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Review

Neurobiological toxicity of radiation in hippocampal cells

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Summary. Worldwide radiation exposure is increasing due to recent nuclear accidents, space travel, atomic weapons testing and use, and medical treatments. In adult animals, ionizing radiation can significantly impact hippocampal neurogenesis and negatively affect hippocampal functions such as cognition. However, there is considerable uncertainty regarding the mechanisms underlying these effects. This article reviews *in vivo* and *in vitro* studies on the effects of irradiation on hippocampal neurogenesis and function in order to gain new mechanistic insights. This information will provide complementary views of our understanding of the normal brain's tolerance to radiation exposure, the potentially serious implications of radiation exposure to cognition, and lead to a discussion of potential strategies for pharmacotherapy and behavioral intervention.

Key words: Cognition, Radiation, Neurogenesis, Hippocampal function, Pharmacotherapy

Introduction

The hippocampus is located within the medial temporal lobe and plays an important role in memory and spatial information processing (Squire, 1993; Zola-Morgan and Squire, 1993). Hippocampal subfields include the dentate gyrus (DG) and *cornu ammonis* (CA) regions CA1 and CA3. The DG, in particular, is a remarkably dynamic structure and a major site of hippocampal neurogenesis in adult mammals (Suzuki and Amaral, 1994). Thousands of new neurons are created every week in the DG (Kempermann et al., 1997), enough to replace the entire granular layer over the course of a lifetime (Monje and Palmer, 2003). Moreover, the basal rate of hippocampal neurogenesis in different mouse strains correlates with performance on hippocampal-dependent behavioral tasks (Kempermann, 2002; Kim et al., 2008a, 2009). Convincing evidence in animal models supports the importance of hippocampal neurogenesis for normal cognitive function (Shors et al., 2001), and various manipulations that inhibit hippocampal neurogenesis impair performance in cognitive and behavioral tasks (Lemarie et al., 2000; Tada et al., 2000; Yang et al., 2010a, 2011a). Therefore, altering hippocampal neurogenesis would be expected to affect hippocampal functions, including cognitive performance.

Inhibition of hippocampal neurogenesis and cognitive impairment have a number of damaging effects on brain tissue both *in vivo* and *in vitro*. These effects can be divided into two main categories: (1) effects on metabolic pathways in vulnerable post-mitotic neurons and glia; and (2) effects on proliferating neural stem/progenitor cells. These pathways are also vulnerable to other cellular stressors such as free radicals (Gobbel et al., 1998; Monje et al., 2002). In principle, all pathways that control either cell division or apoptosis could be affected by age, hormonal status, excitatory input, growth factors, chemical and physiological stimuli, environmental enrichment and irradiation. A number of these pathways have been characterized (Kuhn et al., 1996; Kempermann et al., 1997; Parent et al., 1997; Scott et al., 1998; Young et al., 1999; Lemaire et al., 2000; Tada et al., 2000; Kronenberg et al., 2003; Wojtowicz, 2006; Kim et al., 2008b; Yang et al., 2010b, 2011a). However, additional studies in animal models and in the normal, intact human brain are needed to understand the role these factors play in hippocampal neurogenesis.

Humans are increasingly exposed to radiation from various sources. Nuclear accidents, such as radiation leakage from the Fukushima nuclear power plant in

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Japan in 2011, space travel, atomic weapons testing and use, and medical treatments including cancer therapy have contributed to a rising interest in the effects of human radiation exposure. Ionizing radiation affects multiple organs, which differ in their apparent response. Nevertheless, the adult brain is less vulnerable to radiation than other radiosensitive organs (Task Group on Radiation Quality Effects in Radiological Protection, Committee 1 on Radiation Effects, 2003; Harrison and Streffer, 2007). As a result, there has been less interest in studying the detrimental effects of irradiation on the brain. However, many patients develop progressive deficits in short-term memory, spatial relations, visual motor processing, quantitative skills, and attention months to years after radiation exposure. *In vivo* data demonstrate that the slope of the radiation dose-response curve in the hippocampus is much steeper than that in other radiosensitive organs, such as the intestinal crypt (Kim et al., 2012). Therefore, the hippocampus, particularly the neural progenitor cells in the subgranular zone (SGZ) of the DG, might be radiosensitive to relatively low-dose exposure. Many patients who receive yearly partial large-field or whole-brain irradiation for cancer treatment are surviving longer (Stone et al., 2004). Thus, radiation-induced side effects, including cognitive impairment, will become a major health problem (Coleman et al., 2004; Meyers and Brown, 2006). Although the most commonly reported deleterious effects of irradiation occur directly via DNA damage and subsequent disruption of protein synthesis (Belka et al., 2001), there are also specific effects on biochemical pathways that indirectly affect transcription (Wojtowicz, 2006).

In mammalian brains, severe structural and functional injury can occur after high radiation doses >60 grays (Gy), fractionated (Tofilon and Fike, 2000), but lower doses may produce cognitive impairment, even without any significant morphological alterations (Roman and Sperduto, 1995; Kim et al., 2008b). Table 1 summarizes studies from rodent models, where irradiation from various sources, with altered frequency and dose, impairs performance in one or more cognitive tests (Yoneoka et al., 1999; Martin et al., 2001; Madsen et al., 2003; Raber et al., 2004; Rola et al., 2004; Shi et al., 2006; Winocur et al., 2006; Clark et al., 2008; Kim et al., 2008b; Manda et al., 2008a,b; Achanta et al., 2009; Caceres et al., 2010; Conner et al., 2010; Yang et al., 2012). These results, however, are highly dependent on the protocol applied and the learning task. Furthermore, the precise pathogenesis of the impairment is poorly understood, although involvement of neural precursor cells in the DG has been suggested in some cases (Andres-Mach et al., 2008; Monje and Palmer, 2003; Fike et al., 2007; Kim et al., 2008b). These important issues are being addressed using both *in vitro* and *in vivo* approaches with some of the most pertinent ideas, as well as some new concepts, summarized below.

This review will give an overview of *in vitro* and *in vivo* studies that have explored the effects of irradiation on neurogenesis and cognition, along with those studies examining the differential effects of radiation quality on hippocampal neurogenesis, cytotoxicity and cell viability. This information will provide complementary views of our understanding of the normal brain's tolerance to radiation exposure and lead to a discussion of potential strategies for pharmacotherapy and behavioral intervention.

In vivo approaches to study of neurogenesis and cognitive impairment after irradiation

Several studies have suggested that radiation-induced cognitive deficits are associated with inhibition of hippocampal neurogenesis in adult animals (Madsen et al., 2003; Raber et al., 2004; Snyder et al., 2005; Kim et al., 2008b; Yang et al., 2012). Further, Mizumatsu et al., (2003) suggested that microglia activation may be a critical factor during long-term inhibition of neurogenesis. These studies showed that neural stem cells in the SGZ of the DG are vulnerable to irradiation exposure in a time- and dose-dependent manner and precisely correlated this with hippocampal neurogenesis and cognitive impairment.

There is no consensus regarding the irradiation dose needed to kill proliferating progenitor cells in an adult brain without inducing serious short-term side effects (Rola et al., 2004; Snyder et al., 2005). Although irradiation causes variable amounts of morphological changes in brain structure in pre-/postnatal animals, fewer structural changes are associated with irradiation in adult brains (Sheline et al., 1980; Rola et al., 2004; Snyder et al., 2005; Kim et al., 2008b). A single 10 Gy dose induces apoptosis of the proliferating stem cells in the DG of adult rats, while many remaining cells in the hippocampus are unaffected (Peissner et al., 1999). Similarly, a single 5 Gy dose blocks neurogenesis in only the adult rat hippocampus (Parent et al., 1997) and a 10 Gy X-ray exposure to the mouse brain results in a ~90% loss of proliferating cells in the DG 3-4 months after irradiation (Raber et al., 2004). A relatively low dose of γ-irradiation (0.5-4 Gy) also alters the rate of neurogenesis in adult mice in a time- and dose-dependent manner without changes in hippocampal structure (Kim et al., 2008b). Overall, an irradiation range of 0.5-10 Gy of X- and γ-rays in these studies was sufficiently detrimental to inhibit neurogenesis in the hippocampi of experimental adult animals without causing changes in hippocampal structure. Further hippocampal function studies can now be extended with this information.

The mechanisms underlying radiation-induced cognitive impairment have remained elusive, but important possibilities include alterations in the neurogenic cell populations in the DG (Kim et al., 2008b), loss of neuronal maturity in the DG (Raber et al., 2004), alterations in N-methyl D-aspartate subunits (Shi et al., 2006), and genetic risk factors (Villasana et al., 2006). Factors involved in reversing the effects of

irradiation have also been studied. For example, it has been proposed that microglial activation causes persistent reduction in Arc gene expression (Monje and Palmer, 2003) and blocks the inflammatory reaction after irradiation (Monje et al., 2003). The recovery of neurogenesis in the absence of morphological glial reactions affects hippocampal-dependent learning and memory (Kim et al., 2008b). Therefore, reduced hippocampal neurogenesis correlates in time with the deficit in hippocampal-dependent memory retention, and the recovered hippocampal neurogenesis correlates with the recovery in hippocampal-dependent memory retention. However, the possibility remains that biochemical changes in other regions, including CA1 and CA3, may contribute to radiation-induced cognitive impairment.

In vitro approaches to study the radiation response of immature hippocampal cells

In vitro study of the consequences of radiation-induced hippocampal cell death to cognitive impairment

Table 1. The effects of ionizing radiation exposure on cognition *in vivo*.

Radiation type, frequency and dose	Animals (age)	Cognitive assessment	Cognitive deficit	Reference
0.3 and 3 Gy of X-rays	SD rats (21, 50, 70 days)	Trace fear conditioning	X	Achanta et al. (2009)
		Contextual fear conditioning	X	
		Delay fear conditioning	X	
10 Gy of X-rays	SD rats (21, 50, 70 days)	Trace fear conditioning	O	Achanta et al. (2009)
		Contextual fear conditioning	X	
		Delay fear conditioning	X	
5 Gy of X-rays	Wistar rat (neonatal)	Object recognition	O	Caceres et al. (2010)
		Inhibitory avoidance (ST)	O	
		Inhibitory avoidance (LT)	X	
Three sessions of 5 Gy of γ-rays for a 3- or 4-day interval	Male and female C57BL/6 (50 or 66 days)	Morris water maze	X	Clark et al. (2008)
		Contextual fear conditioning	X	
40 Gy in eight fractions of 5 Gy of γ-rays (twice/week for 4 weeks)	Male F344xBN rats (10-12 weeks)	Object recognition	O	Conner et al. (2010)
2 Gy of γ-rays	Male ICR (7 weeks)	Passive avoidance	O	Kim et al. (2008b)
		Object recognition	O	
24 Gy in eight fractions of 3 Gy of γ-rays (two 4-day periods separated by a 3 -days pause)	Male Wister rat (2 months)	Object recognition	X	Madsen et al. (2003)
		Place recognition memory	O	
		Morris water maze	X	
2 Gy of nucleon 56Fe beams	Male C57BL/6 mice (6 and 8 weeks)	Morris water maze	O	Manda et al. (2008a,b)
1.5 and 4.5Gy of γ-rays	Male CD1 mice (8 weeks)	Passive avoidance (1.5 Gy)	X	Martin et al. (2001)
		Passive avoidance (4.5 Gy)	O	
10 Gy of X-rays	Male C57BL/6 mice (2 months)	Morris water maze	X	Raber et al. (2004)
		Barnes maze	O	
		Plus maze	X	
		Object recognition	X	
		Passive avoidance	X	
5 Gy of X-rays	Male C57BL/6 mice (3 weeks)	Object recognition	X	Rola et al. (2004)
		Morris water maze	O	
		Barnes maze	X	
45 Gy in nine fractions of 5 Gy of γ-rays (twice/week for 4.5 weeks)	Male F344xBN F1 hybrid (12 months)	Morris water maze	O	Shi et al. (2006)
10 Gy of γ-rays on two consecutive days	Male Long Evans rat (4 months)	Contextual fear conditioning	O	Winocur et al. (2006)
		NMTS	X	
		DNMTS	X	
2 Gy of neutrons	Male ICR (8 weeks)	Passive avoidance	O	Yang et al. (2012)
		Object recognition	O	
40 Gy in eight fractions of 5 Gy of γ-rays over 24 days	Male Fisher 344 Rats (6 months)	Morris water maze	X	Yoneoka et al. (1999)
		Passive avoidance	O	

Gy, grays; SD, Sprague Dawley; ST, short-term (1 h) intertrial interval; LT, long-term (24 h) intertrial interval; F344xBN, Fischer 344xBrown Norway; ICR, Institute for Cancer Research; NMTS, non-matching-to-sample task; DNMTS, delayed non-matching-to-sample task.

is difficult, because analysis of cognitive impairments depends on complicated *in vivo* models (Fike et al., 2007). The consensus view holds that non-cycling cells, such as neurons, are more resistant to irradiation than cycling cells, such as astrocytes and vascular endothelial cells. Exposure of non-cycling post-mitotic neurons to ionizing radiation induces neuronal apoptosis via DNA damage and oxidative stress (Gobbel et al., 1998). Moreover, an irradiation study using developing rat brains suggested that the radiosensitive cell population consists primarily of cells nearing cell division or cells that have recently completed mitosis and are beginning to differentiate (Hicks et al., 1961). These conditions could thus be employed to address specific mechanisms associated with irradiation on immature and mature hippocampal cells using *in vitro* approaches.

Seven day *in vitro* (DIV) neurons are susceptible to X-irradiation at a high dose of 30 Gy, whereas 21 DIV neurons are resistant to this type of irradiation (Shirai et al., 2006). Gobbel et al., (1998) reported that 2 Gy of X-irradiation is the threshold irradiation dose at 7 DIV. Moreover, X-irradiation of immature neurons causes structural deficits, such as a loss of cell connections and a reduction in synapse formation in surviving neurons, which are thought to result in their dysfunction (Okamoto et al., 2009). Cell viability declines in a dose-dependent manner within the relative low-range of γ-irradiation applied (0-4 Gy) in 0.5 DIV-cultured hippocampal cells, which is comparable to neural immature progenitor cells before cell connections and synapses are formed (Song et al., 2010; Yang et al., 2011c). However, 14 DIV hippocampal cells are resistant to this irradiation level (Song et al., 2010). Thus, immature hippocampal cells are significantly more radiosensitive than are mature cells, indicating that the susceptibility of hippocampal cells to irradiation depends on their differentiation state.

The effects of caspase inhibitors on radiation-induced cytotoxicity have been examined in several studies. Caspase-3 activation and caspase-specific poly (ADP-ribose) polymerase (PARP) cleavage have been assessed by Western blot in an *in vitro* system (Song et al., 2010), and the effects of caspase inhibitors examined with a lactate dehydrogenase (LDH) release assay (Yang et al., 2011c). Active caspase-3 and cleaved PARP markedly increase in immature hippocampal cells and both the caspase family inhibitor Z-VAD-FMK and the caspase-3 specific inhibitor Z-DEVD-FMK significantly block γ-irradiation-induced cytotoxicity in these cells (Yang et al., 2011c). These results suggest that γ-irradiation-induced cell death in immature hippocampal neurons depends on a pro-apoptotic caspase-3 pathway. *In vitro* systems such as this may thus prove useful to study various neuronal perturbation factors caused by radiation exposure and to screen radioprotectants.

Relative biological effectiveness of radiation sources in the hippocampus

Several reports have compared various radiation quality indices. Equal doses of different types of radiation do not produce equivalent biological effects. If radiation is absorbed by biological material, ionization and excitation occur, which is not distributed randomly but tends to be localized along the tracks of individual charged particles in a pattern that depends on the type of radiation involved. For example, γ-rays give rise to fast electrons that carry a unit of electrical charge and have a very small mass. In contrast, neutrons give rise to recoil photon particles that carry a unit of electrical charge but with a mass nearly 2,000 times greater than that of an electron.

The energy loss per unit length of particle track is called the stopping power in nuclear physics and linear energy transfer (LET) in radiation biology. LET is the energy transferred per unit length of the track. It is a useful way to indicate the quality of different types of ionizing radiation, which is ~0.3 keV/mm for γ-rays and 12 keV/mm for neutrons (Hall, 1972). Heavy charged particles, such as neutrons, are referred to as high-LET radiation, whereas X-ray, γ-rays, and fast electrons are deemed low-LET radiation (Hall, 1972). In humans, exposure to neutrons can occur from nuclear fission reactions usually associated with the production of nuclear energy and from cosmic radiation in the natural environment (Dudkin et al., 1990; Dyer et al., 1996; Keith et al., 1992). Consequently, it is important to study the direct effects of neutrons on human organs and tissues to precisely assess the risk of damage. High-LET components, particularly neutrons, contribute principally to the risk, so that the contribution of even small neutron doses is magnified (Grahn, 1983). Among ionizing radiation types, high-LET radiation is more lethal than low-LET radiation because of its ability to produce more complex double-strand DNA breaks (Gobbel et al., 1998; Fischer et al., 2005). High-LET radiation may favor the production of lesions in which strand breaks occur in proximity to base damage or DNA-protein cross-linkage (Britten et al., 2001). Variations in relative biological effectiveness (RBE) due to dose, oxygenation, cell cycle parameters and tissue characteristics have been well-documented (Britten et al., 2001; Ishida et al., 2006).

Increased apoptotic cell death and decreased neurogenesis in the hippocampal DG of mammalian brains appear to be dose-dependent after exposure to both fast neutrons and γ-rays (Guida et al., 2005; Yang et al., 2010b). Based on dose-response data, the RBE value of fast neutrons is ~1.9 for apoptosis in the hippocampus of adult ICR mice. Fast neutrons inhibit proliferation, as measured by Ki-67 expression, ~3.2x that seen with exposure to γ-rays at the same dose. Fast neutrons also inhibit doublecortin, a marker of immature progenitor cells, ~2.5x that seen with exposure to γ-rays at the same dose. The amount of γ-irradiation exposure, particularly 2 Gy, reduces hippocampal neurogenesis temporally correlated with deficits in hippocampal-dependent memory retention 1-3 days after irradiation. Recovered hippocampal neurogenesis is correlated with the recovery of hippocampus-dependent memory retention 7

days after irradiation (Kim et al., 2008b). However, in mice trained 1 and 7 days after acute exposure to a relatively low-dose of fast neutrons, exposure to 0.8 Gy neutrons is sufficient to induce significant memory deficits in the hippocampal-related learning paradigms, including the object recognition memory test and contextual fear conditioning (Yang et al., 2012). Thus, fast neutrons have a higher RBE for neurogenesis indices compared with those of γ-rays. Additionally, an *in vitro* study compared the detrimental effects and RBE of high-LET fast neutrons on rat immature hippocampal cultured cells with those of low LET γ-rays (Yang et al., 2011b). Fast neutrons dose-dependently increase cytotoxicity and decrease cell viability in immature hippocampal cells with RBEs of ~2.35 as measured by LDH release and ~2.42 by MTT assay indices. Thus, fast neutrons have higher RBE cell death indices compared with γ-rays. Consequently, the *in vivo* and *in vitro* effects on hippocampal neurogenesis, cytotoxicity, and cell viability differ among radiation sources and may be used as indices of RBEs.

Novel drug targets for radiation-induced impairment of hippocampal neurogenesis and cognition

Both *in vivo* and in *vitro* approaches have shown radioprotective effects of various treatments on hippocampal dysfunction, neurogenesis, and cytotoxicity (Lonergan et al., 2002; Fan et al., 2007; Manda et al., 2008a,b, 2009; Lee et al., 2009, 2010; Jenrow et al., 2010, 2011; Conner et al., 2010; Kim et al., 2010a,b; Acharya et al., 2011; Yang et al., 2011c) (Table 2). Usually, radiation-induced neuronal apoptosis is a function of oxidative stress (Gobbel et al., 1998), and antioxidants play an essential role in the survival of neuronal cells exposed to various metabolic and oxidative challenges and may also influence neurogenesis (Sleeper et al., 2002).

Amifostine is a well-known radioprotective agent with free radical scavenging properties (McDonough et al., 1992). *In vitro*, pretreatment with amifostine inhibited γ-ray-induced cytotoxicity of immature hippocampal cells through decreases in intracellular reactive oxygen species (ROS) levels (Yang et al., 2011c). Amifostine suppresses radiation-induced cell death *in vitro* and *in vivo* in developing cerebellar granular cells (Guelman et al., 2003, 2005). Additionally, amifostine significantly attenuates recognition memory defects in adult mice exposed to low-dose radiation, possibly by inhibiting the detrimental effect of irradiation on hippocampal neurogenesis (Lee et al., 2010). However, amifostine use is limited clinically due to its restricted treatment range and toxic side effects (Jagetia, 2007). Thus, identifying additional drugs that are effective, minimally toxic, and affordable has become a focus for pharmaceutical researchers.

Plant-based pharmacological agents have been identified as radioprotective agents that reverse deleterious effects of ionizing radiation in patients

Table 2. Radioprotective treatments affecting hippocampal dysfunction, neurogenesis, and cytotoxicity *in vivo* and *in vitro*.

Radiation type, frequency, and dose	Experimental model	Treatments	Study models	Reference
10 Gy dose with a 6-field IMRT plan	Athymic nude rats	Human neural stem cells	Cognition	Acharya et al. (2011)
40 Gy in eight fractions of 5 Gy, twice/week for 4 weeks of γ-rays	Male F344xBN rats (10-12 weeks old)	AT1RA L-158,809	Cognition, Neurogenesis	Conner et al. (2010)
5 or 10 Gy of X-ray	Male Mongolian gerbils (2 months old)	Environmental enrichment	Neurogenesis	Fan et al. (2007)
10 Gy of γ-rays	Male Fischer 344 rats (200-240 g)	Ramipril	Neurogenesis	Jenrow et al. (2010)
10 Gy of γ-rays	Male Fischer 344 rats (200-240 g)	Atrovastatin and Ramipril	Neurogenesis	Jenrow et al. (2011)
5 Gy of γ-rays	Male C3H/HeN mice (8 weeks old)	G-CSF	Neurogenesis	Kim et al. (2010a,b)
2 Gy of γ-rays	Male C57BL/6 (7 weeks old)	Rolipram	Cognition, Neurogenesis	Kim et al. (2010a,b)
2 Gy of γ-rays	Male ICR (8 weeks old)	Red ginseng	Cognition, Neurogenesis	Lee et al. (2009)
2 Gy of γ-rays	Male ICR (8 weeks old)	Amifostine	Cognition, Neurogenesis	Lee et al. (2010)
10 Gy of γ-rays	Male Wistar rats	Eicosapentaenoic acid	LTP model, ROS, Apoptosis	Lonergan et al. (2002)
1.5 Gy of nucleon ^{56}Fe beams	Male C57BL/6 (8 weeks old)	α-lipoic acid	Cognition, ROS	Manda et al. (2008a)
2 Gy of nucleon ^{56}Fe beams	Male C57BL/6 (6 weeks old)	AFMK	Cognition, ROS, Neurogenesis	Manda et al. (2008b)
6 Gy of X-ray	Male C57BL/6 (6 weeks old)	Melatonin	Neurogenesis, ROS	Manda et al. (2009)
2 Gy of γ-rays	Immature hippocampal neurons	Amifostine, Epigallocatechin gallate, Z-DEVD-FMK	Cytotoxicity, ROS	Yang et al. (2011c)

Abbreviations: Gy, grays; F344xBN, Fischer 344 x Brown Norway; ICR, Institute for Cancer Research; AT_1RA, angiotensin II type 1 receptor antagonist; G-CSF, granulocyte-colony stimulating factor; LTP, long-term potentiation; ROS, reactive oxygen species; AFMK, N1-acetyl-N2-formyl-5-methoxykynuramine; Z-DEVD-FMK (selective caspase-3 inhibitor), Z-D(OMe)-E(OMe)-V-D(OMe)-fluoromethyl ketone.

exposed to radiation from nuclear accidents (Jagetia, 2007). Epigallocatechin gallate is the main ingredient of green tea polyphenols and significantly blocks increased LDH level and intracellular ROS levels in immature hippocampal cells *in vitro* (Yang et al., 2011c). Red ginseng, which possesses antioxidant activities, protects against radiation-induced cognitive impairment and suppression of hippocampal neurogenesis after whole body exposure (Lee et al., 2010). Therefore, since irradiation has a detrimental effect on immature progenitor and developing neurons, possibly through a cytotoxic mechanism that includes free radical stress, antioxidant substances have potential therapeutic utility in brain irradiation.

Anti-inflammatory therapy is the most obvious treatment strategy to address the inflammatory component of radiation injury. The chronic microglial inflammation observed after irradiation negatively regulates neurogenesis (Hwang et al., 2006). Thus, anti-inflammatory therapy should help restore endogenous neurogenesis, as well as heighten the efficacy of cell-replacement strategies. Pre-treatment with anti-inflammatory drugs, such as indomethacin, or a peroxisome proliferator-activated receptor-α agonist combined with fenofibrate, partially prevents microglial activation and the decrease in neurogenesis induced by irradiation exposure (Monje et al., 2003; Ramanan et al., 2009). Eicosapentaenoic acid, which has anti-inflammatory properties and inhibits age-related increases in interleukin-1 in the hippocampus (Babcock et al., 2000), effectively protects hippocampal neurons from damage induced by whole body irradiation (Lonergan et al., 2002). Treatment with the angiotensin converting enzyme inhibitors AT1RA L-158,809 and ramipril ameliorates radiation-induced cognitive deficits in rats and also reduces apoptosis among SGZ progenitors and inflammatory disruption within the SGZ microenvironment (Conner et al., 2010; Jenrow et al., 2010). The administration of atorvastatin combined with ramipril appears to synergistically ameliorate radiation-induced inhibition of neurogenesis (Jenrow et al., 2011). Therefore, anti-inflammatory therapy may be a potential therapeutic approach for brain irradiation.

More refined strategies could target specific ligand-receptor interactions, such as cytokines and their effective target cells. The administration of hematopoietic cytokines during brain injury is effective for recovering functional memory through the proliferation of intrinsic neural stem/progenitor cells (Kawada et al., 2006). The hematopoietic cytokine granulocyte-colony stimulating factor (G-CSF) induces bone marrow stem cell proliferation and mobilization, and activates endothelial cell proliferation, which could help establish a vascular niche for neural stem cells (Jung et al., 2006). The basic cellular functions of G-CSF appear conserved in the central nervous system; i.e., inhibition of apoptosis and stimulation of cell differentiation (Komine-Kobayashi et al., 2006). Astrocyte-produced G-CSF contributes to neurosphere generation and decreases the frequency of neuronal death (Ding et al., 2009). Moreover, administration of G-CSF during brain injury is effective for recovering functional memory through the proliferation of intrinsic neural stem/progenitor cells (Kawada et al., 2006; Sanchez-Ramos et al., 2009). G-CSF pre-treatment of the irradiated brain ameliorates the suppression of hippocampal neurogenesis, suggesting that G-CSF plays a neuroprotective role against irradiation-induced ablation of hippocampal neurogenesis (Kim et al., 2010b). Thus, hematopoietic cytokines like G-CSF may be potential therapeutic approaches for brain irradiation.

Radiation injures neural stem cells and limits their growth potential. The stem/progenitor pool in irradiated patients is thus likely depleted over time. Neither recruitment of endogenous neural stem/precursor cells nor cell transplantation strategies to restore hippocampal neurogenesis are possible until the neurogenic microenvironment is restored. Stem/precursor cells can subsequently be grafted back into the neurogenic environment, where they have the capacity to differentiate into neurons (Suhonen et al., 1996). A recent study demonstrated the direct cognitive benefits derived from engraftment of human stem cells in rodents, suggesting that this procedure may afford a promising strategy for the long-term functional restoration of cognition in individuals subjected to cranial radiotherapy (Acharya et al., 2011). Additionally, drugs stimulating neuronal stem cell proliferation in the brain, which would improve cognitive ability in patients with brain injury (Silva et al., 1992; Finkbeiner, 2000; Nakagawa et al., 2002; Fujioka et al., 2004), may need to be studied. These agents include activators of the cAMP-response element binding protein (CREB) (Kim et al., 2010a). CREB is a basic-domain leucine zipper transcription factor, which regulates transcription via a DNA sequence known as the cAMP-response element (Brindle and Montminy, 1992). Previous studies have demonstrated that activating CREB plays an important role in cellular and behavioral models of learning and memory, as well as in neuronal survival and activation (Finkbeiner, 2000; Fujioka et al., 2004). The temporal pattern of reduced hippocampal neurogenesis caused by irradiation corresponds to that of CREB phosphorylation (Kim et al., 2010a), supporting the hypothesis that CREB phosphorylation is associated with neurogenesis in the DG of the mouse hippocampus. Rolipram, a specific phosphodiesterase type 4 isoform inhibitor, restores activity of the cAMP/CREB pathway (Nakagawa et al., 2002; Vitolo et al., 2002). In a radiation-induced hippocampal dysfunction model, promoting CREB activity by rolipram treatment ameliorates neurogenesis-dependent hippocampal functions, including learning and memory, and also increases the rate of hippocampal neurogenesis by activating CREB (Kim et al., 2010a). Therefore, rolipram may not only immediately counteract reduced CREB phosphorylation in the DG of the adult hippocampus, but also protect against decreased

hippocampal neurogenesis in adult mice after irradiation, suggesting it to be a potential therapeutic agent against irradiation-induced hippocampal dysfunction. Therefore, drugs and stem cell grafting that target increased neuronal stem cell proliferation represent potential therapeutic approaches for brain irradiation.

Two additional agents have been suggested as potential therapeutics. The first is α-lipoic acid, an endogenously produced coenzyme that plays an essential role in ketoacid dehydrogenase reactions. Its properties as an antioxidant have recently been reviewed (Bilska and Wlodek 2005), and α-lipoic acid is also a potent neuroprotective antioxidant that mitigates radiation-induced decline in memory (Manda et al., 2008a). The second is the neurohormone melatonin, a chief secretory product of the pineal gland rhythmically synthesized in synchrony with the dark phase of the circadian cycle (Reiter, 1991). Melatonin and its metabolites N1-acetyl-N2-formyl-5methoxykynuramine (AFMK) are well-known direct free radical scavengers. Melatonin pretreatment significantly reduces radiation-induced damage of hippocampal neurogenesis and oxidative stress (Manda et al., 2009). AFMK also protects against high-LET radiation-induced impairment of memory and hippocampal neurogenesis (Manda et al., 2008b).

The development of novel drugs is important for protecting patients from the radiation-induced impairment of hippocampal neurogenesis and cognition. Despite extensive research, there is no approved drug to protect or attenuate radiation injury. It is further necessary to design suitable delivery models to reduce side effects. More recent studies have focused on development of drugs with moderate efficacy, low toxicity, and that can be administered easily (Jagetia, 2007). These novel drugs may be effective protective or attenuating agents that safeguard the cognitive health of those potentially affected by radiation, such as astronauts during long space missions and soldiers in postwar rescue work (Manda and Reiter, 2010). Moreover, it seems likely that supplementing brain tumor patients with adjuvant therapy may benefit successful cranial-radiotherapy or at least overcome the side effects of cranial radiotherapy. Therefore, more advanced studies are required to address the precise mechanisms of the radioprotection in these experimental animals and to develop effective novel drugs as a potential therapeutic approach to treatment of irradiation-induced brain dysfunction.

Conclusion

While extensive data showing that neurogenesis/cognitive functions are significantly affected by ionizing irradiation have appeared over the past few years, there remains considerable uncertainty regarding exactly how those changes evolve. *In vitro* techniques for study of neural stem/progenitor cells will provide a unique way to address mechanistic elements associated with the radiation response. Genetic models and novel approaches provide new tools with which the complexities of whole animal models can be dissected to better understand how cellular radiation injury evolves into deficits affecting behavioral performance. Therefore, the information combined from both *in vitro* and *in vivo* models provides novel quantitative methods to address normal brain tolerance and how to protect neural progenitor cells against exposure to ionizing irradiation. Furthermore, equal doses of different types of irradiation produce different biological effects. For example, high-LET radiation is several times more effective than low-LET radiation at generating hippocampal lesions. Novel drugs have been studied to reduce hippocampal neurogenesis and the cognitive deficits produced by radiation. Although much research has been promising, novel agents have not been very effective and, with limited exceptions, are not the standard of care in radiation medicine. Further studies are needed to develop an optimized protector against and attenuator of radiation-induced hippocampal injury and cognitive impairment.

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