EXOGENOUS FACTORS INDUCED APOPTOSIS IN THE ADULT RAT BRAIN

*** Soňa Bálentová, ** Enikő Račeková, * Marian Adamkov**

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

*** Institute of Neurobiology, Slovak Academy of Science, Košice, Slovak Republic*

INTRODUCTION

The mammalian subventricular zone (SVZ) of the brain lateral ventricles retains the capacity of neurogenesis and gliogenesis till adulthood. In adult brain, the fate of newly generated neurons and glial cells depends on their location. Progenitor cells of the SVZ migrate along the clearly defined pathway, the rostral migratory stream (RMS) into the olfactory bulb, where functional connections are established. Ionizing radiation, currently used in the radiotherapy, can cause significant damage to normal mammalian brain. The dose rate that can be administered is limited by potential injury to brain tissues. The most evident brain response following exposure to ionizing radiation is induction of apoptosis.

To determine if acute post-irradiation changes can be converted into long-term alterations, we investigated in the present study the proliferating activity and apoptosis in the RMS of adult rats after whole-body irradiation with sublethal dose (3 Gy) of gamma rays.

MATERIAL AND METHODS

Animals. The experiment was performed on adult male rats of the Wistar strain 2 – 4 months old at the beginning of experiment. The animals were irradiated with a single whole-body dose of 3 Gy of gamma rays using a ^{60}Co source (apparatus CHISOSTAT, CHIRANA, Prague) and survived 1, 5 and 10 days after exposure.

Tissue processing. To label proliferating cells, the thymidine analogue 5-bromo-2-deoxyuridine-BrdU (Sigma, St. Lois, MO) was administered to control and irradiated rats i.p. at the dose of 100 mg/kg body weight 24 h and repeatedly 4 h before sacrifice. The animals were anesthetized and perfused transcardially with 4% paraformaldehyde. After sacrifice the brains were immediately removed from the skull, postfixed in the same fixative overnight at 4°C and cryoprotected with 30% sucrose. Serial sagittal cryostat 42 µm thick sections were processed using the BrdU immuhistochemistry to label proliferating cells. For visualisation of dying neurons in the adult rat forebrain via fluorescence asay, another brain samples were formalin fixed and paraffin embedded. Serial sagittal 10 µm thick paraffin sections were cut on microtome and stained using by Fluoro-Jade C fluorescent dye (Chemicon Inc.).

Image analysis and quantification. The sections were observed and scanned under a 40x oil immersion objective using a digital camera (DP50) mounted on microscope (Olympus BX51) and displayed to a PC using DP Imaging software, version 3.0. We counted the number of $BrdU^+$ cells per cubic millimeter in sections containing the entire extent of the RMS (4 – 6 sections per animal) individually in vertical arm, elbow and horizontal arm of the RMS by using Dissector program version 2.0 (Tomori et al., 2001). Fluorescein-labeled Fluoro-Jade C positive cells were detected with fluorescence microscope equipped with FITC filter (green fluorescence), which allow to visualise areas of apoptotic cells. The quantitative analysis of tissue slides has been still under evaluation.

RESULTS AND DISCUSSION

Light microscopic examination of sagittal sections in various parts of the RMS (Fig. 1) of irradiated rats (3 Gy of gamma rays) showed that the initial decrease in number of $BrdU^+$ cells was similar in the whole extent of the RMS (by 50 – 63%) suggesting similar radiosensitivity of these cells along the migratory pathway. In later time intervals, however, a non uniform increase in number of $BrdU^+$ cells was seen; the highest increase occurred in the caudal part of the RMS, in the vertical arm (by 50 – 72% at the 5th – 10th days), and the smallest increase in the rostral part of the RMS, in the horizontal arm (by 33% at the 10th day) (Fig. 2). These results suggest that the BrdU labeled cells originate mainly from the SVZ and during their migration from the caudal to rostral part of the RMS they dye or cease to proliferate, or, most probably, they accumulate in the caudal parts of the RMS due to slackening of migration.

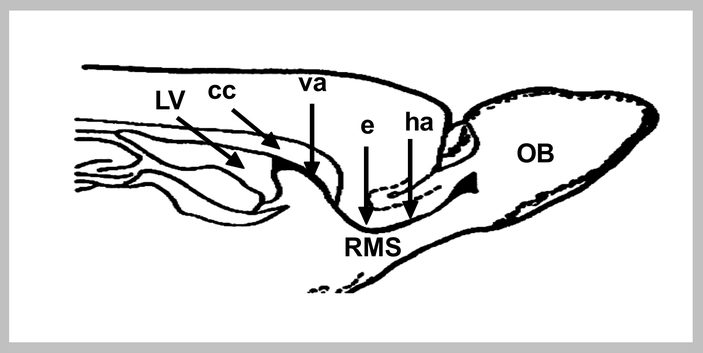

Fig. 1. Schematic sagittal view of the rat forebrain. Vertical arrows point the individual parts of the RMS, which the BrdU+ cells and Fluoro-Jade C+ cells were counted. cc — corpus callosum; LV — lateral ventricle; va — vertical arm; e — elbow; ha — horizontal arm of the RMS; OB — olfactory bulb.

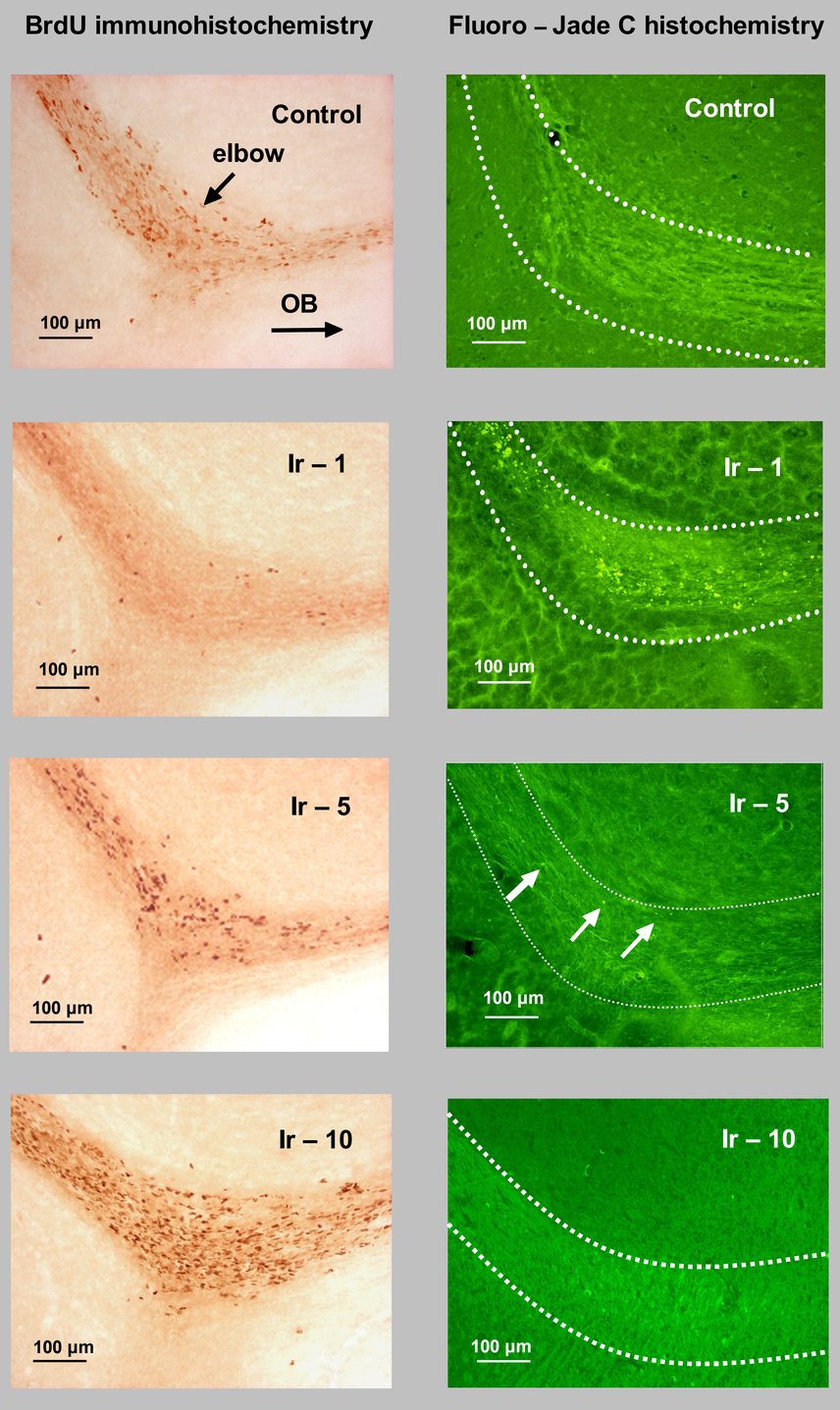

Fig. 2. BrdU immunohistochemistry and Fluoro-Jade C staining. Photomicrographs of the sagittal sections through the forebrain of adult male rats showing the labeled (dark brown) nuclei of BrdU-positive cells and apoptotic Fluoro-Jade C-positive cells (green fluorescent; white arrows) in the RMS elbow of control and experimental rats 1, 5 and 10 days after irradiation (Ir) with the dose of 3 Gy of gamma rays.

Results of our investigation correspond with the findings of Smith and Luskin (1998) concerning the different cell cycle length of neuronal progenitors in the RMS.

Exposure to single or fractionated doses of ionizing radiation significantly decreases the numbers of stem or precursor cells via apoptosis (Shinohara et al., 1997). In our pilot study, aimed to analysis of dying precursor cells in the RMS following irradiation, evidence for apoptosis was obtained by the use of Fluoro-Jade C. Recently, this fluorescent dye has been confirmed to label cells undergoing apoptosis under physiological conditions in the RMS of neonatal rats (Martončíková et al., 2003) as well as adult animals (Mitrušková et al., 2005). Our preliminary results showed that the reduction of $BrdU^+$ cells 24 hrs after the irradiation was accompanied by simultaneous striking increase in apoptotic Fluoro- Jade C positive cells. In course of the following five days after the exposure, the number of apoptotic cells significantly decreased but it was still higher than in control animals.

On the other hand, we dont observe any Fluoro-Jade C positive cells ten days after exposure contrary to simultaneous striking increase of $BrdU^+$ cells at the same interval. However, the quantitative analysis of Fluoro-Jade C positive cells in the forebrain are still evaluated, we can speculate that irradiation rather induces slowing down of proliferating cells migration than increasing the cells division. Regarding the radiation-induced damage of DNA and resulting inhibition of cell-cycle progress (Dasika et al., 1999; Melo and Toczyski, 2002), we suppose that the DNA damage might contribute to slowing down of cell migration and thus induces accumulation of $BrdU^+$ cells in the RMS.

Supported in part by AV 4/2026/08 Grant.

MVDr. Soňa Bálentová, PhD.
Institute of Histology and Embryology
Jessenius Faculty of Medicine in Martin
Comenius University
Malá Hora 4
037 54 Martin
Slovak Republic
Tel: + 421 043 4131531
E-mail: balentova@jfmed.uniba.sk

REFERENCES

Dasika, G. K., Lin, S.C. J., Zhao, S., Sung, P., Tomkinson, A., Kee, E. Y. H. (1999) Oncogene 18, 7883–7899.
Martončíková, M., Račeková, E., Orendáčová, J., Poušová, B. (2003) Physiol. Res. 52, 10P.
Melo, J., Toczyski, D. (2002) A unified view of the DNA-damage checkpoint. Curr. Opin. Cell Biol. 14, 237–245.
Mitrušková, B., Orendáčová, J., Račeková, E. (2005) Cell. Mol. Neurobiol. 8, 1255–1264.
Shinohara, C., Gobbel, G.T., Lamborn, K.R., Tada, E., Fike, J.R. (1997) Cancer Res. 57, 2694–2702.
Smith C. M., Luskin, M. B. (1998) Dev. Dyn. 213, 220–227.
Tomori, Z., Krekule, I., Kubínová, L. (2001) Image Anal. Stereol. 20, 119–130.