

New Measurements for Hadrontherapy and Space Radiation: Biology

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Abstract

The dual goals of optimizing clinical efficacy of hadrontherapy and determining radiation risk estimates for space research have intersected to a common focus for investigation of the biological effects of charged particles. This paper briefly highlights recent international progress at accelerator facilities engaged in both biological and clinical studies of the effects of particle beams, primarily protons, carbon and iron ions. Basic mechanisms of molecular, cellular and tissue responses continue under investigation for radiations with a range of ionization densities. Late normal tissue effects, including the risk of cancer in particular, are of importance for both research fields. International cooperation has enhanced the rate of progress as evidenced by recent publications. Specific areas of biomedical research related to the biological radiotoxicity of critical organs (especially the central nervous system), individual radiosensitivities to radiation carcinogenesis, and the analysis of effects in mixed radiation fields still require more research. Recommendations for addressing these issues are made.

KEYWORDS: Hadrontherapy, space radiation, biological effects, clinical data.

1. Introduction

Space exploration has captivated the world's attention. It also poses a unique set of risks for the pioneers who engage in space travel. Acute and late effects from radiation exposure are among the occupational hazards to be considered. Much is known about the effects of conventional radiations (such as photons) from human therapeutic exposures, and there is an emerging literature on biological and clinical effects from components of the cosmic radiation spectra that are used in hadrontherapy. The man-made radiations used in hadrontherapy (such as protons and carbon ions) are produced in accelerators that, in a few cases, are also capable of accelerating iron ion beams. Iron is a heavy-ion component of space radiation that has drawn attention due to its prevalence and potential for enhanced biological effectiveness. The dose and dose-rate range of primary interest, however, is different for radiotherapy and space radioprotection. This fact has challenged investigators to develop novel methods to adequately address the measurement of effects at the dose of interest. Excellent summaries of each research area are available [1, 2]. This paper will provide a broad-brush overview of recently published biological and clinical work from both disciplines. These two research areas are entwined in a yin-yang relationship that will continue to foster contributions important to each field.

2. Clinical contributions to understanding radiation response

Radiotherapy for cancer has enlightened our under-

standing of normal tissue tolerances to radiation in numerous ways, including the identification of the enhanced effectiveness of highly ionizing radiations (e.g. [3]), late effects on normal tissues [4], radiosensitive human syndromes [5, 6], and the high radiosensitivity of embryonic development [7]. The atomic bomb survivors continue to be the major source of information on radiation-induced cancer incidence [8, 9], noncancer mortality due to diseases of the circulatory, digestive and respiratory systems [10], and changes in total serum cholesterol [11]. Genetic aspects of biological risk of chronic multifactorial diseases (e.g. coronary heart disease, essential hypertension and diabetes mellitus) due to radiation exposure also have been reported [12]. Lipoprotein modifications that appear following radiation exposure may result from an induced inflammatory state and may further contribute to vascular damage [13, 14]. Epidemiological data on specific radiation accidents are also becoming available (e.g. [15, 16]) and may contribute to our understanding of the significance of the mode and rate of the radiation received. This is important since most occupational radiation exposures are at considerably lower doses and dose rates.

A new generation of accelerators available worldwide is providing hadrontherapy biological characterization and clinical results. Table I is a partial list of recent proton and carbon papers contributing to our understanding of beam-dependent variables that affect biological parameters used to clinically implement these radiations. In addition, clinical confirmation of the benefits of the tight three-dimensional conformal tumor treatment field in reducing normal tissue toxicities and improving clinical outcome of the particle radiotherapies are included.

Table I – New Hadrontherapy Characterization and Clinical Results.

Beam	Type	Description	Selected Citations
Protons	Clinical:	Pediatric optic pathway gliomas, compared to photon techniques. Cranial tumors	Fuss (1999) [50] Austin-Seymour (1990) [51]; McAllister (1997) [52] Slater (1998) [53]
		Prostate carcinoma Ocular or choroidal malignant melanoma	Habrand (1996) [54]; Chauvel (1996) [55]; Romani (1998) [56]; Miralbell (1997) [57] Krengli (1998) [58] Courdi (1999) [59]; Munzenrider (1999) [60]; Gragoudas (1993) [61] Paganetti (2000) [62]
	Radiobiology	Radiobiological significance of energy distribution in a spread-out Bragg peak RBE-LET relationships for cell inactivation and mutations Review of radiobiology and uncertainties Studies of physiology and morphology of cat lateral geniculate nuclei after exposure Mutation spectrum in human-hamster hybrid cell line Behavioral effects mediated by peripheral or central systems in rats 5-HT ₃ receptor antagonists ameliorate emesis in ferrets Lipid peroxidation in low density lipoproteins DNA double strand breaks and complex lesions Acute effects on immune systems in mouse	Belli (1997) [63]; (1998) [64] Yang (1999) [65] Reder (2000) [66] Kraemer (2000) [67] Rabin (1998) [22]; Joseph (1998) [24]; King (1999) [68] Ziegler (1998) [69] Ottolenghi (1997) [70] Kajioka (2000) [71]
Carbon	Clinical:	Phase I/II therapy results Phase I/II for uterine cervical cancer	Tsujii (1997) [72] Nakano (1999) [73]
		Radiobiology	Biological verification of heavy ion treatment planning Review of biophysical characteristics of HIMAC irradiation system DNA damage and repair in human embryo fibroblasts Cytogenetic damage in human lymphocytes Chromosome breakage and cell lethality in human hepatoma cells Mouse intestinal response to spread-out human hepatoma cells Clonogenic and cell cycle analysis in p53 mutated glioblastoma cell lines Induced WAF1 gene expression in human glioblastoma cells Induced vascular endothelial growth factor (VEGF) in lung carcinoma cells DNA damage and repair in human fibroblast and glioblastoma cells RBE comparisons in cells with different repair capacities Response of pig lung Review: tumor therapy and track structure Tumor therapy Physics & biology of treatment planning
Heavy ions	Clinical	Biological verification of heavy ion treatment capacities Computation of cell survival for heavy ion beam therapy	Mitaroff (1998) [74] Mitaroff (1998) [74]
		Radiobiology	Strand breaks determined by pulse field gel electrophoresis Lipid peroxidation in liposomes Radiation effects on biological membranes
Fe	Radiobiology	Frequency of incomplete exchanges in human lymphocytes	Wu (1999) [90]
Fe/He/ γ	Radiobiology	Tumor potential of high Z, high LET charged particle radiation	Alpen (1993) [36]
He/C/Ne	Radiobiology	LET, RBE and OER spectrum on cell lines	Furusawa (2000) [91]

3. Common concerns for radiation therapy and space research

Cancer therapy and space radiation health have several common concerns. Understanding the effects of mixed radiation fields, the significance of dose-rate, concern for acute effects on rapidly dividing tissues and on the peripheral or central nervous system, and late effects such a cancer-induction or

delayed tissue changes are particularly of interest. For the International Space Station (ISS) orbit of 51.6° and 210 n.m. altitude for solar minimum at 10 g/cm² shielding, calculated organ dose equivalents show approximately not quite equal doses from the GCR and from trapped protons, although there are organ-to-organ variances [17]. Estimated equivalents dose rates for space activities in low earth orbit (LEO) show progressively higher dose rates

from Space Shuttle, to Mir Space Station, to the orbit for the ISS [18]. The bone marrow equivalent dose rate on ISS is 16-20 cSv y^{-1} , and for skin it is estimated to be 18-25 cSv y^{-1} [19].

4. Biomarkers

The identification of biological markers of exposure to drugs, toxins or radiation, and inherent human sensitivity to these agents, as well as indicators of human disease states, has been a quest of many. An excellent recent review by Brooks [20] subdivides biomarkers depending on the application. The review demonstrates that markers of sensitivity and disease often have little usefulness in dose-reconstruction and also that many markers of dose or exposure may not be applicable for prediction of

sensitivity or risk. Nevertheless, the growing field of molecular epidemiology has contributed an array of endpoints and molecular and cellular mechanisms for responses to exposures.

Biomarkers of exposure or dose are relevant both to the need for radiation risk estimation during space travel, and to the identification of radiosensitive patients undergoing therapy. For particle exposures at low doses, the concept of dose loses its meaning. It may be more appropriate to consider the number of particle traversals per individual cell, or the fraction of a cell population traversed within a specific timeframe. Exposure variables such as linear energy transfer (LET), dose distribution and dose-rate have been usefully studied with specific biomarkers. Table II summarizes recent data on biological markers pertinent to particle radiation exposures. Most notable among the data listed are

Table II – Biomarkers and biodosimetry.

			Selected Citations
Review		Biological monitoring of radiation exposure	Horneck (1998) [92]
Radiation exposure	Cytogenetic endpoints	γ - irradiation	Thieren (1999) [93]; Heimer (1999) [94]; Wu (1999) [90]
LET range 31 – 1435keV/ μ m		Fe R-banded chromosome rearrangements in human lymphocytes	Testard (1997) [95]
LET range 0.3 – 140 keV/ μ m		Rejoining and misrejoining of chromatin breaks	Durante (1998) [96]
Radiation accidents		Stable chromosome aberrations in haemopoietic breaks	Kreja (1999) [97]; Tawn (2000) [98]
Space radiation		Prediction of dicentric frequencies in manned missions to Mars	Kreja (1999) [97]; Tawn (2000) [98]
North Atlantic route flight		Chromosomal alterations in female cabin missions to Mars	Wolf (1999) [101]
Background radiation		Chromosome translocation	Lucas (1999) [102]
Iron		Analysis of mutant quality and quantity in human-hamster hybrid cells	Wolf (1999) [101]
Alpha	Genetic marker	Hpr	Schwartz (1997) [104]
Long haul flights		Assessing exposure	Bottollier-Depois (2000) [105]
	Micronuclei		Boreham (2000) [106]
	Micronuclei	p53 genotype dependent MN frequency after Fe exposure	Chang (2000) [107]
Argon, Carbon and Uranium	Comet assay	DNA damage	Testard (2000) [108]
Radiation sensitivity or susceptibility		Apoptosis in normal, AT and breast cancer patient samples	Barber (2000) [109]
		Intrinsic radiosensitivity of healthy and cancer donors as determined by micronucleus assay	Slonina (1997) [110]
X	Human lymphocytes	Nucleic acid-based biodosimetry using	Blakely (in press) [111]
Argon	V79	Fluorogenic 5'-nuclease PCR assay	
		Chromosome aberrations in 1 st and 2 nd post irradiation metaphases	Ritter (2000) [112]
Space environment		Fluorescence in situ hybridization (FISH) on Mir-18 flight crew	Yang (1997) [113]
Alpha		PCC	Greinert (1999) [114]
	Human lymphocytes	Estimation of frequency of true incomplete exchanges	Wu (1999) [90]
	Light ions	Monte Carlo simulations of chromosome aberrations based on a mechanistic model	Wu (1999) [90]

measurements of biomarkers from human space travelers and parallel experiments from accelerator-based studies, clinical radiotherapy patients and ground-based radiation workers. Significant features of the data include discrete qualitative and quantitative differences in biomarkers from radiations of increased ionization density, as well as differences in the time course of appearance depending on the endpoint examined.

5. Particle-induced gene expression

Radiation-induced changes in gene expression are known to be dependent on the ionization density of the radiation since the pioneering work of Woloschak and Chang-Liu [21] and others. There are a number of interesting recent papers in this area, and they are summarized in Table III. Dose, radiation type and the time course examined remain key variables in the analysis of these data.

6. Current status of relevant knowledge of biological effects of space radiations: molecular, cellular and tissue effects

Overall, the message emerging from biological studies of the effects of particle radiations is that novel types of molecular and cellular damage and repair responses are produced by radiations with increased LET values compared to damage and repair produced by radiations with less ionization density. This also translates into novel tissue effects. It is difficult to review the contributions to this area concisely. Due to space limitations in this brief report, representative recent literature can be found in Table I.

Of particular importance for tissue and organ studies are the studies of particle effects on the neuromuscular and central nervous systems. The work of Rabin and Joseph [22-28] with low particle fluences of several particle beams have shown decrements on motor function and increased vulnerabilities to oxidative stress. In addition, they show dose- and LET-dependent conditioned taste aversion. Lett et al. [29, 30] have reported late degenerative changes in photoreceptor cells due to heavy-ion exposure.

7. Cell signalling to cells and extracellular matrix

DNA damage is a key focus of radiobiological studies. Non-DNA targets and signaling pathways are also important in the sequelae following particle radiation exposures, as epitomized by the pioneering work of Barcellos-Hoff [31] reporting particle-induced activation of the latent form of TGF- β and radiation-induced remodeling of extracellular matrix. Another example is the recent work of Zhao et al. ([32] showing changes in proteins of the extracellular matrix. My own laboratory has begun work in this area as evidenced by the paper by McNamara et al. [33] showing changes in cell adhesion molecules induced by proton irradiation. Cell communication by endocrine, autocrine and paracrine signaling will continue to be revealed as our molecular tools increasingly allow us to explore internal communication networks [34].

8. Radiation-induced cancer

The chief somatic effect of ionizing radiation of concern at low doses is the induction of cancer. Ionizing radiation at dose levels of interest for ground-based radiation protection is generally considered to be a weak carcinogen. There is, however, firm evidence of radiation-induced cancer risk in humans at moderate and high acute doses of ionizing radiation, but with a wide range of tissue- and organ-dependent sensitivity to radiation-induced cancer [35]. The molecular mechanisms underlying radiation-induced carcinogenesis are not well established. The low dose or low dose rate region of dose response curves are confounded by statistical fluctuations of normal cancer incidence, and by intriguing radiobiological observations of potentially relevant phenomena such as hormesis, adaptive response, gene induction, cytokine activation, bystander effect and genomic instability appearing in irradiated cohorts many generations after exposure. The tumorigenic potential of high-energy hadrons has been surveyed systematically with only one model system, the mouse hardierian [36, 37]. A careful analysis of the probability of traversal at the lowest doses of 600 MeV/nucleon iron ions demonstrated that for doses up to 0.4 Gy (25% tumor pre-

Table III – Radiation induced gene expression.

Radiation	Gene Expression	Selected Citations
Fission neutrons	Modulation of isotype-specific actin expression	Woloschak (1991) [116]
X-rays, Fission neutrons and Fe	Delayed hpS2 and prolonged CIP1/WAF1/SD11 expression	Balcer-Kubiczek (1999) [117]
X-rays and Carbon	Dose-dependent increase in VEGF gene and protein expression	Ando (2000) [118]
Protons and Helium	FGF-2 gene and protein expression	Chang (2000) [119]
X-rays, α -particles and Carbon	All 3 radiations induce p53 dependent WAF1 accumulation	Takahashi (2000) [81]

valence), practically no cells have been traversed by two or more tracks [37]. Storer and Fry [38] exposed male and female mice to single or multiple doses of fission neutrons and measured the subsequent incidence of fatal tumors. Comparison of the dose-effect results from single low (0.025 Gy to 0.2 Gy) doses, with the results of fractionated doses of 0.06 Gy to 0.48 Gy over a period of 24 weeks, showed no differences. They concluded that, for most tumors and for life shortening, the single dose-effect relationship for fission neutrons is close to linear at low doses. Induction of skin tumors by electrons or hadrons has been investigated in rats [39-41]. Although cancer induction by low LET radiation is subject to repair or recovery in the sense that multiple exposures produce fewer cancers than the same single dose, this recovery is not seen following exposure to high LET radiation. Miller et al. [42] report that the measured oncogenicity from exactly one alpha particle was significantly lower than for a Poisson-distributed mean of one alpha particle, implying that cells traversed by multiple alpha particles contribute most of the risk. They concluded that if this result was applied generally, extrapolation from high-level radon risks (involving cellular traversal by multiple alpha particles) may overestimate low-level radon risks involving only one single alpha particle. Work presented by Hall et al at this meeting [43] demonstrate that immortalized human epithelial cells irradiated with graded doses of ^{56}Fe ions show a step-wise malignant transformation within several months after irradiation and, finally, tumorigenicity in nude mice. At lower ^{56}Fe doses, no transformed cells were produced and no tumors were formed in nude mice. However, cells exposed to the lower doses of ^{56}Fe ion were found to be susceptible to tumorigenesis many months later by a subsequent challenge dose of low LET radiation that alone is ineffective in inducing tumors. The authors concluded that this indicates a heritable instability induced by heavy ions that persists for many generations. Up to now, there is no evidence that cancers develop after the exposure of humans to hadrons, and the validity of extrapolating to humans from animal models is not well established.

9. Radiation-induced cataract

A considerable number of studies of proton-induced cataract in animals has been investigated for proton beam energies from 50 MeV to 9 GeV and with a range of dose [44]. It is shown that the relative biological effectiveness (RBE) of 50 MeV and 645 MeV protons is the same as that of standard radiation, and that the RBE of charged particles accelerated to high and relativistic energies is higher. A dose rate dependence was demonstrated to be greatest for exposures delivered at 18 Gy/min, and it decreased at 1.8 Gy/min and was even lower at 0.18 Gy/min. Late ophthalmological complications after total body irradiation in non-human primates have been reported up to 25 years after single high doses

of protons [45]. Cataracts induced in uveal melanoma patients undergoing proton [46] or helium-ion therapy [47, 48] are dependent on the volume of lens in the treatment field. Earlier animal studies reported enhancement of cataractogenesis by exposure to ^{56}Fe ions and other heavy ions. The use of subjective and nonsubjective methodologies to evaluate lens radiation damage in exposed populations has been recently reviewed [49].

10. Some unknown factors in assessing heavy-ion damage

There are a number of unknown factors in assessing biological effects of hadrons. The consequences of persistent chromosomal rearrangements in human lymphocytes from individuals (or cells *in vitro*) that have been exposed to particle beams is unknown. The enhanced role of high LET radiations in inducing genomic instability is also a topic that requires further investigation. The significance of individual genetic susceptibility to risk assessment of both space workers and cancer patients must be evaluated. More work is needed on the late effects of low particle fluences, and rates on tissues, and studies of the consequences of the induction of remodeling of the basement membrane in the extracellular matrix of tissues. An assessment must also be made of the importance of the particle-radiation-induced enhanced sensitivity of neural behavior, and the combined effects of other multiple stressors (for example, radiation plus microgravity). There are still major gaps in our knowledge of the influence of age of particle-radiation exposure, gender, inherent susceptibility and the role of diet and other environmental factors. We need to know how to extrapolate high dose rate data to low dose rate, project estimates of risk over time, and transfer risk estimates from one population to another.

11. Summary

Radiation therapy for cancer using particle beams from accelerators has led to careful studies of the underlying molecular, cellular and tissue radiobiology leading to the radiation effects on the tumor target, as well as on surrounding normal tissues. These normal tissue data provide a base-line for extrapolation of the effects to the generally lower dose and dose-rates of components of the GCR spectrum expected for occupational exposures of crews in space flight. Additional space radiation health studies are in progress for heavier components of the GCR such as iron ions that are not used in hadrontherapy. Although much of the research for these two fields is published in a few key radiation journals, many relevant data are also scattered through an eclectic array of journals and institutional publications. This fact has hampered the realization that many common research themes link hadron therapy

with space radiation health. Several of these current research themes are briefly highlighted in this paper, and foretell a burgeoning future of new scientific contributions to both research areas.

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