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Hippocampal dysfunction during the chronic phase following a single exposure to cranial irradiation

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ABSTRACT

Ionizing radiation can significantly affect brain functioning in adults. The present study assessed depression-like behaviors in adult C57BL/6 mice using the tail suspension test (TST) at 30 and 90 days following a single cranial exposure to γ-rays (0, 1, or 10 Gy) to evaluate hippocampus-related behavioral dysfunction during the chronic phase following cranial irradiation. Additionally, hippocampal neurogenesis, inflammatory cytokines, inducible nitric oxide synthase (iNOS), cyclooxygenase-2 (COX-2), brain-derived neurotrophic factor (BDNF), and glial cell line-derived neurotrophic factor (GDNF) were analyzed. At 30 and 90 days following irradiation with 10 Gy, mice displayed significant depression-like behaviors. We observed a persistent decrease in the number of cells positive for doublecortin, an immunohistochemical marker for neurogenesis, in the hippocampus from 1 to 90 days after irradiation with 10 Gy. Changes in the mRNA expression of inflammatory cytokines, including interleukin (IL)-1β, tumor necrosis factor-α, IL-6, and interferon-γ, were not correlated with the decrease in hippocampal neurogenesis or the appearance of depression-like behavior during the chronic phase following irradiation. However, at 30 and 90 days after irradiation with 10 Gy, the number of microglia was significantly decreased compared with age-matched sham-irradiated controls. The reduction in the chronic phase was consistent with the significant down-regulation in the mRNA expression of iNOS, COX-2, BDNF, and GDNF in the hippocampus. Therefore, hippocampal dysfunction during the chronic phase following cranial irradiation may be associated with decreases in the neurogenesis- and synaptic plasticity-related signals, concomitant with microglial reduction in the hippocampus.

Introduction

The hippocampus is located within the ventromedial aspect of the temporal cortex in the brain and plays important roles in memory and emotional regulation (Richardson et al., 2004; Richter-Levin, 2004; Squire, 1992; Zola-Morgan and Squire, 1993). It is established that new neurons are produced in the subgranular zone (SGZ) of the dentate gyrus (DG) within the hippocampus and that neural stem cells are produced throughout life (Altman and Das, 1965). When necessary, stem cells from these specific areas of the brain can migrate and repopulate damaged areas following brain stimulation (Doetsch et al., 1999; Morshead et al., 1994). The rate of neurogenesis in the hippocampus likely plays a pivotal role in hippocampus-dependent functions, including learning and memory and regulation of emotion (Bruel-Jungerman et al., 2005; Fike et al., 2009; Sahay and Hen, 2007; Shors et al., 2001), and it may be altered by several factors, including chemicals (Seo et al., 2010; Yang et al., 2010), radiation (Kim et al., 2008), and environmental enrichment (van Praag et al., 1999).

The brain may be exposed to ionizing irradiation following nuclear accidents or during space travel, atomic weapon testing and use, and medical treatment. Although the adult brain is less vulnerable to irradiation than other organs, it is likely that even relatively low doses of irradiation can induce cognitive impairment (Butler et al., 2006; Kim et al., 2008; Meyers and Brown, 2006). Moreover, in adults, cranial irradiation can induce a variety of side effects, including long-lasting declines in cognitive functioning (Abayomi, 1996; Robison et al., 2005), even if few structural changes occur (Sheline et al., 1980; Snyder et al., 2005). In experimental animals, acute irradiation has resulted in the transient or prolonged loss of proliferating cells in the DG, as well as in learning and memory impairment (Kim et al., 2008;

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Raber et al., 2004). The acute effects of 2 and 4 Gy irradiation on adult neurogenesis, and the associated memory deficits, were fully reversed 2 weeks and 1 month later, respectively (Ben Abdallah et al., 2007; Kim et al., 2008). However, radiation-induced reductions in hippocampal neurogenesis have also been associated with cognitive deficits in young adult rodents 3 months after a single exposure to whole-brain irradiation with 5 or 10 Gy (Raber et al., 2004; Rola et al., 2004). Thus, cranial irradiation with relatively high doses (≥5 Gy) may result in the prolonged reduction of hippocampal neurogenesis and may also be related to delayed cognitive impairments. It has been suggested that this process is associated with neuroinflammation (Hong et al., 1995; Kyrkanides et al., 2002; Monje et al., 2003; Rola et al., 2008). Previous studies have reported that excessive high-dose irradiation (25–35 Gy) induced an up-regulation of pro-inflammatory cytokines such as interleukin (IL)-1β, tumor-necrosis factor (TNF)-α, IL-6, and interferon (IFN)-γ during the hyper-acute phase of 4 or 6 h (Hong et al., 1995; Kyrkanides et al., 2002). In contrast, a loss of microglia was detected in the subacute phase 7 days following a single dose of 8 Gy to immature rat brain (Kalm et al., 2009) and at 1 and 10 weeks following a single 10 Gy dose to adult rats (Schindler et al., 2008). Thus, there is some controversy regarding the effects of inflammation on the reduction in hippocampal neurogenesis and associated hippocampal functions following irradiation, requiring further investigations of specific time-point and radiation-dose analyses. Nevertheless, the precise mechanisms associated with hippocampal dysfunction following cranial irradiation remain unknown.

Neurotrophic factors play critical roles in the synaptic activity and plasticity of mature neurons (Murer et al., 2001), as well as in the proliferation, differentiation, and survival of neurons in the central nervous system (McAllister, 2001). Brain-derived neurotrophic factor (BDNF) and glial cell line-derived neurotrophic factor (GDNF) genes are abundantly expressed in the mouse hippocampus (McCarthy, 2006) and have important roles in the development and function of dopaminergic neurons and the regulation of a variety of functions (Lapchak et al., 1996; Lewin and Barde, 1996; Shen et al., 1997). Decreased BDNF expression in the hippocampus is related to cognitive deficits (Gooney et al., 2004; Hattiangady et al., 2005; Linnarsson et al., 1997), and previous research suggests that depression and neurodegenerative diseases, such as Parkinson's disease and Alzheimer's disease, are linked to a lack of neurotrophic factors, including BDNF and GDNF (Altar, 1999; Shirayama et al., 2002; Siegel and Chauhan, 2000). However, there is a relative lack of knowledge concerning the possible involvement of BDNF and GDNF in hippocampal dysfunction following cranial irradiation.

Thus, the present study assessed depression-like behavior using the tail suspension test (TST) and measured levels of doublecortin (DCX), an immature progenitor neuron marker, using immunohistochemistry in adult mice following cranial exposure to γ-rays (1 or 10 Gy). Additionally, changes in the expression levels of inflammatory cytokines (IL-1β, TNF-α, IL-6, and IFN-γ), pro-inflammatory enzymes, including inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2), markers for microglia (Iba-1) and astrocytes (glial fibrillary acidic protein [GFAP]), and neurotrophic factors, including BDNF and GDNF were examined in the mouse hippocampus.

Materials and methods

Animals

Male C57BL/6 mice (8 weeks of age; Daihan-Biolink Co.; Chungbuk, Korea) were housed in a room maintained at 23 ± 2 °C with a relative humidity of 50 ± 5%, artificial lighting from 08:00 to 20:00 h, and 13–18 air changes every hour. The animals were given *ad libitum* access to tap water and commercial rodent chow (Jeil Feed Co.; Daejeon, Korea). After acclimatization, mice were randomly divided into three groups ($n = 36$ mice for sham-irradiated group, $n = 60$ mice for the 1- and 10-Gy-irradiated groups), and further subdivided into 13 groups ($n = 12$ mice/group) based on the days after irradiation (1, 2, 8, 30, and 90 days). To minimize the number of mice used in this study, the same group was used for the acute phase sham-irradiated control (1, 2, and 8 days). The Institutional Animal Care and Use Committee of Chonnam National University approved the protocols used in this study (CNU IACUC-YB-2012-38), and the animals were cared for in accordance with internationally accepted principles for laboratory animal use and care as found in the National Institutes of Health Guidelines (USA). The number of animals used and the suffering caused was minimized in all experiments.

Irradiation and tissue sampling

The mice were anesthetized with tiletamine/zolazepam (Zoletil 50®; Virak Korea; Seoul, Korea) and immobilized, and single radiation fractions of 1 and 10 Gy apiece were delivered through the whole brain. The brain received whole-brain irradiation using six MV high-energy photon rays (ELEKTA; Stockholm, Sweden) at a dose rate of 3.8 Gy/min. A radiation dosimeter (Semiflex Ionization Chamber 31013, PTW Co., Freiburg, Germany) was used to determine that the radiation doses ranged from 99 to 100% at a point 3 cm below the surface of the simulated mouse head. We aligned the center of the head to the beam line center using the mouse holder. The distance of the skin from radiation source was 1 m. Sham-irradiated mice were anesthetized with tiletamine/zolazepam (Zoletil 50®) and immobilized for the same period of time without irradiation. Mice from each group were subdivided (1 and 2 experiments, Fig. 1): (1) Eight mice per group were used for hippocampal collection to extract mRNA. In the chronic phase, mice were subjected to TST, followed by hippocampal collection for mRNA extraction 4 h later; (2) Four mice per group were sacrificed, and the brains were quickly removed and divided at the midline. The left hemisphere was fixed in 4% paraformaldehyde in phosphate-buffered saline (PBS, pH 7.4) for immunohistochemistry and the hippocampus was removed from the right hemisphere and immediately stored at −70 °C for mRNA extraction.

Tail suspension test (TST)

The TST was conducted as reported previously (Steru et al., 1985). Briefly, mice were suspended from a plastic rod mounted 50 cm above a surface by fastening the tail to the rod with adhesive tape. Immobility was measured for 6 min using the SMART video-tracking system (Panlab; Barcelona, Spain).

Immunohistochemistry

The immunohistochemical procedures were performed as described previously (Kim et al., 2008; Yang et al., 2011; 2010). Briefly, after 0-, 1-, or 10-Gy irradiation ($n = 4$ mice/group), 5-μm thick sagittal sections were deparaffinized, hydrated, and allowed to react with polyclonal rabbit anti-DCX (1:400; Cell Signaling Technology; Beverly, MA, USA), anti-GFAP (1:2000; Dako; Glostrup, Denmark), and anti-Iba-1 (1:1000; Wako; Osaka, Japan) for 2 h at room temperature (RT). Then, the sections were reacted with biotinylated goat anti-rabbit IgG (Vector ABC Elite Kit; Vector; Burlingame, CA, USA) for 60 min at RT. Immunoreactivity was performed for 60 min at RT using the avidin–biotin peroxidase complex (Vector ABC Elite Kit) prepared according to the manufacturer's instructions, and the peroxidase reaction was developed using a diaminobenzidine substrate kit (SK-4100; Vector). As a control, the primary antibodies were omitted for a few test sections in each experiment. All sections were counterstained with Harris' hematoxylin prior to being mounted.

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