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Full Length Article

Effect of whole-brain irradiation on the specific brain regions in a rat model: Metabolic and histopathological changes

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ABSTRACT

Effect of ionizing radiation on the brain affects neuronal, glial, and endothelial cell population and lead to significant morphological, metabolic, and functional deficits. In the present study we investigated a dose- and time-dependent correlation between radiation-induced metabolic and histopathological changes. Adult male Wistar rats received a total dose of 35 Gy delivered in 7 fractions (dose 5 Gy per fraction) once per week in the same weekday during 7 consecutive weeks. Proton magnetic resonance spectroscopy (^{1}H MRS), histochemistry, immunohistochemistry and confocal microscopy were used to determine whether radiation-induced alteration of the brain metabolites correlates with appropriate histopathological changes of neurogenesis and glial cell response in 2 neurogenic regions: the hippocampal dentate gyrus (DG) and the subventricular zone-olfactory bulb axis (SVZ-OB axis). Evaluation of the brain metabolites 18–19 weeks after irradiation performed by ^{1}H MRS revealed a significant decrease in the total N-acetylaspartate to total creatine (tNAA/tCr) ratio in the striatum and OB. A significant decline of gamma-aminobutyric acid to tCr (GABA/tCr) ratio was seen in the OB and hippocampus. MR revealed absence of gross inflammatory or necrotic lesions in these regions. Image analysis of the brain sections 18–21 weeks after the exposure showed a radiation-induced increase of neurodegeneration, inhibition of neurogenesis and strong resemblance to the reactive astrogliosis. Results showed that fractionated whole-brain irradiation led to the changes in neurotransmission and to the loss of neuronal viability in vivo. Metabolic changes were closely associated with histopathological findings, i.e. initiation of neuronal cell death, inhibition of neurogenesis and strong response of astrocytes indicated development of late radiation-induced changes.

1. Introduction

Our view of radiation-induced response of the brain has changed remarkably over the last several decades as preclinical and clinical research has been progressed. Radiation-induced brain injury has been often observed after radiotherapy of brain primary tumors and metastases. Based on the timeline and clinical relevance, radiation-induced brain injury is described in terms of acute, early delayed and late injury. Since both of early injuries are reversible and can resolve spontaneously, current efforts are focused on the structural and functional consequences of late radiation-induced brain injury. Late symptoms were viewed as due to decrease of proliferating capacity of glial or vascular endothelial cells and these events could ultimately produce white mater necrosis. Nowadays, there is a growing awareness that patients receiving partial or whole brain radiotherapy can develop a significant cognitive impairment at more than 6 months after irradiation, frequently in the absence of detectable anatomical abnormalities (Sundgren and Cao, 2009). Since the population of patients with late symptoms is growing rapidly, the current effort is focused on prevention/mitigation of the radiation-induced brain injury.

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Radiation-induced late brain injury is believed to reflect dynamic interactions between multiple cell types within the brain including neuronal, glial and endothelial cell populations. Very important component of radiation injury to the brain is the well-known observation that irradiation can inhibit neurogenesis. Preclinical studies conducted over the past two decades revealed that irradiation alters adult neurogenesis in two major regions: in the subgranular zone (SGZ) of the hippocampal dentate gyrus (DG) and in the subvetricular zone (SVZ) lining the brain lateral ventricles (LV) (Doetsch et al., 1997; Kempermann, 2002; Lledo et al., 2006). Rodent studies revealed wide range of radiation-induced changes on molecular, cellular and functional levels, including disruption of blood-brain barrier, microglial activation, enhanced expression of astrocytes, apoptosis of oligodendrocyte type 2 astrocyte (O-2A), high expression of inflammation-related molecules (tumor necrosis factor alpha; TNF α, interleukin-1 beta; IL-1β, intercellular adhesion molecule-1; ICAM-1, cyclooxygenase 2; COX 2), changes of synaptic plasticity and neuronal gene expression (immediate-early genes; IEG, glutamatergic N-methyl-D-aspartic acid; NMDA receptor subunits), reduction in both hippocampal-dependent and perirhinal-cortex dependent cognitive function at least 6 months post-irradiation (Cicciarello et al., 1996; Kyrkanides et al., 1999; Gaber et al., 2003; Rola et al., 2004; Brown et al., 2005; Yuan et al., 2006; Ramanan et al., 2009; Rosi et al., 2008; Kalm et al., 2009; Wilson et al., 2009; Machida et al., 2010; Zhou et al., 2011).

In the present study, we investigated whether expected radiation-induced histopathological changes in the brain neurogenic regions correlate with appropriate metabolic changes. Using morphometric measurement of brain sections and non-invasive magnetic resonance spectroscopic technique we analyzed specific cell phenotypes and quantified brain metabolites in the brain of rats, survived 18–21 weeks after irradiation.

2. Materials and methods

2.1. Animals

Adult Wistar male rats (*n = 14*) (Velaz, Prague, Czech Republic) 3–4 months old at the start of the experiment and weighing approximately 362 g were used in this study. The animals were housed in a climate controlled conditions (temperature of 22–24 °C, light-controlled environment with 12/12 h light/dark cycle) and provided with food and water *ad libitum*. The methods for animal's experiments were approved by the Animal Care and Use Committee, Jessenius Faculty of Medicine in Martin, Comenius University in Bratislava, Slovak Republic (approval number Ro 4204/14-221 for animal experiments). Experiment was carried out in accordance with Directive 2010/63/EU of the European Parliament and of the Council on the protection of animals used for scientific purposes.

2.2. Irradiation procedure

The animals were lightly anesthetized by intraperitoneal injection of ketamine hydrochloride (a dose of 1–2 ml/kg body weight) and by subcutaneous injection of xylazine hydrochloride (0.1-0.2 ml/kg body weight). All subjects received the whole brain irradiation with a total dose of 35 Gy delivered in 7 fractions (dose 5 Gy per fraction) once per week in the same weekday during 7 consecutive weeks. Equivalent 2 Gy per fraction dose EQD_2 is 60 Gy in 30 fractions (5 fractions per week) which result in the same biological effective dose BED = 117 Gy. For this BED calculation was used a linear quadratic model with a value of $\alpha/\beta = 2.1$ taken from QUANTEC (Lawrence et al., 2010). BioGrayPlus program version 2.0.3.1023 was used for radiobiological calculations (Matula and Končik, 2013). Radiation was delivered to anesthetized subjects by Teragam KO-2 device (UJP, Prague, Czech Republic) using radioactive isotope 60Co with the energy of 1.17 and 1.33 MeV. Animals were placed at the border of the radiation field. Using single posterior open field the irradiation of the whole brain and medulla oblongata was ensured without the use of additional shielding blocks (Fig. 1). The irradiated *(n = 9)* and sham irradiated animals *(n = 5)* survived 18–21 weeks after the last dose per fraction.

2.3. ^1H MRS

Proton magnetic resonance spectroscopy (^1H MRS) used a 7 T Bruker BioSpec small animal MR scanner (Bruker BioSpin MRI, Ettlingen, Germany) with an inner magnet bore diameter of 114 mm and water cooled gradients (B-GA 12S2, total gradient strength of 440 mT/m, slew rate of 3440 T/m/s). The ^1H volume resonator (Bruker BioSpin MRI, Ettlingen, Germany) was used for RF transmission and the 4-elements ^1H surface array coil (Bruker BioSpin MRI, Ettlingen, Germany) was used for signal reception. Control *(n = 5)* and irradiated *(n = 9)* animals (18–19 weeks after irradiation) were anesthetized with sevoflurane (6% sevoflurane and O_2 for induction of anesthesia and then 3.5–4.5% sevoflurane for anesthesia maintenance) and stabilized with tooth holder and nose mask in a dedicated water heated bed (Bruker BioSpin MRI). Body temperature and respiratory rate were monitored during the scanning procedure.

To ensure similar head positioning, 3D T_1-weighted reference images were acquired via an FLASH (fast low angle shot) sequence within 12.8 s measurement time. For precise anatomical imaging, T_2-weighted MRI in tree orthogonal directions were obtained with a turbo spin echo (RARE; rapid acquisition with relaxation

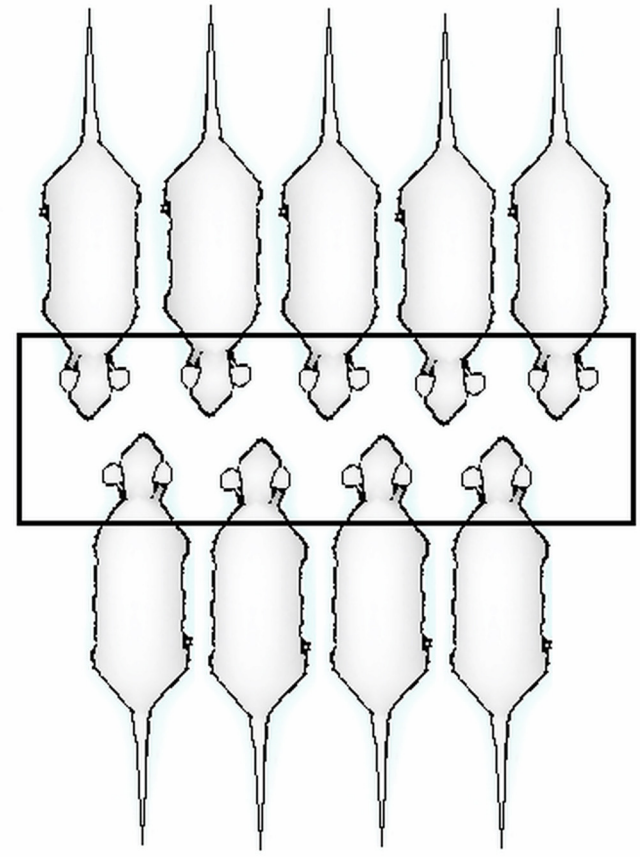

Fig. 1. Schematic drawing of the irrradiation field covered the entire head and neck of irradiated animals *(n = 9)*.