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Ionizing radiation induced long-term alterations in the adult rat rostral migratory stream

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ABSTRACT

Ionizing radiation can induce significant injury to normal brain structures. To assess radiation-induced late effects, adult male Wistar rats received whole-body exposure with fractionated doses of gamma rays (a total dose of 4 Gy) and were investigated thirty, sixty and ninety days later. Immunohistochemistry and confocal microscopy were used to determine the density of neuroblasts derived from the anterior subventricular zone (SVZa) and brain resident microglia distributed along and/or adjacent to subventricular zone–olfactory bulb axis (SVZ–OB axis). Cell counting was performed in four anatomical parts along the well defined pathway, known as the rostral migratory stream (RMS) represented by the SVZa, vertical arm, elbow and horizontal arm of the RMS. Strong overdistribution of neuroblasts was seen in the SVZa thirty and sixty days after irradiation replaced by a steep decline in the following parts of the RMS and the highest decrease ninety days after radiation treatment along the entire SVZ–OB axis. Radiation treatment led to a decline or loss of microglia in almost all counted parts through the entire experiment. Results showed that ultimate decline of the SVZa descendants and loss of microglia suggests a contributory role of reduced neurogenesis in the development of radiation-induced late effects.

Introduction

The adult mammalian brain contains highly specialized sources of neural stem cells, called neurogenic niches. Descendants of these multipotent neuronal progenitors, neuroblasts are produced in two discrete regions of the adult brain, the subventricular zone (SVZ) lining the brain lateral ventricles (LV) and the subgranular zone (SGZ) of the hippocampal dentate gyrus (DG) (Doetsch et al., 1997; Kempermann, 2002; Alvarez-Buylla and Lim, 2004). Progenitor cells of the hippocampal formation migrate into the granular cell layer (GCL) of the dentate gyrus, whereas those derived from the anterior SVZ (SVZa) migrate along a well defined pathway known as the rostral migratory stream (RMS) toward the olfactory bulb (OB), where they differentiate into granule or periglomerular interneurons and integrate into preexisting functional circuits (Carleton et al., 2003; Abrous and Koehl, 2005; Lledo et al., 2006). A variety of approaches, including morphological and immunohistochemical analyses, cell lineage or cell transplantation suggest that the SVZ contains a group of stem cells, progenitor cells and cell types of glial, endothelial and microglial origin (Doetsch et al., 1997; Alvarez-Buylla and Lim, 2004).

Radiation-induced brain injury can damage the neuronal, glial and vascular compartments of the brain and may lead to anatomical and functional deficits. Actively proliferating stem/progenitor cells are more vulnerable to radiation damage than mature cells, such as neurons. Research of irradiation effects in normal brain tissue has mostly focused on studies of single-dose irradiation (Peissner et al., 1999; Amano et al., 2002; Mizumatsu et al., 2003; Balentova et al., 2006; Lazarini et al., 2009; Balentova et al., 2011). Single moderate whole brain irradiation led to reduced numbers of neural stem and progenitor cells from the SVZ in a dose-dependent pattern, up to several months after treatment, however these changes were reversible (Tada et al., 1999; Amano et al., 2002). On the other hand, cellular responses to single exposure are rapid and massive, within hours after treatment, whereas the fractionated response is delayed and surpassed the end of radiation treatment. However,

Abbreviations: BMDCs, bone marrow-derived cells; BSA, bovine serum albumin; CD11b, cluster of differentiation molecule 11b; CD11b-IR, CD11b immunoreactive; Cox, cyclooxygenase; DCX, doublecortin; DCX-IR, DCX immunoreactive; DG, dentate gyrus; e, elbow; GCL, granular cell layer; ha, horizontal arm; ICAM-1, intercellular adhesion molecule-1; IL-1-ß, interleukin-1 beta; IL-6, interleukin-6; LV, lateral ventricle; NDPase, nucleoside diphosphatase; OB, olfactory bulb; PB, phosphate buffer; PBS, phosphate buffer saline; RMS, rostral migratory stream; SGZ, subgranular zone; SVZ, subventricular zone; SVZa, anterior horn of the SVZ; SVZ–OB, subventricular zone–olfactory bulb axis; TNF-α, tumor necrosis factor-alpha; va, vertical arm.

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a large single dose of radiation is not commonly used in clinical treatment. Fractionated treatment led to vascular structural changes, increased blood–brain barrier permeability, microglial activation, enhanced expression of astrocytes and high expression of inflammation-related molecules (cyclooxygenase, Cox; intercellular adhesion molecule-1, ICAM-1; tumor necrosis factor-alpha, TNF-α) up to six months post-irradiation (Cicciarello et al., 1996; Gaber et al., 2003; Yuan et al., 2006; Wilson et al., 2009; Zhou et al., 2011).

Microglial cells are considered to be the CNS tissue-resident macrophages and act as principal immune effector cells of the CNS responding to any pathological event. They have a considerable capacity to expand in response to injury and acute or chronic diseases in the CNS (Hailer et al., 1999; Lewis et al., 2012). Microglia become activated after injury and this process involves morphological transformation with increased expression of pro-inflammatory genes (Cox, interleukin-1 beta [IL1-β], interleukin 6 [IL-6], TNF-α) (Hwang et al., 2006; Lee et al., 2010). Rodent and human studies also suggest that activation of microglia is possibly associated with decreased hippocampal neurogenesis and cognitive function (Monje et al., 2002; Raber et al., 2004; Schindler et al., 2008).

In a previous experiment we characterized the short-term effects of fractionated irradiation on the spatio-temporal cell distribution in the neurogenic rat forebrain (Balentova et al., 2013). Based on previous analysis and by using a different schedule of radiation treatment we analyzed selected cell phenotypes resident in the SVZ–OB axis up to ninety days after the radiation treatment.

Methods

Animals

Adult Wistar male rats ($n = 15$) (SAV Dobra Voda, SR) 7–8 months old at the start of the experiment and weighing approximately 380 g were used in this study. The animals were kept in standard conditions (temperature of 22–24 °C, light-controlled environment with a12/12 h light/dark cycle) and provided with food and water *ad libitum*. The methods for the use of experimental animals were approved by the Animal Care and Use Committee, Jessenius Faculty of Medicine in Martin, Comenius University in Bratislava, Slovak Republic (approval number Ro 1663/08-221/3 for animal experiments).

Irradiation

For the irradiation procedure the animals were anesthetized by i.p. injection of ketamine (1–2 ml/kg body weight) and s.c. injection of xylazine (0.1–0.2 ml/kg b.w.). The rats were whole-body irradiated using a ^{60}Co radiation source (apparatus Teragam 02 UJP, Prague, Czech Republic) at an average dose rate of 2.47 Gy min^{-1}. Irradiated rats received 4 Gy of gamma rays (1 Gy × 4) given at seven days intervals; half of the dose per fraction (0.5 Gy) was delivered to each side of the body to ensure equal midline radiation. The animals survived thirty, sixty and ninety days after the last exposure (three animals at each time interval). Control animals were euthanized on day 30 ($n = 2$), day 60 ($n = 2$) or day 90 ($n = 2$).

Immunohistochemistry

Animals were overdosed by inhalation of a mixture containing 3% sevoflurane, 68% N_2O and 30% O_2 and transcardially perfused with saline followed by fixative 4% paraformaldehyde in 0.1 M phosphate buffer (PB) and decapitated. Brains were removed from the skull, postfixed overnight in the same fixative at 4 °C and cryoprotected in 30% sucrose for 18 h. Samples were immersed in

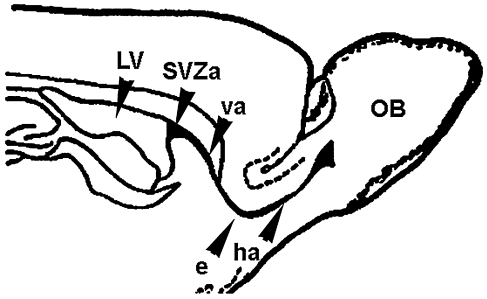

Fig. 1. Schematic sagittal view of the rat forebrain. Arrowheads indicate the individual parts along the SVZ–OB axis, where the DCX-IR and CD11b-IR cells were counted. SVZa, anterior horn of the subventricular zone; LV, lateral ventricle; va, vertical arm; e, elbow; ha, horizontal arm of the RMS; OB, olfactory bulb.
Adapted from Martoncikova, 2004.

embedding medium (Killik, Bio Optica, Milano, Italy) and immediately frozen by rapid cooling boost in cryobar (Shannon Cryotome E, Thermo Scientific, Waltham, MA, USA). Serial sagittal 30 μm frozen sections were cut, collected on lysine coated slides and air-dried. To minimize non-specific binding of the secondary antibody, sections were incubated for 1 h at room temperature (RT) in goat blocking solution (10% goat serum, 1% BSA, 0.5% Tween 20 in PBS) and then covered overnight at 4 °C with rabbit anti-doublecortin (DCX; 1:50, Cell Signaling Technology, Danvers, MA, USA) or mouse anti-cluster of differentiation molecule 11b (CD11b; 1:100, Millipore, Temecula, CA, USA), transmembrane protein, expressed on the surface of macrophages. Microtubule associated protein doublecortin has been found in cell bodies and leading processes of migrating neuroblasts. After rinsing, the sections were incubated for 2 h at RT with goat anti-rabbit secondary antibody labeled with Alexa Fluor 594 or goat anti-mouse labeled with Alexa Fluor 488 (1:100, diluted in 0.3% Triton X-100 and 1% BSA in PBS, Molecular Probes, Eugene, OR, USA) and finally coverslipped with Fluoromount (Serva, Heidelberg, Germany). The slides were viewed with an Olympus FluoView FV10i confocal laser scanning microscope (Olympus, Japan), objective of 10× with zoom up to 20× and 60× magnification equipped with Alexa Fluor 488 (excitation: 499 nm; emission: 520 nm) or Alexa Fluor 594 (excitation: 590 nm; emission: 618 nm). The image capture was performed with Olympus Fluoview FV10–ASW software, version 02.01 (Olympus) and further processed in Adobe Photoshop CS3 Extended, version 10.0 for Windows (Adobe Systems, San Jose, CA, USA).

Image analysis

Quantitative assessment was performed in a standardized counting area which included 30 μm thick serial sagittal sections from four different areas along the SVZ–OB axis *i.e.* anterior horn of the SVZ (SVZa), vertical arm, elbow and horizontal arm representing the individual parts of the RMS (Fig. 1). The vertical arm of the RMS begins in the anterior horn of brain lateral ventricles (LV) and curves ventrally between the *corpus callosum* and *corpus striatum*. Then the RMS turns in a prominent angle, the elbow located half the distance from the rostral tip of the LV to the OB and the horizontal arm, which presents the rostral half of the RMS. The numbers of DCX immunoreactive (DCX-IR) or CD11b immunoreactive (CD11b-IR) cells (red and green fluorescent cytoplasm and processes, respectively) were counted in each stained section throughout the RMS (10–15 sections per animal). Quantitative analysis was performed using ImageJ software (National Institutes of Health, Bethesda, MD, USA), a public domain image processing and analysis program. A total of 161 fields of view using 10× or 20× objective were analyzed. First RGB channels were converted to 8bit gray scale images. Threshold levels were adjusted from 27 pixels (min) to 221 pixels