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Effects of Neutron Radiation on Apoptotic Cell Death and Cell Proliferation in Dentate Gyrus of Rats

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ABSTRACT

Radiations are known to cause death of neuronal precursors and neuronal cells in the brains. Neutrons differ from photons in the mode of their interactions with tissues. Moreover, neutrons have a higher linear energy transfer and generate more dense ionization, resulting in inducing severe breakage of double-stranded DNA and generation of more free radicals than photons. In the present study, we investigated the effects of fast neutron radiation on apoptotic neuronal cell death and cell proliferation in the hippocampal dentate gyrus of the rats. For this study, immunohistochemistry for caspase-3 and 5-bromo-2-deoxyuridine (BrdU), and terminal deoxynucleotidyl transferase-mediated nick end-labeling (TUNEL) staining were performed. In the present results, high-dose of fast neutron radiation (1 Gy) increased apoptotic neuronal cell death and suppressed cell proliferation in the dentate gyrus. The present study shows that high-dose of fast neutron radiation may exert negative effects on the cognitive functions, especially hippocampal-dependent learning tasks by enhancing apoptosis and by suppressing cell proliferation of hippocampal neuronal cells.

Key words: fast neutron radiation, dentate gyrus, apoptosis, cell proliferation

INTRODUCTION

High linear-energy-transfer (LET) radiations, such as neutrons and α-particles, have been reported to have a greater biological effectiveness than low-LET radiations such as X-rays and γ-rays. Although the biological aspects and mechanisms of the effects of high-LET radiation have not been clarified (Goodhead, 1999; Ishida et al., 2006), radiations are known to cause apoptosis. Radiation-induced apoptosis depends on the radiation source as well as the radiation dose (Aref et al., 1999; Goodhead, 1999).

Apoptosis, programmed cell death, plays a crucial role in the development and the maintenance of homeostasis in all multi-cellular organisms, such as cell replacement, tissue remodeling, and removal of

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damaged cells (Thompson, 1995; Yuan and Yanker, 2000). On the other hand, inappropriate or excessive apoptosis has been implicated in many diseases including neurodegenerative disorders, cancers, autoimmune diseases, acquired immunodeficiency syndrome (AIDS), and stroke (Thompson, 1995).

Terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL) staining is an assay that detects the characteristic of apoptotic cell death e.g. DNA fragmentation (Gavrieli et al., 1992). Many studies showed that high-dose of radiation increased the number of TUNEL-positive cells representing apoptotic cell death (Peiβner et al., 1999; Tada et al., 2000). Another important characteristic of apoptosis is the activation of caspase-3. Caspase-3 is the most widely studied member of the caspases family and one of key executors of apoptosis (Cohen, 1997). Lonergan et al. (2002) demonstrated that the activation of caspase-3 is implicated in apoptotic neuronal cell death.

Neutrons, non-charged particles, differ from photons in the mode of their interactions with tissues and they generate more dense ionization. These properties of fast neutrons induce many free radicals and result in more irreversible DNA breakage than photons (Kantor and Simon, 1996). Ionizing radiation-induced DNA damage results in cell death. Traditionally, radiation-induced cell death is divided into two types: one is interphase death and the other is reproductive death (Shinohara and Nakano, 1993). Following an exposure to ionizing radiations, the cells undergo apoptotic cell death at various cell cycle stages (Shinomiya, 2001) and the forms of apoptosis are different according to the timing of radiation-induced cell death in relation to cell cycle progression (Radford and Murphy, 1994; Shinomiya et al., 2000).

The dentate gyrus of the hippocampus is critical brain area related to neurogenesis. Neurogenesis occurs in discrete regions of the brain such as the rostral subventricular zone (SVZ) of the lateral ventricles and the subgranular zone (SGZ) of the dentate gyrus. Neurogenesis in the dentate gyrus continues throughout adulthood in a variety of species including humans (Kuhn et al., 1996; Eriksson et al., 1998; Gould et al., 1999; Kunlin et al., 2001). Neurogenesis is known to play an important role in learning processes, and there are several interesting correlations between the number of new neurons and performance on hippocampal-dependent learning tasks (Barnea and Nottebohm, 1994; Gould et al., 1999). Shors et al. (2002) reported that hippocampal neurogenesis in the dentate gyrus is connected to the specific type of learning.

Since fast neutrons were introduced in the 1970s, fast neutron therapy has been suggested to be effective for some carcinomas (Douglas et al., 2000; Tokumitsu et al., 2000). While X-rays-induced apoptosis has been reported (Barber et al., 2000), the effect of fast neutrons on apoptosis has not been documented. In the present study, the effects of fast neutron radiation on apoptotic neuronal cell death and cell proliferation in the hippocampal dentate gyrus of the rats were investigated. For this study, immunohistochemistry for caspase-3 and 5-bromo-2-deoxyuridine (BrdU), and terminal deoxynucleotidyl transferase-mediated nick end-labeling (TUNEL) staining were performed.

MATERIALS AND METHODS

Animals and treatments

Male Sprague-Dawley rats weighing 250±10 g (8 weeks in age) were used for the experiment. The experimental procedures were performed in accordance with the animal care guidelines of the National Institutes of Health (NIH) and the Korean Academy of Medical Sciences. The rats were housed under the controlled temperature (20±2°C) and the lighting (07:00～19:00 h) conditions with food and water made available *ad libitum*. The animals were divided into four groups: the control group; 0.01 Gy radiation group; 0.1 Gy radiation group; 1 Gy radiation group (n=5 in each group).

Exposure to radiations

All animals were situated in close-fitting Perspex boxes (22×11×4 cm) and irradiated by fast neutrons generated by a cyclotron (MC-50, Scanditronix, Sweden). The rats in the radiation were exposed to 0.01, 0.1, and 1 Gy whole body fast neutron radiation at the rate of 0.09 cGy/min (35 MeV, 20 μA) from source-axial distance 150 cm for one time.

The rats were sacrificed 6 h after radiation. The rats in the control group were left on the linear accelerator without exposure to radiation.

Tissue preparation

The animals were weighed and anesthetized using Zoletil 50® (10 mg/kg, i.p.; Vibac Laboratories, Carros, France). After a complete lack of response was observed, the rats were transcardially perfused with 50 mM phosphate-buffered saline (PBS) and fixed with a freshly prepared solution consisting of 4% paraformaldehyde in 100 mM phosphate buffer (PB, pH 7.4). The brains were dissected and postfixed in the same fixative overnight and transferred into a 30% sucrose solution for cryoprotection. Serial coronal sections of 40 μm thickness were made with a freezing microtome (Leica, Nussloch, Germany).

BrdU immunohistochemistry

For visualization of cell birth in the hippocampal dentate gyrus, BrdU-specific immunohistochemistry was performed as previously described (Kim et al., 2002). In brief, the brain sections were permeabilized by incubation in 0.5% Triton X-100 in PBS for 20 min, then incubated in 50% formamide-2 X standard saline citrate (SSC) at 65°C for 2 h, denaturated in 2 N HCl at 37°C for 30 min, and rinsed twice in 100 mM sodium borate (pH 8.5). These processes were executed in due order. Afterwards, the sections were incubated overnight at 4°C with BrdU-specific mouse monoclonal antibody (1 : 600; Roche, Mannhein, Germany). The sections were then washed three times with PBS and incubated for 1 h with the biotinylated mouse secondary antibody (1 : 200; Vector Laboratories, Burlingame, CA, USA). Then the sections were incubated for additional 1 h with avidin-biotin-horseradish-peroxidase complex (1 : 100; Vector Laboratories). For visualization, the sections were incubated for 5 min in 50 mM Tris-HCl (pH 7.6) containing 0.02% 3,3-diaminobenzidine (DAB; Sigma Chemical Co., St. Louis, MO, USA), 40 mg/ml nickel chloride, and 0.03% hydrogen peroxide. Subsequently, the slides were air-dried overnight at room temperature, and cover slides were mounted using Permount® (Fisher Scientific, New Jersey, USA).

Caspase-3 immnunohistochemical staining

In order to detect caspase-3 expression, free-floating tissue sections were washed three times in 50 mM PBS and were then permeabilized in 0.2% Triton X-100 for 30 min. After washed three times with PBS, the sections were incubated overnight with mouse anti-caspase-3 antibody (Santa Cruz Biotechnology, Santa Cruz, CA, USA) at a dilution of 1 : 500. The sections were washed three times in PBS and incubated for 1 h with biotinylated anti-mouse antibody (1 : 200; Vector Laboratories). Bound secondary antibody was then amplified using the Vector Elite ABC® kit (Vector Laboratories). The antibody-biotin-avidin-peroxidase complexes were visualized using 0.02% DAB. The sections were mounted onto gelatinized glass slides, air dried, and cover slides were mounted using Permount® (Fisher Scientific).

TUNEL staining

In order to detect apoptotic cell death, a TUNEL staining was performed using the In Situ Cell Death Detection Kit (Roche) according to a previously described method (Kim et al., 2002). Briefly, the sections were mounted onto gelatin-coated slides and air-dried overnight at room temperature. The sections were then incubated with proteinase K (100 μg/ml), rinsed, incubated in 3% H_2O_2, permeablilized with 0.5% Triton X-100, rinsed again, and incubated in the TUNEL reaction mixture. The sections were rinsed and visualized using a converter-POD with nickel-DAB. The slides were air-dried overnight at room temperature, and cover slides were mounted using Permount® (Fisher Scientific).

Data analysis

The area of the granular layer of the dentate gyrus was measured using Image-Pro®Plus image analyzer (Media Cybernetics Inc., Silver Spring, MD, USA). The number of BrdU-positive, caspase-3-positive and TUNEL-positive cells in the hippocampal dentate gyrus was counted hemilaterally throughout the entire extent of the hippocampal dentate gyrus. BrdU-positive, caspase-3-positive and TUNEL-positive cells were expressed as the mean number of cells per mm^2 of the cross sectional area of the granular layer of the dentate

gyrus.

All data were analyzed using the statistical software SPSS (version 12.0). The data are expressed as the mean±standard error of the mean (SEM). For the comparison among the groups, one-way ANOVA and Duncan's post-hoc test were performed and differences among the groups were considered statistically significant at $p<0.05$.

RESULTS

Effect of fast neutron radiation on the number of TUNEL-positive cells in the hippocampal dentate gyrus

Photomicrographs of TUNEL-positive cells in the dentate gyrus of each group are presented in Fig. 1. The number of TUNEL-positive cells in the dentate gyrus was 4.71±0.98/mm^2 in the control group, 7.53±1.69/mm^2 in the 0.01 Gy radiation group, 10.10±1.69/mm^2 in the 0.1 Gy radiation group, and 35.41±3.23/mm^2 in the 1 Gy radiation group (Fig. 1).

The present results showed that low-doses of radiation (0.01 Gy and 0.1 Gy) did not exert significant effect on apoptotic neuronal cell death. On the other hand, high-dose of radiation (1 Gy) significantly increased apoptosis in the dentate gyrus of the rats.

Effect of fast neutron radiation on the caspase-3 expression in the hippocampal dentate gyrus

Photomicrographs of caspase-3-positive cells in the dentate gyrus of each group are presented in Fig. 2. The number of caspase-3-positive cells in the dentate gyrus was 1.56±0.63/mm^2 in the control group, 7.35±2.84/mm^2 in the 0.01 Gy radiation group, 8.14±2.48/mm^2 in the 0.1 Gy radiation group, and 60.07±12.52/mm^2 in the 1 Gy radiation group (Fig. 2).

The present results showed that low-doses of radiation (0.01 Gy and 0.1 Gy) did not exert significant effect on the caspase-3 expression. On the other hand, high-dose of radiation (1 Gy) significantly increased the capase-3 expression in the dentate gyrus of the rats.

Effect of fast neutron radiation on cell proliferation in the hippocampal dentate gyrus

Photomicrographs of BrdU-positive cells in the dentate gyrus of each group are presented in Fig. 3. The mean number of BrdU-positive cells in the dentate gyrus was 63.99±6.13/mm^2 in the control group, 68.17±4.50/mm^2 in the 0.01 Gy radiation group, 64.73±6.04/mm^2 in the 0.1 Gy radiation group, and 47.64±3.26/mm^2 in the 1 Gy radiation group (Fig. 3).

The present results showed that neuronal cell

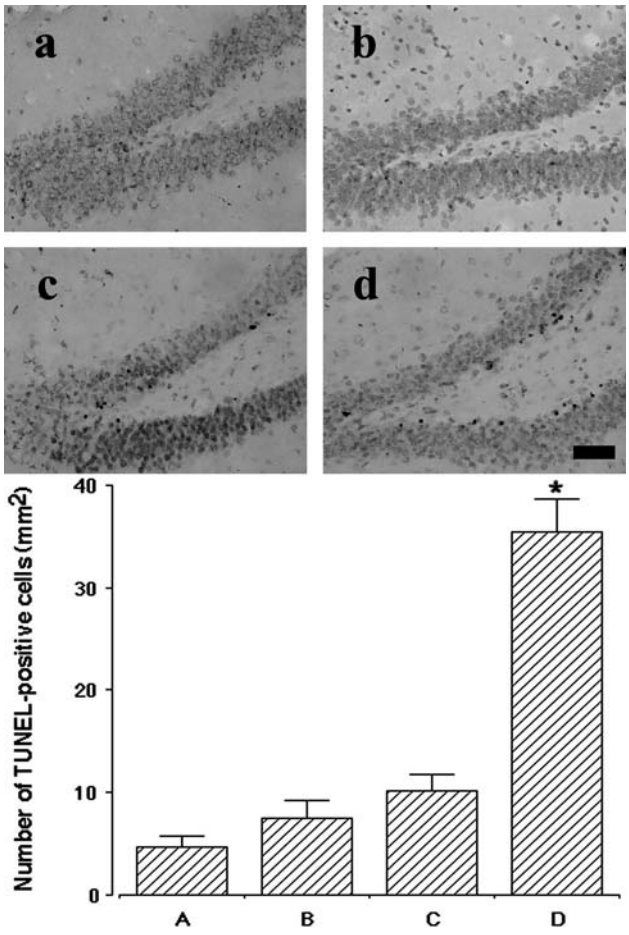

Fig. 1. Effect of fast neutron radiation on the apoptosis. Upper: Photomicrographs of the terminal deoxynucleotidyl transferase-mediated dUTP nick end-labeling (TUNEL)-positive cells in the dentate gyrus of the hippocampus in each group. (a) Control group, (b) 0.01 Gy radiation group, (c) 0.1 Gy radiation group, (d) 1 Gy radiation group. The scale bar represents 25 μm. Lower: Number of TUNEL-positive cells in the dentate gyrus of the hippocampus in each group. (A) Control group, (B) 0.01 Gy radiation group, (C) 0.1 Gy radiation group, (D) 1 Gy radiation group. The data are represented as mean±S.E.M. *$p<0.05$ compared to control group.

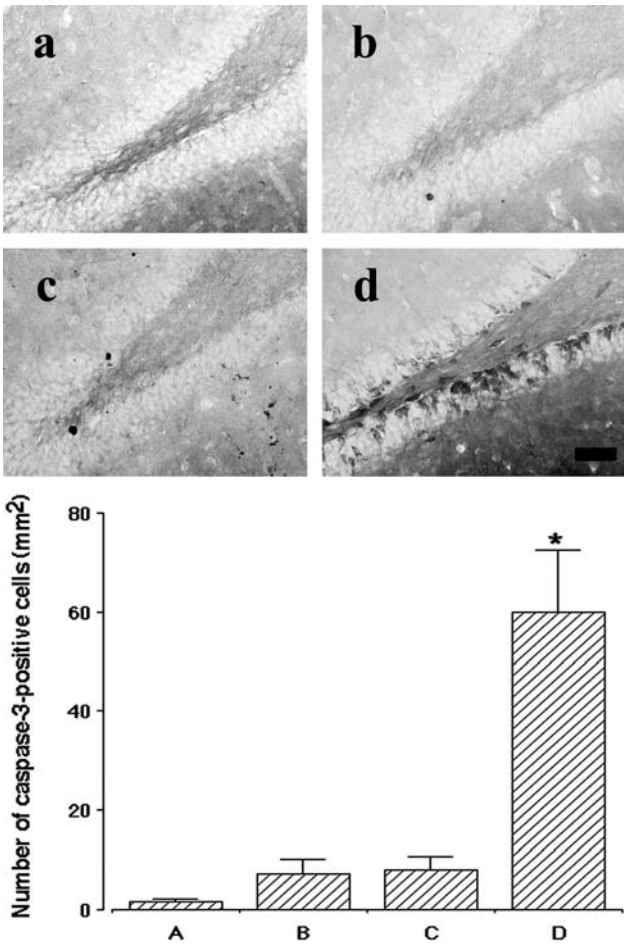

Fig. 2. Effect of fast neutron radiation on the caspase-3 expression. Upper: Photomicrographs of caspase-3-positive cells in the dentate gyrus of the hippocampus in each group. (a) Control group, (b) 0.01 Gy radiation group, (c) 0.1 Gy radiation group, (d) 1 Gy radiation group. The scale bar represents 25 μm. Lower: Number of caspase-3-positive cells in the dentate gyrus of the hippocampus in each group. (A) Control group, (B) 0.01 Gy radiation group, (C) 0.1 Gy radiation group, (D) 1 Gy radiation group. The data are represented as mean±S.E.M. *p<0.05 compared to control group.

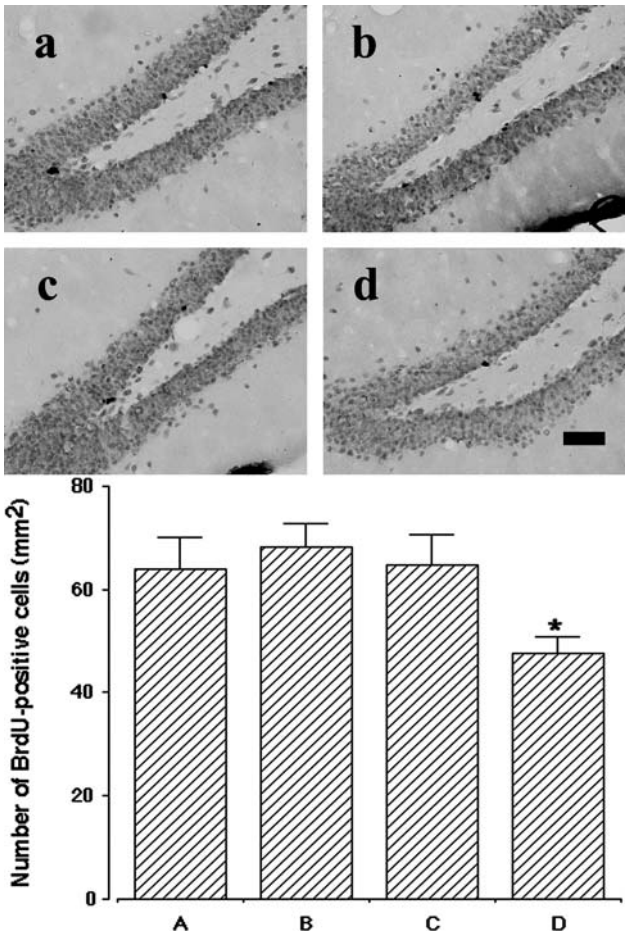

Fig. 3. Effect of fast neutron radiation on cell proliferation. Upper: Photomicrographs of 5-bromo-2'-deoxyuridine (BrdU)-positive cells in the dentate gyrus of the hippocampus in each group. (a) Control group, (b) 0.01 Gy radiation group, (c) 0.1 Gy radiation group, (d) 1 Gy radiation group. The scale bar represents 25 μm. Lower: Number of BrdU-positive cells in the dentate gyrus of the hippocampus in each group. (A) Control group, (B) 0.01 Gy radiation group, (C) 0.1 Gy radiation group, (D) 1 Gy radiation group. The data are represented as mean± S.E.M. *p<0.05 compared to control group.

proliferation in the dentate gyrus was suppressed by high-dose of fast neutron radiation (1 Gy), whereas low-doses of radiation (0.01 Gy and 0.1 Gy) did not significantly increase new cell proliferation in the dentate gyrus of the rats.

DISCUSSION

Radiations are known to cause death of neuronal precursors and neuronal cells (Peiβner et al., 1999; Lonergan et al., 2002; Nowak et al., 2006), reduce neurogenesis, and impair cognitive functions (Raber et al., 2004; Rola et al., 2004b). The major studies on the radiations have focused on X-rays. In the present study, we investigated the effects of fast neutron radiation on apoptotic neuronal cell death and cell proliferation in the hippocampal dentate gyrus of the rats.

TUNEL-positive cells are an independent marker for apoptosis representing DNA degradation (Cooper-Kuhn and Kuhn, 2002). Terminal deoxynucleotidyl transferase (TdT) enzyme tags newly generated free 3'OH DNA ends by double- and single-stranded DNA breaks with a modified dUTP nucleotide. As mentioned above, ionizing radiation damages DNA, and induces apoptotic cell death.

Many studies reported that the appearance of TUNEL-positive cells in the dentate gyrus of the rats was significantly increased after X-ray-radiation (Peiβner et al., 1999; Tada et al., 2000). Fast neutrons have been known to be more lethal than X-rays because neutron radiation can produce more complex DNA damage (Goodhead, 1999; Coelho et al., 2002).

In the present study, high-dose of radiation (1 Gy), not low-doses of radiation (0.01 Gy and 0.1 Gy), significantly increased the number of TUNEL-positive cells in the dentate gyrus of the rats. These results mean that high-dose of fast neutron radiation damaged DNA and induced DNA strand break in the dentate gytus of the rats.

Shinomiya (2001) classified radiation-induced apoptosis into premitotic and postmitotic apoptosis. During premitotic apoptosis, caspase-3 is promptly activated and the mitochondrial transmembrane potential is decreased, resulting in a rapid and strong apoptotic cell death. Although the precise mechanisms on how the caspases are rapidly activated following the exposure to ionizing radiation are not clarified, radiation-induced apoptosis is known to be triggered by a prompt activation of caspases (Shinomiya et al., 2000; Shinomiya, 2001; Lonergan et al., 2002).

Many studies have suggested that fast neutron radiation induces apoptosis through two pathways: p53-dependent and p53-independent events (Goodhead, 1999; Fischer et al., 2003). Some studies reported that fast neutron radiation induces apoptosis through p53-dependent pathway (Fischer et al., 2005). Fischer et al. (2003) reported that fast neutron radiation induces rapid cell death through various factors such as p53, caspase-8, and Bcl-2 family.

In the present study, high-dose of radiation (1 Gy) significantly increased capase-3-expression in the dentate gyrus of the rats while low-doses of radiation (0.01 Gy and 0.1 Gy) did not exert significant effect on the caspase-3 expression.

Rola et al. (2004a) reported that neurogenesis in the hippocampus is vulnerable to the radiotherapy. Radiation actually induces learning and memory deficits (Roman and Sperduto, 1995; Abayomi, 1996; Surma-aho et al., 2001), and even low-dose radiation can lead to progressive cognitive decline and memory deficits in both animals and humans (Abayomi, 1996; Surma-aho et al., 2001; Monje and Palmer, 2003; Raber et al., 2004; Byrne, 2005). Many studies have indicated that postnatal neurogenesis is necessary for the normal functions of brain, and have suggested that radiation-induced cognitive dysfunction is related to impaired neurogenesis in the dentate gyrus, because hippocampal precursor cells are extremely radiosensitive (Yoneoka et al., 1999; Madsen et al., 2003; Mizumatsu et al., 2003; Monje and Palmer, 2003; Rola et al., 2004b; Winocur et al., 2006). Radiation-induced impairment in neurogenesis induces defect in the proliferative capacity of the precursor cells (Bauer and Patterson, 2005).

The present results showed that the number of BrdU-positive cells, a marker of cell proliferation, was decreased in high-dose of fast neutron radiated group (1 Gy). On the other hand, low-doses of radiation (0.01 Gy and 0.1 Gy) were shown to increase new cell formation in the dentate gyrus of the rats; however, the augment was not statistically significant. This means that high-dose of fast neutron radiation suppressed cell proliferation in the dentate gyrus of the rats, therefore it can be suggested that high-dose of fast neutron radiation affects hippocampal-dependent learning tasks.

Here, in this study, we have shown that high-dose of neutron radiation (1 Gy) significantly increases apoptotic neuronal cell death and suppresses cell proliferation in the dentate gyrus of the rats. The present study shows that high-dose of fast neutron radiation may exert negative effects on the cognitive functions, especially hippocampal-dependent learning tasks by enhancing apoptosis and by suppressing cell proliferation of the hippocampal neuronal cells.

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REFERENCES

Abayomi OK (1996) Pathogenesis of irradiation-induced cognitive dysfunction. *Acta Oncol* 35:659-663.

Aref A, Yudelev M, Mohammad R, Choudhuri R, Orton C and Al-Katib A (1999) Neutron and photon clonogenic survival curves of two chemotherapy resistant human intermediate-grade non-Hodgkin lymphoma cell lines. *Int J Radiat Oncol Biol Phys* 45:999-1003.

Barber JBP, West CML, Kiltie AE, Roberts SA and Scott D (2000) Detection of individual differences in radiation-induced apoptosis of peripheral blood lymphocytes in normal individuals, ataxia telangiectasia homozygotes and heterozygotes, and breast cancer patients after radiotherapy. *Radiat Res* 153:570-578.

Barnea A and Nottebohm F (1994) Seasonal recruitment of hippocampal neurons in adult free-ranging black-capped chickadees. *Proc Natl Acad Sci USA* 91:11217-11221.

Bauer S and Patterson PH (2005) The cell cycle-apoptosis connection revisited in the adult brain. *J Cell Biol* 171: 641-650.

Byrne TN (2005) Cognitive sequelae of brain tumor treatment. *Curr Opin Neurol* 18:662-666.

Coelho D, Fischer B, Holl V, Jung GM, Dufour P, Bergerat JP, Denis JM, Gueulette J and Bischoff P (2002) Involvement of TP53 in apoptosis induced in human lymphoblastoid cells by fast neutrons. *Radiat Res* 157:446-452.

Cohen GM (1997) Caspases: the executioners of apoptosis. *Biochem* J 326:1-16.

Cooper-Kuhn CM and Kuhn HG (2002) Is it all DNA repair? Methodological considerations for detecting neurogenesis in the adult brain. *Brain Res Dev Brain Res* 134:13-21.

Douglas JG, Laramore GE, Austin Seymour M, Koh WJ, Stelzer K and Griffin TW (2000) Treatment of locally advanced adenoid cystic carcinoma of the head and neck with neutron radiotherapy. *Int J Radiation Oncology Biol Phys* 46:551-557.

Eriksson PS, Perfilieva E, Bjork-Eriksson T, Alborn AM, Nordberg C, Peterson DA and Gage FH (1998) Neurogenesis in the adult human hippocampus. *Nat Med* 4:1313-1317.

Fischer B, Benzina S, Jeannequin P, Dufour P, Bergerat JP, Denis JM, Gueulette J and Bischoff PL (2005) Fast neutrons-induced apoptosis is Fas-independent in lymphoblastoid cells. *Biochem Biophys Res Commun* 26:533-542.

Fischer B, Coelho D, Dufour P, Bergerat JP, Denis JM, Gueulette J and Bischoff P (2003) Caspase 8-mediated cleavage of the pro-apoptotic BCL-2 family member BID in p53-dependent apoptosis. *Biochem Biophys Res Commun* 306:516-522.

Gavrieli Y, Sherman Y and Ben-Sasson SA (1992) Identification of programmed cell death in situ via specific labeling of nuclear DNA fragmentation. *J Cell Biol* 119:493-501.

Goodhead DT (1999) Mechanisms for the biological effectiveness of high-LET radiations. *J Radiat Res* 40:1-13.

Gould E, Tanapat P, Hastings NB and Shors TJ (1999) Neurogenesis in adulthood: a possible role in learning. *Trends Cog Sci* 3:186-192.

Ishida Y, Ohmachi Y, Nakata Y, Hiraoka T, Hamano T, Fushiki S and Ogiu T (2006) Dose-response and large relative biological effectiveness of fast neutrons with regard to mouse fetal cerebral neuron apoptosis. *J Radiat Res (Tokyo)* 47:41-47.

Kantor G and Simon JM (1996) Treatment of bone metastases. *Bull Cancer Radiother* 83:275-276.

Kim SH, Kim HB, Jang MH, Lim BV, Kim YJ, Kim YP, Kim SS, Kim EH and Kim CJ (2002) Treadmill exercise increases cell proliferation without altering of apoptosis in dentate gyrus of Sprague-Dawley rats. *Life Sci* 71:1331-1340.

Kuhn HG, Dickinson-Anson H and Gage FH (1996) Neurogenesis in the dentate gyrus of the adult rat: age-related decrease of neuronal progenitor proliferation. *J Neurosci* 16:2027-2033.

Kunlin J, Manabu M, Jing QL, Xian OM, Sophie B, Roger PS and David AG (2001) Neurogenesis in dentate subgranular zone and rostral subventricular zone after focal cerebral ischemia in the rat. *Proc Natl Acad Sci USA* 98:4710-4715.

Lonergan PE, Martin DS, Horrobin DF and Lynch MA (2002) Neuroprotective effect of eicosapentaenoic acid in hippocampus of rats exposed to gamma-irradiation. *J Biol Chem* 277:20804-20811.

Madsen TM, Kristjansen PE, Bolwig TG and Wortwein G (2003) Arrested neuronal proliferation and impaired hippocampal function following fractionated brain irradiation in the adult rat. *Neuroscience* 119:635-642.

Mizumatsu S, Monje ML, Morhardt DR, Rola R, Palmer TD and Fike JR (2003) Extreme sensitivity of adult neurogenesis to low doses of X-irradiation. *Cancer Res* 63: 4021-4027.

Monje ML and Palmer T (2003) Radiation injury and neurogenesis. *Curr Opin Neurol* 16:129-134.

Nowak E, Etienne O, Millet P, Lages CS, Mathieu C, Mouthon MA and Boussin FD (2006) Radiation-induced H2AX phosphorylation and neural precursor apoptosis in the developing brain of mice. *Radiat Res* 165:155-164.

Pei β ner W, Kocher M, Treuer H and Gillardon F (1999) Ionizing radiation-induced apoptosis of proliferating stem cells in the dentate gyrus of the adult rat hippocampus. *Brain Res Mol Brain Res* 71:61-68.

Raber J, Rola R, LeFevour A, Morhardt D, Curley J, Mizumatsu S, VandenBerg SR and Fike JR (2004) Radiation-induced cognitive impairments are associated with changes in indicators of hippocampal neurogenesis. *Radiat Res* 162:39-47.

Radford IR and Murphy TK (1994) Radiation response of mouse lymphoid and myeloid cell lines. Part III. Different signals can lead to apoptosis and may influence sensitivity to killing by DNA double-strand breakage. *Int J Radiat Biol* 65:229-239.

Rola R, Otsuka S, Obenaus A, Nelson GA, Limoli CL, VandenBerg SR and Fike JR (2004a) Indicators of hippocampal neurogenesis are altered by 56Fe-particle irradiation in a dose-dependent manner. *Radiat Res* 162: 442-446.

Rola R, Raber J, Rizk A, Otsuka S, VandenBerg SR, Morhardt DR and Fike JR (2004b) Radiation-induced impairment of hippocampal neurogenesis is associated with cognitive deficits in young mice. *Exp Neurol* 188:316-330.

Roman DD and Sperduto PW (1995) Neuropsychological

effects of cranial radiation: current knowledge and future directions. *Int J Radiat Oncol Biol Phys* 31:983-998.

Shinohara K and Nakano H (1993) Interphase death and reproductive death in X-irradiated MOLT-4 cells. *Radiat Res* 135:197-205.

Shinomiya N (2001) New concepts in radiation-induced apoptosis: 'premitotic apoptosis' and 'postmitotic apoptosis'. *J Cell Mol Med* 5:240-253.

Shinomiya N, Kuno Y, Yamamoto F, Fukasawa M, Okumura A, Uefuji M and Rokutanda M (2000) Different mechanisms between premitotic apoptosis and postmitotic apoptosis in X-irradiated U937 cells. *Int J Radiat Oncol Biol Phys* 47:767-777.

Shors T, Townsend DA, Zhao M, Kozorovitskiy Y and Gould E (2002) Neurogenesis may relate to some but not all types of hippocampal-dependent learning. *Hippocampus* 12:578-584.

Surma-aho O, Niemela M, Vilkki J, Kouri M, Brander A, Salonen O, Paetau A, Kallio M, Pyykkonen J and Jaaskelainen J (2001) Adverse long-term effects of brain radiotherapy in adult low-grade glioma patients. *Neurology* 56:1285-1290.

Tada E, Parent JM, Lowenstein DH and Fike JR (2000) X-irradiation causes a prolonged reduction in cell proliferation in the dentate gyrus of adult rats. *Neuroscience* 99:33-41.

Thompson CB (1995) Apoptosis in the pathogenesis and treatment of disease. *Science* 267:1456-1462.

Tokumitsu H, Hiratsuka J, Sakurai Y, Kobayashi T, Ichikawa H and Fukumori Y (2000) Gadolinium neutron-capture therapy using novel gadopentetic acid-chitosan complex nanoparticles: in vivo growth suppression of experimental melanoma solid tumor. *Cancer Lett* 150:177-182.

Winocur G, Wojtowicz JM, Sekeres M, Snyder JS and Wang S (2006) Inhibition of neurogenesis interferes with hippocampus-dependent memory function. *Hippocampus* 16:296-304.

Yoneoka Y, Satoh M, Akiyama K, Sano K, Fujii Y and Tanaka R (1999) An experimental study of radiation-induced cognitive dysfunction in an adult rat model. *Br J Radiol* 72:1196-1201.

Yuan J and Yanker BA (2000) Apoptosis in the nervous system. *Nature* 407:802-809.