IONIZING RADIATION INDUCED APOPTOSIS IN THE ADULT RAT FOREBRAIN

Soňa Bálentová [1], Eva Hajtmanová [2], Yvetta Mellová [3], Marian Adamkov [1]

[1] *Institute of Histology and Embryology, Jessenius Faculty of Medicine, Comenius University, Martin*

[2] *Department of Radiotherapy and Oncology, Martin Faculty Hospital, Martin*

[3] *Institute of Anatomy, Jessenius Faculty of Medicine, Comenius University, Martin, Slovak Republic*

INTRODUCTION

Ionizing radiation commonly used in the radiotherapy of brain tumours can cause adverse side effects to surrounding normal brain tissue. The most significant response of adult brain to radiation damage is induction of apoptosis. Generally, proliferating stem and progenitor cells are highly vulnerable to ionizing radiation. The adult mammalian subventricular zone (SVZ) of the brain lateral ventricles retains the ability to generate neurons and glial cells throughout life. The progenitor cells arisen in the SVZ migrate along the well defined pathway - the rostral migratory stream (RMS) to the olfactory bulb (OB), which can establish some functional connections.

In the present study we investigated the occurence of radiation-induced apoptosis in the forebrain using animal model of radiosurgery. Adult male Wistar rats received single whole-body gamma irradiation with the dose of 3 Gy and survived 1, 5 or 10 days after exposure.

MATERIAL AND METHODS

Animals. The experiment was performed on adult male rats of the Wistar strain 2 – 3 months old at the beginning of experiment. The animals were anesthetized for irradiation procedure by i.p. injection of ketamine (1 - 2 ml/kg b.w.) and a s.c. injection of xylasine (0,1 - 0,2 ml/kg) and fixed in animal holder to obtain the reproducible body position. The irradiation was done with a single whole-body dose of 3 Gy of gamma rays using a ^{60}Co source (apparatus TERAGAM 02 UJP Prague) and the animals survived 1, 5 or 10 days after exposure.

Fluorescence in situ labeling of DNA strand breaks. The animals were overdosed with ether anaesthesia and after sacrifice and decapitation the brains were immediately removed from the skull and immersed in the 4 % formalin solution for 3 days and paraffin embedded according to standard protocol. Serial sagittal 10 μm thick sections were cut on microtome and after deparaffinization and pretreatment with Proteinase K, the tissue sections were processed using by In Situ Cell Death Detection Kit, POD (Roche Diagnostics). This assay allow to labeling DNA strand breaks by terminal deoxynucleotidyl transferase (TdT), which catalyzes polymerization of fluorescein labeled nucleotides to free 3`– OH DNA ends in a template-independent manner (TUNEL-reaction). Incorporated fluorescein (FITC) was detected by anti-FITC antibody Fab fragments, conjugated with horse-radish peroxidase (POD). After substrate reaction, fluorescein-labeled DNA has been detected with fluorescence microscope OLYMPUS BX 41 equipped with FITC filter, which allow to visualise areas of apoptotic cells in situ in the rat forebrain. Quantification of serial digital images obtained by digital camera OLYMPUS and the following cumputer image analysis was made of all TUNEL positively labeled cells (green fluorescence) in the selected areas of the rat forebrain and recently has been still analysed.

RESULTS AND DISCUSSION

Analysis of apoptotic cell death determined by in situ labeling of DNA strand breaks with fluorescence microscope in course of several days after exposure showed the different distribution of apoptotic cells. Considerable increase of apoptotic TUNEL-positive cells was observed 24 hrs after irradiation predominantly in caudal parts of RMS; i.e. in anterior subvetricular zone (SVZa) and vertical arm of RMS (Fig. 1D, D-a, D-b). The appearance of apoptosis following caudo-rostral gradient decreased and in olfactory bulb (OB) the apoptotic cells have been occurred only rarely (Fig. 1E). Contrary to these findings, in the following five days after exposure the numbers of apoptotic cells strongly reduced in the whole extent of RMS and decreased near to control level (Fig. 1G-I). On the other hand, major incidence of apoptotic TUNEL-positive cells was observed in animals, investigated ten days after exposure. The striking increase of apoptosis was located not only adjacent to SVZa and vertical arm of RMS, the marked increase simultaneosly affect also the OB (Fig . 1J,K). Given the previous reported evidence for high sensitivity of adult forebrain neurogenesis to ionizing radiation and our earlier works, which showed the extented cell proliferation in adult RMS with simultaneous changes in apoptosis at various time intervals after exposure, we suppose, that radiation-induced apoptosis clearly affects proliferating cell population in the adult rat forebrain.

This work was supported by AV 4/2026/08 Grant.

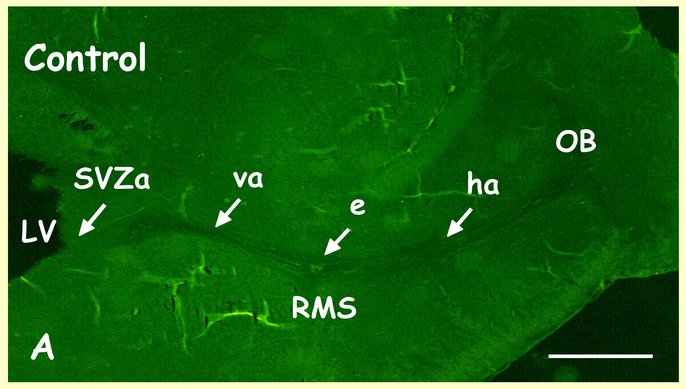

Fig.1. Distribution of apoptotic cells in the adult rat forebrain stained by fluorescence in situ labeling of DNA strand breaks (TUNEL technique). Photomicrographs of the sagittal sections through the forebrain of adult male control rat (A) showing whole extent of the RMS and the RMS individual parts (arrows), which the TUNEL-positive cells have been counted. (B – K) Details of labeled nuclei of TUNEL -positive cells (green fluorescence) in selected parts of rat forebrain (SVZa of LV, va, e, OB) of control and exposed rats 1, 5 or 10 days after irradiation with the dose of 3 Gy of gamma rays. In course of ten days after irradiation the appearance of apoptosis increased following caudo-rostral gradient. LV – lateral ventricle; SVZa – anterior subventricular zone; va – vertical arm; e - elbow; ha – horizontal arm of RMS; RMS – rostral migratory stream; OB – olfactory bulb. Calibration bars: A = 500 μm; B, C, D, E – K = 200μm; D-a = 100 μm; D-b = 50 μm.