in an unfavorable way for an unlucky cell traversed by a heavy charged particle and lead to an enhanced probability of malignant transformation or loss of function.
SUMMARY
The structured energy deposition of charged particles leads to unique patterns of damage at the molecular, cellular and tissue levels that is dependent on the particle identity and energy. Cellular responses to such damage reflect slower and less complete repair capacities. The consequences are unique gene regulation reprogramming, activation of signal transduction cascades, effects on neighboring unirradiated cells, remodeling of the microenvironment and delayed expression of genetic damage. Cellular targets outside the nucleus may be susceptible to ion track damage in ways that could couple cellular reactions to radiation exposure and altered gravity environments. Future efforts at determination of health risks for astronauts will need to integrate these diverse and unique cellular responses to radiation exposure.

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ABBREVIATIONS AND DEFINITIONS
Dose Energy absorbed per unit mass. (Gray)
Fluence (planar) Particles per unit area. (cm⁻²)
Cross section Probability per unit fluence.
(usually expressed as µm²)
LET Linear energy transfer.
Energy deposited per track length.
(usually expressed as keV/µm)
RBE Relative biological effectiveness.
Ratio of doses of test radiation to x-rays that produce the same biological effect.
HZE High charge (Z) and energy (E) particles.
ECM Extracellular matrix.
LD₅₀ Lethal dose to 50% of population.
NSRL NASA Space Radiation Laboratory at Brookhaven National Laboratory.
SPE Solar particle event.
Q Quality factor. A legally defined RBE.

REFERENCES


