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ADVANCES IN RADIATION BIOLOGY – HIGHLIGHTS FROM 16TH ICRR SPECIAL FEATURE: COMMENTARY

Can a comparison of clinical and deep space irradiation scenarios shed light on the radiation response of the brain?

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ABSTRACT

Not surprisingly, our knowledge of the impact of radiation on the brain has evolved considerably. Decades of work have struggled with identifying the critical cellular targets in the brain, the latency of functional change and understanding how irradiation alters the balance between excitatory and inhibitory circuits. Radiation-induced cell kill following clinical fractionation paradigms pointed to both stromal and parenchymal targets but also defined an exquisite sensitivity of neurogenic populations of newly born cells in the brain. It became more and more apparent too, that acute (days) events transpiring after exposure were poorly prognostic of the late (months-years) waves of radiation injury believed to underlie neurocognitive deficits. Much of these gaps in knowledge persisted as NASA became interested in how exposure to much different radiation types, doses and dose rates that characterize the space radiation environment might impair central nervous system functionality, with possibly negative implications for deep space travel. Now emerging evidence from researchers engaged in clinical, translational and environmental radiation sciences have begun to fill these gaps and have uncovered some surprising similarities in the response of the brain to seemingly disparate exposure scenarios. This article highlights many of the commonalities between the vastly different irradiation paradigms that distinguish clinical treatments from occupational exposures in deep space.

INTRODUCTION

Exposure of the central nervous system (CNS) to multiple radiation types comes from a variety of sources, including environmental (background), occupational (nuclear workers), and from medical procedures (diagnostic and therapeutic). The most significant data regarding the radioresponse of the CNS have been derived from the clinical experience, where therapeutic treatments using cranial irradiation have been used to forestall primary and secondary CNS malignancies.[1–5] Typical doses involve 60 Gy of photon-based (X-ray, gamma-ray) radiation modalities delivered in multiple small fractions aimed at minimizing normal tissue damage while eliciting certain levels of tumor growth delay if not control.[1] More recently, hadron-based (charged particles generated in cyclotrons) therapies have been used to control head and neck and CNS tumors, and most often involve protons[6,7] while outside the USA (largely in Germany and Japan), heavier ions (carbon) have been utilized.[8,9] Charged particles of specified energy and mass can be directed such that energy deposited can be localized to the tumor, where the density of ionizations (Bragg peak) can be superimposed over the tumor volume while minimizing damage to the collateral normal tissue bed.[10] Interestingly in space, a wider range of charged particles exist, that include protons and helium ions derived from the sun as well as minor contributions from heavier charged species ranging up to iron ions (atomic number $Z \leq 26$) that define the isotropic field of **G**alactic **C**osmic **R**ays (GCR).[11] **H**igh atomic number (**Z**) and **Energy** (HZE) particles are charged nuclei in the GCR that possess an electrical charge greater than +2. The multiple fluences and energies of these HZE particles traveling at near relativistic speeds highlight the complexities of the radiation fields in space, and provide a launching point for our discussions highlighting the nuances of the CNS radiation response to the vastly disparate radiation exposure scenarios encountered terrestrially and in deep space.

WHAT HAPPENS TO YOUR BRAIN FOLLOWING CLINICAL RADIOTHERAPY OF BRAIN TUMORS

Clinicians have known for decades that brain tumor survivors (adult and pediatric) suffer from progressive and debilitating cognitive impairments resulting from their cranial radiotherapy.[3–5,12] These unintended normal tissue toxicities limit the dose that can be safely administered to the tumor bed, and severely compromise quality of life. For the treatment of glioblastoma multiforme (GBM), the most aggressive primary brain tumor, radiotherapy protocols are used in combination with temozolomide (TMZ). The use of TMZ as a concurrent and adjuvant chemotherapeutic agent, has been shown to increase both overall and progression-free survival in patients.[2] Furthermore, GBM patients typically receive whole-brain fractionated x-irradiation to a total dose of 60 Gy delivered in 2 Gy fractions over 6 weeks.[1,13] Such clinical radiation exposures have clearly been shown to induce cognitive impairments,[4,14] and combined treatment using radiotherapy and TMZ have similarly been shown to elicit significant adverse neurocognitive side effects.[2,13] Despite acknowledgment of the cognitive problems, interpretation of the literature is hampered by numerous confounding factors (*e.g.,* differences in disease status, inter patient variation, treatment regimen, psychological reactions to diagnosis and treatment, baseline cognitive reserve and differences in test administration).[15–18] In addition, it is difficult to delineate the specific brain regions most sensitive to radiation and cytotoxic drug exposure and thus the mechanisms underlying effects on cognitive function. While it is beyond the scope of the present manuscript to review the clinical literature, several comprehensive reviews have elaborated on the radiation response of the CNS and the many potential causes and consequences of radiation-induced cognitive dysfunction.[3,19–23] Here, the focus will be on data derived from pre-clinical models, able to provide deeper mechanistic insight regarding the potential parallels between clinical and space radiation exposure of the CNS.

While the mechanisms underlying the unintended side effects of cranial radiation exposure remain to be completely elucidated, rodent studies using single dose and fractionated irradiation protocols, designed to approximate clinical treatments, have pointed to certain underlying mechanisms that characterize the CNS response to irradiation. Some insight has been provided by structural MRI studies, where white matter necrosis transpiring at protracted times after the cessation of treatments appears more dependent on total dose rather than a particular fractionation schedule.[24] Neuroinflammation has also been shown to be a persistent problem, and in many instances can be linked to microglial activation.[25–31] Adverse effects can result from a pro-inflammatory environment, where disruptions in synaptic transmission and secretion of growth factors may result.[32,33] Astrocytes also play a role in neuropathological conditions, including neurodegeneration and neuroinflammation.[34,35] In the context of brain injury, persistent modifications that characterize reactive astrogliosis have been described over acute and protracted post-irradiation time frames.[25,36]

Following cranial exposures (≤10 Gy) rodents exhibit a number neurocognitive decrements spanning multiple regions of the brain that persist (perhaps indefinitely) and over protracted post-irradiation times.[37–40] Hippocampal and cortical-based deficits in learning and memory, along with the emergence of mood disorders are temporally coincident with changes in the structural plasticity of neurons and inflammation as documented after cosmic radiation exposures.[39–41] Clinical irradiation paradigms elicit marked reductions in neurogenesis, dendritic complexity, spine density, and elevations in neuroinflammation that are hallmarks of the CNS radiation response.[29,42–46] Data derived from both space and clinical irradiation scenarios suggest that compromised neurocognitive functionality goes in step with reductions in the structural complexity of neurons and increased inflammation in the brain. Faced with this reality, much of the work from my laboratory has focused on interventions able to ameliorate radiation-induced cognitive dysfunction, and identifying common mechanistic themes for resolving radiation-induced pathology in the brain.

TREATMENTS FOR RADIATION-INDUCED COGNITIVE DYSFUNCTION

Despite the growing acknowledgment that cognitive outcome is a major criterion for assessing therapeutic outcome, cognitive dysfunction following cancer treatments remains an unmet medical need. To address this unresolved normal tissue complication, our lab was the first to pioneer stem cell and stem-cell derived **E**xtracellular **V**esicle (EV)-based strategies for the potential resolution of such normal tissue toxicities.[38] Using several models of radiation-induced cognitive dysfunction, we have shown that intrahippocampal transplantation of several human stem cell sources were capable of ameliorating cognitive deficits following clinical irradiation paradigms.[47–51] Transplanted stem cells were shown to engraft, adopt neural cell fates and functionally integrate into hippocampal circuitry.[47] Longer term effects pointed to trophic support mechanisms. In the relative absence of engrafted cells, expression of the **A**ctivity **R**egulated **C**ytoskeleton-associated protein (ARC) known to facilitate synaptic transmission, was elevated in irradiated brains previously transplanted with **H**uman Neural **S**tem Cells (hNSC).[51] Importantly, neurocognitive benefits associated with stem cell grafting included a preservation of host neuronal morphology and an attenuation of neuroinflammation.

Success of these preclinical studies prompted efforts to circumvent certain limitations associated with cellular transplantation strategies, namely immune rejection and teratoma formation. To this end, we evaluated the therapeutic benefits of hippocampally grafted EV derived from human stem cell sources following cranial irradiation. In a proof of principal study, we found that hNSC-derived EV afforded similar neuroprotective properties as grafted stem cells, where neurocognitive benefits of EV were again associated with significant protection of host neuronal morphology and a reduction of neuroinflammation.[52] More recent work has now extended these findings by demonstrating that systemic administration of hNSC-derived EV can resolve radiation-induced cognitive dysfunction and inflammation in wild-type mice through an miR-124-based mechanism.[53] The similar protective benefits found after either stem cell or EV grafting suggested that strategies able to preserve

neuronal structure, myelination, and limit inflammation in the brain would have a positive impact on multiple facets of cognition following CNS exposure to ionizing radiation.[54–57] Indeed, related data from us and others have found that in general, interventions able to curtail oxidative and/or inflammatory signaling in the brainprovide an overall beneficial outcome for the CNS following radiation exposure.[31,44,58–60]

A NEW TWIST FOR REDUCING NORMAL TISSUE TOXICITIES IN THE IRRADIATED BRAIN: THE "FLASH EFFECT"

Data highlighted to this point indicate that radiation-induced toxicities in the brain involving changes to neuronal structure and inflammation seem to be contributory if not causal to resultant cognitive dysfunction. It therefore stands to reason that potentially any strategy able to preserve neuronal structure, myelination state and minimize inflammation in the irradiated brain may have long-lasting neurocognitive benefits. In this regard, FLASH radiotherapy (FLASH-RT) does precisely that, and represents a burgeoning radiation modality that implements ultra-high mean dose rates in excess of 100 Gy/s. The idea that dose rate could be an adjustable parameter for therapeutic gain has caught the field of radiation oncology by surprise, and its capability to spare normal tissue toxicities without compromising tumor treatments points to one of the more exciting developments in radiation biology in years.[61] Recent reports from our group highlight the tremendous potential of this innovative radiation modality. When compared to conventional dose rate irradiation, FLASH-RT eliminated short- and long-lasting cognitive deficits (1–6 months post-exposure), minimized astrogliosis and the activation of microglia and preserved the structural integrity of mature neurons in the brain.[62,63] Implications for radiation oncology are significant and suggest that if properly implemented, dose escalation to the tumor bed can be safely achieved while maintaining and/or reducing normal tissue toxicities to acceptable levels.[64] While the benefits of FLASH-RT are only starting to be realized, such advantages are certainly not limited to the CNS, as normal tissue sparing has now been demonstrated in multiple tissues using several preclinical animal models[65–69] including the first human subject.[70]

SPACE RADIATION

Despite the fact that clinical treatments involve much different types (low LET) and higher doses of ionizing radiation (~60 Gy) used to control CNS malignancies,[1,2] it behooves an examination of NASA-related research investigating space radiation effects on CNS functionality. As highlighted below, research emerging over the last decade has revealed some surprising parallels regarding the response of the CNS to these disparate irradiation scenarios.

The Earth is protected by the magnetosphere, a shield that deflects charged particles and prevents them from reaching the surface of our planet. This protection is sacrificed during deep space travel where the highly energetic charged particles of the GCR can penetrate the hull of the spacecraft and bodies of the astronauts. HZE particles are characterized in part by their mass and **L**inear **E**nergy **T**ransfer (LET, expressed as keV/μm) a term used to distinguish the microdosimetric properties of specific charged species.[11] HZE particles of higher LET have higher ionizing capacity, since they produce an increased density of ionized species per unit volume at a given dose. Thus, they produce more complex "clustered" cellular damage that challenges the repair and regenerative reserve of cells traversed by these damaging charged particles.

The realization that deep space radiation exposures are far below those used therapeutically or diagnostically in medical practice is also important, where total doses from all radiation types are accrued at dose rates of ~1 mGy/day or one-thousandth of a Gray.[11] While dose rates are low, biological damage will depend on the nature and extent of specific ionizations incurred from all particle traversals, and the sum of these events over a projected round-trip transit to Mars is not expected to exceed a combined dose 0.5 Gy (depending of course on mission duration).[71] Given this brief backdrop, we will now explore how terrestrial simulations of the space radiation environment have contributed to our knowledge regarding the space radiation response of the brain.

WHAT HAPPENS TO YOUR BRAIN WHEN YOU TRAVEL TO MARS: THE CNS RESPONSE TO SPACE RADIATION

Our understanding of the CNS response to charged particle exposures that typify the space radiation environment have made tremendous strides over this last decade. This progress has come in large part, through the efforts of NASA-funded investigators carrying our ground-based simulations of the radiation fields in space at the **N**ASA

Space **R**adiation **L**aboratory (NSRL) at **B**rookhaven **N**ational **L**aboratory (BNL). Here, investigators expose a variety of biological samples to selected beams and scrutinize the consequences of such exposures at various times post-irradiation. For the CNS, rodent models have proven invaluable, as the need to assess neurobiological outcomes in an intact brain are an absolute necessity. In this light, mice and rats exposed to low doses (≤0.5 Gy) of single beams or combinations of multiple beams including protons, helium ions, and various HZE particles have revealed marked neurocognitive decrements that persist for months to years after exposure.[72–74] Over the past few years, NASA and the team at the NSRL have developed a 33-beam complex GCR simulation that can sequentially deliver a complex spectrum of space radiations (**Table**). Investigators having access to this advanced GCR simulation have been able to expose their samples to pre-selected total doses (≤50 cGy) over a chronic 6 day/week, 4-week interval (2.08 cGy/day), and compare data to that obtained when the same total dose and sequential ion combination is delivered in a single day (*i.e.,* 2 h). Results derived from these ongoing experiments will undoubtedly shed further light on the multifaceted consequences of space radiation exposure on CNS functionality.

Armed with a battery of behavioral tasks able to interrogate multiple cognitive domains, research has clearly identified space radiation-induced impairments in learning and memory that suggest astronauts engaged in deep space travel are at an elevated risk for manifesting mission critical performance

Table 1. Definition of the Full GCR Simulation (In order of delivery)

Ion	Energy (MeV)
H	1000
He	1000
Si	600
H	20
H	23
He	20
He	23
Ti	1000
He	27
He	32
H	27
H	32
H	37
H	43
He	37
He	43
O	350
He	50
He	59
H	50
H	59
H	69
H	80
He	69
He	80
C	1000
He	100
H	100
H	150
He	150
Fe	600
He	250
H	250

decrements.[75–78] Past work with rodents has shown that whole-body exposure to charged particles can disrupt behavioral performance that can be linked to impairments in the hippocampus,[76,79–81] amygdala,[73] basal forebrain,[75] mPFC,[72,73,75,82] and other regions.[74,83,84] Furthermore, individual animals subjected to charged particles often exhibit deficits in multiple behavioral paradigms, and many of these decrements transpire over 6–52 weeks after exposure and are temporally coincident with a marked structural plasticity of neurons and glia and elevated neuroinflammation.[72–74] This realization has triggered a surge of research designed to evaluate further the potential CNS health risks associated with such exposures. While rodent-based studies have clearly uncovered many intriguing mechanisms impacting space radiation-induced cognitive dysfunction, disruptions to the integrity of mature neuronal structure and chronic neuroinflammation represent two salient features shared in common with clinical exposure paradigms.

Studies have shown that space relevant doses of various charged particles elicit a robust and persistent deterioration of dendritic structure.[72,73,80,85] These changes have now been documented in various types of neurons throughout the brain and include reductions in dendritic arborization and overall dendritic complexity that point to the capability of charged particles to significantly compromise neuronal morphology over extended times. Coincident with these more macroscopic alterations to neuronal structure are more microscopic changes to the dendritic spines.[72,73] Dendritic spines are the structural correlates of learning and memory and contain the synaptic machinery that mediates neurotransmission. Space radiation exposure elicits significant reductions in dendritic spine density, essentially stripping these structures off the dendritic shaft. Individual animals showing the greatest reductions in dendritic spine density routinely exhibit the poorest performance on select behavioral tasks, suggesting a defined structure function relationship between neuronal structure and cognition.[73] At higher resolution, electron micrographs reveal that similar charged particle exposures lead to significant reductions in axonal myelination,[86] implicating the capability of space radiation exposure to compromise conduction velocity and neurotransmission at multiple levels.

Superimposed on the structural alterations detailed above, are indications of a persistent inflammation in the brain that serves to perpetuate the signature of radiation injury over extended post-irradiation times.[73,74] Multiple reports have now shown that space relevant exposures elicit a persistent neuroinflammation involving significant increases in activated microglia, the innate immune cells of the brain.[73,74,87–89] Chronic increases in activated microglia can trigger inflammatory cascades in the irradiated brain that can elicit signaling changes to disrupt the balance between excitatory and inhibitory neurotransmission. Microglia also play active roles in re-shaping the synaptic landscape, where they can prune dendritic arbors and spines to remodel the connectivity of the irradiated brain.[90–92] Studies showing that microglial elimination can restore neurocognitive function after space and clinical radiation exposures support this idea,[59,93] and point to importance of neuroinflammation in dictating the long-term radiation response of the brain.

Interestingly, our group has recently completed studies undertaken to simulate chronic, space relevant low dose (≤0.20 Gy) and low dose rate (1 mGy/day) exposures using neutrons and photons derived from a ^{252}Cf source.[94] Results have shown that rodents develop significant cognitive decrements and reductions in **L**ong-**T**erm **P**otentiation (LTP), a form of synaptic plasticity that facilitates learning and memory.[94] These new findings point to the marked susceptibility of the CNS, where critical functional outcomes show surprising sensitivity to low dose and low dose

rate radiation exposures. While these data sets portend several potential problems associated with radiation exposure in space, this is not an insurmountable problem or a deal breaker for space travel.[95,96] Advanced shielding and biological interventions designed to combat the adverse effects of space radiation exposure on the brain are areas of active investigation.

SUMMARY AND PERSPECTIVES

The response of the CNS to either clinical or cosmic irradiation scenarios involves a complex and multifaceted series of events that never completely resolve and invariably compromise the functional connectivity of neural circuits and synaptic transmission. While much is now known regarding the radioresponse of the CNS, much remains to be resolved, including the identity of critical cellular and subcellular targets that exhibit differential radiosensitivities. Elucidating meaningful parallels between clinical and space radiation-induced CNS responses is confounded further by the paucity of human data regarding exposure to combined spaceflight stressors. A longitudinal MRI study of astronauts pre- and post-flight uncovered volume changes in various regions of the brain[97] and the highly publicized NASA twin study found that compared to his terrestrial bound brother, spaceflight compromised certain indices of cognitive performance.[98] These recent studies point to spaceflight-induced changes to the brain, but in each instance, it remains difficult to attribute such changes to the effects of radiation exposure alone.

Our capability to intervene on the adverse cascade of signaling events triggered by irradiation will ultimately depend on a more thorough understanding of how ionizing radiation damages and disrupts the delicate balance between excitatory and inhibitory neurotransmission. Protecting the brain against the structural degradation of neurons and the ensuing cascades of oxidative and inflammatory processes triggered by irradiation seem critical to preserve CNS functionality. In support of this rationale, three distinct interventions were highlighted that have been shown to be neuroprotective in the irradiated rodent brain, namely, 1) cranial grafting of human stem cells, 2) cranial grafting and systemic injection of human stem cell-derived EV, and 3) FLASH irradiation. Noteworthy here was that for each of these treatments, neurocognitive sparing was temporally coincident with reductions in neuroinflammation and a preservation of mature neuronal morphology. Thus, it stands to reason, that potential treatments targeting the adverse neurocognitive effects of cranial radiotherapy in humans should, in part, consider agents capable of impacting pathways known to minimize inflammation and protect neuronal structure. Similar logic would apply to the protection of astronauts exposed to the deep space radiation environment. For long-term human health, the brain is likely the last frontier, and protection of our neural circuitry must be a priority for those trying to resolve the neurocognitive side effects resulting from brain tumor radiotherapy and for those destined to engage in deep space travel.

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