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Induction and repair of DNA double-strand breaks in hippocampal neurons of mice of different age after exposure to ^{60}Co γ-rays *in vivo* and *in vitro*

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Abstract. One of the central problems of modern radiobiology is the study of DNA damage induction and repair mechanisms in central nervous system cells, in particular, in hippocampal cells. The study of the regularities of molecular damage formation and repair in the hippocampus cells is of special interest, because these cells, unlike most cells of the central nervous system (CNS), keep proliferative activity, i.e. ability to neurogenesis. Age-related changes in hippocampus play an important role, which could lead to radiosensitivity changes in neurons to the ionizing radiation exposure. Regularities in DNA double-strand breaks (DSB) induction and repair in different aged mice hippocampal cells *in vivo* and *in vitro* under the action of γ-rays ^{60}Co were studied with DNA comet-assay. The obtained dose dependences of DNA DSB induction are linear both *in vivo* and *in vitro*. It is established that in young animals' cells, the degree of DNA damage is higher than in older animals. It is shown that repair kinetics is basically different for exposure *in vivo* and *in vitro*.

1 Introduction

Space journey will imminently affect the astronauts' health, because they will be exposed to Solar Cosmic Rays and Galactic Cosmic Rays in deep space, which include a wide spectrum of different quality radiation: from protons and gamma-rays to heavy charged particles [1].

Studies in recent years have shown that a lot of radiation-induced physiological impairment to CNS functions is due to damage formed in the hippocampus, which plays a key role in the formation of short-term memory and further transition into long-term memory, as well as spatial memory [2]. Moreover, the formation of new neuronal elements is carried out in hippocampus region, which is called dentate gyrus. Neurogenesis also plays a pivotal role in hippocampus function. So, the structure of the hippocampus is most radiosensitive compared to the neurons of other parts of the brain [3]. The majority of adult brain neurons are highly differentiated cells, which have lost the ability to divide and, therefore, are adequate resistant to radiation (the Bergonie and Tribondeau law, 1906).

DNA DSBs are the hardest damage by ionizing radiation, which could cause neurons death and involve disorders of the higher integrative functions of the CNS later [4-5]. Also not repaired DNA damage lead to a loss in the fidelity of information transferred from DNA to proteins [6].

The purpose of this research is to study the regularities of formation and repair of DNA DSB in hippocampal cells of different aged mice under the action of ^{60}Co γ-rays *in vivo* and *in vitro*. Because age plays a major role in susceptibility to radiation, and as it established, young aged mice brains are more radiosensitive [3, 7]. So, hippocampus is one of the brain regions most susceptible to aging [8].

2 Materials and methods

Female CBAxC57Bl, F1 mice were purchased from nursery of laboratory animals "Pushchino" (Pushchino, Russian Federation). The age of the animals in *in vivo* experiments was 8 weeks; the body weight was from 26 to 35 grams. Mice were exposed totally to Co^{60} doses from 1 to 5 Gy of γ-rays. During the experiments animals were kept in individual cages (maximum 6 animals/cage) and were provided with standard food and water *ad libitum*. At the end of experiments, animals were sacrificed by cervical dislocation. After cervical dislocation the hippocampus was dissected from the whole brains.

The cell suspension was isolated from the hippocampus of mature female hybrid mice CBAxC57Bl, F1 for irradiation with Co^{60} γ-rays *in vitro*. The age of the animals was 8 and 48 weeks; the body weight was from 26 to 35 grams. Irradiation of hippocampal cells was carried out in 0.5 ml microtubes (Eppendorf); the volume of the resulting suspension was 200 μl per dose. For irradiation, the Rocus-M radiation therapy facility (LNP, JINR) was used, delivering 0.01 Gy/s. Upon irradiation, samples were placed in an ice bath (0–4°C) for the inhibition of repair.

The procedure isolation and homogenization of mouse hippocampal cells of Seibenhener [9] was followed, with some modifications. All procedures were performed at room temperature. The extracted hippocampus was placed in a balanced HBSS (Mg [-] and Ca [-]) (Gibco). For making single-cell suspension the hippocampus was cut into small pieces (1-2 mm^3) and placed in a collagenase solution (340 U/ml) (Paneco) in HBSS(Ca [+], Mg [+]), after which the tissue samples were incubated for 15 minutes at 37°C. After dissociation, the tissue pieces were carefully pipetted until a homogeneous cell suspension was obtained. The resulting suspension was washed several times by centrifugation in HBSS (200 g), to which veal embryonic serum (10% of the volume) was added. After washing, the pellet was resuspended in 1 ml into L-15 Medium (Leibovitz) (Sigma) culture medium. Comet-assay was performed by the method described earlier [10, 11].

An Axiolab fluorescent microscope (Carl Zeiss) with an AxioCam ICc3 was used for comet image registration. 100 comets were registered for each slide. The scanning of each comet provided its fluorescence intensity profile; the comet head and tail were easily identified on this profile. The comet image was processed using CASP code [12]. The results were analyzed and the plots were constructed using OriginPro2015. Statistical analysis was performed using the Mann-Whitney U-test at $p < 0.05$.

3 Results and discussions

Figure 1 shows the dose dependence of the DNA DSB yield in hippocampal cells of mice (8-week-old) formed under the action of Co^{60} γ-rays *in vitro* and *in vivo*. The dependence is linear in both cells irradiated *in vivo* and irradiated *in vitro*. In this case, the number of lesions formed *in vitro* is greater. It was observed that each tissue of a living organism exposed *in vitro* is 2 to 5 times more radiosensitive than that irradiated *in vivo*. These effects are the result of interstitial differences in the repair of DNA strand damage that occur within 3 minutes necessary for irradiation and tissue preparation *in vivo*, these effects disappear when cells are irradiated *in vitro* on ice [13]. In response to *in vivo* irradiation, repair systems work, and there is also a certain contribution of the immune system.

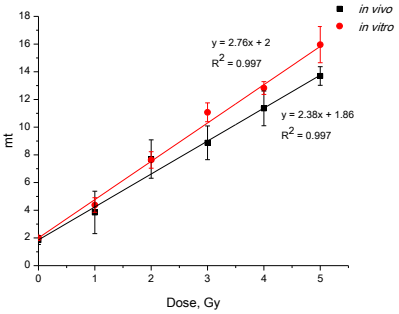

Fig. 1 Induction of DNA DSB in hippocampal cells *in vivo* and *in vitro*.

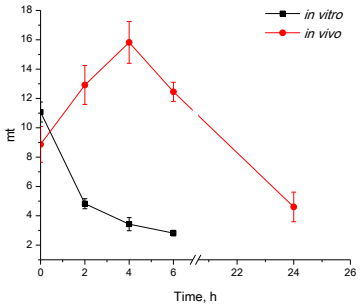

Fig.2 Kinetics of DNA DSB repair in hippocampal cells *in vivo* and *in vitro*.

Figure 2 shows the repair kinetics of DNA DSB in mice (8-week-old) hippocampal cells after exposure to Co^{60} γ-rays at the dose of 3 Gy *in vivo* and *in vitro*. *In vivo* and *in vitro* repair kinetics is significantly different. Repair kinetics *in vivo* has a complex character: after irradiation, a sharp increase of DNA DSB yield is observed up to 4 hours of post-irradiation incubation; then DSB are eliminated, and a majority is repaired; and in 24-hours, their repairs are almost completed. While repair kinetics *in vitro* is characterized by an exponential decrease in the number of lesions in the post-radiation period. These results are consistent with those obtained earlier in other authors *in vivo* [14] and *in vitro* on hippocampal neurons of lines HT22 and HN33 [15]. It can be assumed that in the formation and repair of DNA DSB *in vivo*, an important role is played by the immune and other body systems that affect the features of the course of reconstructive processes in the brain after exposure to ionizing radiation. The repair of most of the induced DNA strand breaks in the culture of mammalian cells is performed within 2 hours after irradiation (X-ray radiation) [13].

The DNA DSB induction in hippocampal cells of different aged mice (8-week-old and 48-week-old) *in vitro* was studied (Fig.3). The amount of DNA DSB, which formed in young adult mice (8-week-old), is dramatically more, that is formed in aged ones (48-week-old). This may be due to the fact that the number of immature and proliferating neurons in the hippocampus of young animals exceeds the number of mature neurons of adult mice approximately tenfold [3]. Casciati et al. [7] showed that the age of mice influences the reaction to irradiation and to neurogenesis in the hippocampus. It has been established that

the dynamic interaction of different cell types, such as neural progenitor cells, neurons, astrocytes, endothelial cells, can be associated with the negative consequences of ionizing radiation exposure to the hippocampus. Moreover, changes in proliferative activity lead to memory dysfunction. It was found that a low level of proliferation and differentiation of stem cells correlated with memory impairment [16]. This susceptibility is also explained by the well-known Bergonier and Tribondo law that cells able to proliferate are much more sensitive to radiation than already differentiated cells. This means that the hippocampal cells of older animals are more radioresistant than younger ones.

Repair kinetics of DNA DSB induced by the action of Co^{60} γ-rays at the dose of 4 Gy was researched. A comparative analysis of DNA DSB repair kinetics in hippocampal cells of different aged mice *in vitro* is shown in the Figure 4. Both dependences are characterized by continuous elimination of damage. The greater damage level induced in the young hippocampal cells once again confirms the fact that they are more radiosensitive than aged (48-week-old) animals. The efficiency of repair processes in adult aged animals is reduced in comparison with the efficiency of repair of 8-week-old mice [5, 17].

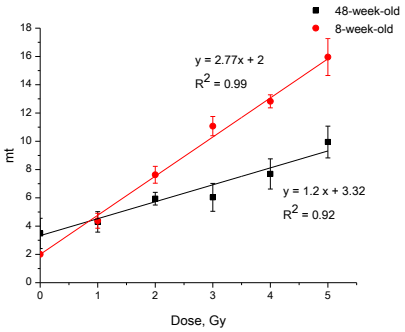

Fig.3 Induction of DNA DSB in hippocampal cells of different aged mice *in vitro*.

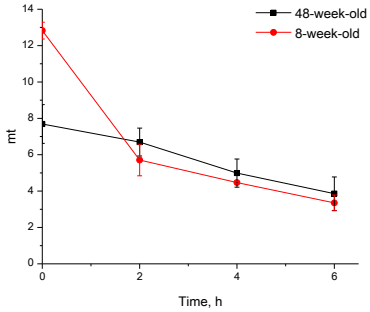

Fig.4 Kinetics of DNA DSB repair in hippocampal cells of different aged mice *in vitro*.

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