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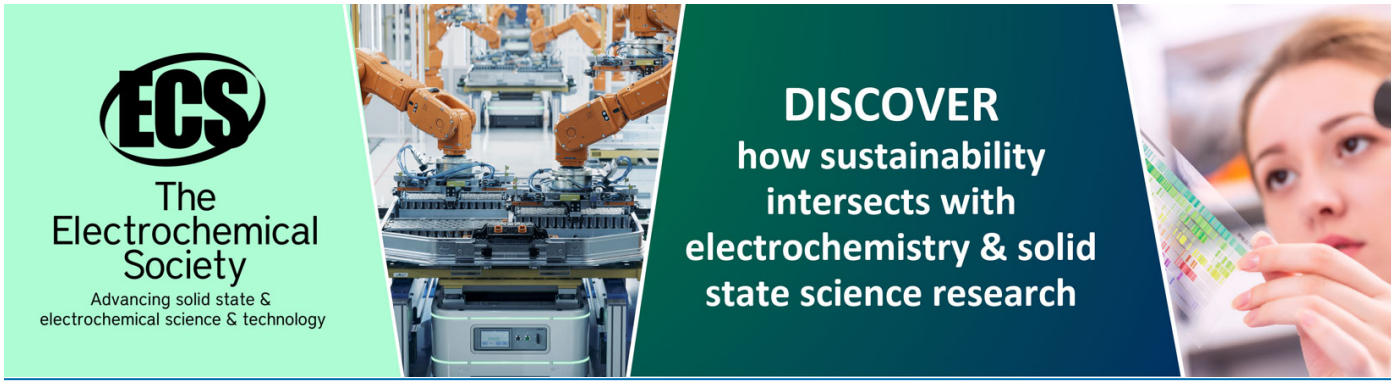

Activation of the body's natural defenses reserve of mice treated with various physico-chemical agents

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Abstract. The purpose of this work is to reveal the activation of natural defenses reserve in mice after treatment with different physico-chemical agents *in vivo* using previously developed technology of adaptive response induction. Physical agents were represented by X-rays, carbon ions, infrared light, He-Ne laser light, famine and chemical agents – by immunomodulator $CaCl_2$ and anti-inflammatory drug ibuprofen. The following tasks were set: assessment of cytogenetic damage using a micronucleus test, the weight index of lymphoid organs (thymus and spleen) and the level of ROS production in whole blood through the method of luminol-dependent zymosan-induced chemiluminescence. SHK mice were irradiated according to the scheme of adaptive response. Analysis of data on the number of cytogenetic damage in bone marrow showed that pretreatment of the animals with all investigated agents and subsequent exposure to X-rays or carbon ions at a dose of 1.5 Gy has led to a decrease in radiosensitivity compared to the nontreated animals. Similar results were observed when analyzing weight index of lymphoid organs. Determination of level of ROS production has shown that the activation index calculated according to the relation of induced to spontaneous light area, was significantly higher in all groups of mice, indicating activation of the natural defenses reserve as compared to the group exposed only at a dose of 1.5 Gy. The obtained results confirm the assumption of revealing activation of the natural defense of the organism with the help of the adaptive response induction technology.

1. Introduction

The search for effective protective methods from the damaging effects of ionizing radiation is still a pressing task of radiobiology. All known methods of radiation protection are divided according to the time of introduction into radioprotectors, mitigators and therapeutic agents. Radioprotectors have a protective effect when treating animals not more than 30 minutes before or during exposure. Mitigators are introduced immediately after exposure, and the third group concerns the protection with different chemical or physical agents that can reduce radiation damage after 5 or more hours after

treatment by activating the body's natural defenses reserve [1]. Scientists are currently intensively researching non-pharmacological treatments for various diseases for the activation of the biological reserve of resistance to various influences that can lead to genetic disorders malignant transformation of cells and emergence of developmental defects in offspring.

We hypothesized that the most promising way for revealing and study this defenses reserve is to use our technology of induction of radiation adaptive response (AR), since radiation damages induced by small exposures are virtually undetectable and can only be detected using large doses of revealing exposure. The AR phenomenon is that prior irradiation of an object in small adjusting doses of low-LET ionizing radiation (γ and X-ray) leads to reduction of sensitivity by 1.5-2 times the subsequent revealing effects of large doses of the same exposure. The data we obtained earlier, mainly with the induction of an AR with the help of radiation exposure, showed that it depends on various conditions: the magnitude and dose rate, the time parameters between the adapting and revealing doses, the radiation quality, and also the individual sensitivity of the organism [2].

With regard to the high-LET ionizing radiation (protons or carbon ions) used in radiotherapy, there is almost no data on their induction of activation of natural protection. We have shown that, in contrast to γ-radiation, preliminary irradiation of mice with secondary radiation from protons with an energy of 70 GeV and π-mesons did not induce a radiation AR [3]. These results supplemented known data obtained in vitro studies on the inability of high- LET ionizing radiation to induce a radiation AR, since it was shown in vitro that secondary radiation induces difficult and unrepaired DNA damage and, to some extent, itself inhibits DNA repair [4]. In addition, the phenomenon when adapting and revealing exposures are factors of various nature is called cross-adaptive response, which can also be seen as one way to detect activation of natural defenses reserve [5, 6].

In recent decades, medical practice has adopted many various instruments based on the use of non-ionizing low-intensity electromagnetic radiation. Infrared light (IRL) and He-Ne laser (HNL) radiation that have bio-stimulating effect are most commonly application [7–12]. Despite these there are few dates on genotoxic effects of these radiations with certain operating parameters, they are controversial and not classified according to power and damage criteria [13–16]. The existing data do not allow concluding about the optimal doses of different radiations for activation of natural protection reserve. Therefore, the problem of finding different agents of physical or chemical nature capable of activation of the body's natural defense, same as small doses of ionizing radiation, is very relevant.

Therefore, the aim of this work is to detect activation of body's natural defenses reserve through radiation and cross adaptations induction technology when treating mice with different physical and chemical adaptogens and revealing doses of low- and high-LET ionizing radiation. The following tasks were set: assessment of cytogenetic damage using a micronucleus test in bone marrow cells, the weight index of lymphoid organs (thymus and spleen) and the level of reactive oxygen species (ROS) production in whole blood.

2. Materials and methods

Experiments were performed with male outbred albino SHK mice (body weight 26–30 g) at an age of two months. The animals were kept under the standard conditions in the vivarium of the Institute of Theoretical and Experimental Biophysics (Russia). The experiments conformed to the regulations and legal acts concerning the procedures of animal experiments and the humane treatment of animals.

2.1. Experimental condition

Physical agents for adaption of animals were the following: famine during 24 hours, ionizing radiation – carbon ions with the energy of 450 MeV/nucleon at a dose of 0.1 Gy (uniform pulse beam, 1 times in 8 sec, release duration 0.8 sec / Protvino) and non-ionizing radiation (IRL diode matrix – irradiation of the whole body of the animal for 10 min (850 nm, 22 mW/cm^2) and HNL – for 15 and 100 sec (632.8 nm, 0.7 mW, 0.16 mW/cm^2), at which the nose of the animal was irradiated.

Chemical agents were represented by immunomodulator $CaCl_2$ (1 g/250 ml of water was given to animals during 6 days) and pharmacological anti-inflammatory drug ibuprofen (i.p. 10 mg/kg once).

One day after the treatments, all groups of animals were additionally irradiated with carbon ions or X-rays at doses of 1.5 Gy according to the earlier used scheme (0.1 Gy + 1.5 Gy) [17].

As a positive control mice were irradiated with X-rays at doses of 0.1 and 0.5 Gy for the radiation AR induction (RUT setup, Mosrentgen, Russia; 0.1 Gy/min, 200 kV, 8 mA, filters 1.0 mm Cu and 1.0 mm Al / Pushchino).

2.2. Experimental methods

Animals were taken from the experiment through decapitation 28 h after exposure to X-rays or carbon ions at a dose of 1.5 Gy, and the bone marrow samples were prepared in order to count polychromatic erythrocytes (PCE) with micronuclei (MN) using standard technique [18]. The experimental point included 5 mice and at least 20000 analyzed PCE. The relative weight of the thymus and spleen were calculated against average absolute organ weight to the average weight of an animal in the group (weight index). Level of ROS production was measured in the whole blood using the method of luminol-dependent zymosan-induced chemiluminescence (ChL) with 12-channel device Chemilum-12 (Russia). Light area was determined under the curve at spontaneous and induced ChL (S_{sp}, S_{ind}), as well as the activation index (AI), which is the ratio of the S_{ind}/S_{sp} and characterizes the strengthening of ChL. Measurements of ChL in the blood of control and irradiated animals were conducted in parallel. The significance of differences between groups was estimated using the Student's t-test.

3. Results and discussion

Table 1 contains data on the number of PCE with MN in the bone marrow of mice treated to IRL, HNL, carbon ions, X-rays, $CaCl_2$ and ibuprofen, with subsequent exposures at a dose of 1.5 Gy of carbon ions or X-rays.

Table 1. The number of polychromatic erythrocytes with micronuclei in the bone marrow of mice treated to IRL, HNL, carbon ions, X-rays, $CaCl_2$ and ibuprofen, with subsequent exposures at a dose of 1.5 Gy of carbon ions or X-rays.

Experimental condition	Number of mice	Number of PCE	Number of PCE with micronuclei	% of PCE with micronuclei
0.1 Gy X-ray + 1.5 Gy C ions	5	20000	2768	6,9±0,72*
0.1 Gy C ions + 1.5 Gy C ions	5	20000	1136	5,7±0,36*
HNL 15 sec + 1.5 Gy C ions	5	20000	1213	6,1±0,27*
IRL 10 min + 1.5 Gy C ions	5	20000	1140	5,7±0,42*
Famine + 1.5 Gy C ions	4	20000	1124	5,6±0,35*
$CaCl_2$ + 1.5 Gy C ions	5	20000	1359	6,8±0,46*
Ibuprofen + 1.5 Gy C ions	5	20000	1297	6,5±0,35*
1.5 Gy C ions	5	20000	1947	9,7±0,65
0.1 Gy C ions + 1.5 Gy X-ray	5	20000	1195	5,9±0,41*
0.1 Gy X-ray + 1.5 Gy X-ray	5	20000	837	4,19±0,72*
HNL 15 sec + 1.5 Gy X-ray	5	20000	1213	6,10±0,45*
IRL 10 min + 1.5 Gy X-ray	5	20000	1140	5,70±0,5*
Famine + 1.5 Gy X-ray	5	20000	899	4,50±0,37*
$CaCl_2$ + 1.5 Gy X-ray	5	20000	1402	6,6±0,47*
Ibuprofen + 1.5 Gy X-ray	5	20000	1245	6,2±0,32*
0.5 Gy X-ray + 1.5 Gy X-ray	5	20000	1606	8,0±0,42
He-Ne 100 sec + 1.5 Gy X-ray	5	20000	1444	7,2±0,28
1.5 Gy X-ray	5	20000	1681	8,4±0,55

* Difference from a group of mice irradiated at a dose of 1.5 Gy, $p < 0.05$

Table 1 shows that the pretreatment of animals with all investigated agents and subsequent exposure to X-rays or carbon ions has led to a decrease in radiosensitivity compared to non-treated animals, which may indicate activation of the body's natural defenses using the cross AR. Preliminary exposure of animal to X-ray at higher doses of 0.5 Gy or HNL radiation during 100 sec and subsequent X-ray exposure at a dose of 1.5 Gy do not lead to a decrease in the number of cytogenetic damage, i.e. activation of the natural protection depends on the dose, as well as the induction of the AR according to the scheme (0.1 Gy + 1.5 Gy). These data are consistent with the results on the induction of AR obtained on mice irradiated with HNL [19].

The weight index of the thymus and spleen were measured simultaneously with the measurement of cytogenetic damage in bone marrow of mice. Thymus and spleen, along with bone marrow, are blood-forming organs with actively proliferating tissue. Figure 1 presents the weight index of thymus and spleen of mice treated to IRL, HNL, carbon ions, X-rays, $CaCl_2$ and ibuprofen, with subsequent exposures at a dose of 1.5 Gy of carbon ions or X-rays. It can be seen that in case of exposure of the untreated mice, thymus and spleen weight index decreases only at a dose of 1.5 Gy. Pretreatment of animal groups with 0.1 Gy of carbon ions, 0.1 Gy of X-ray, HNL 15 sec and IRL10 min has protected thymus from decrease of weight index only when exposed to 1.5 Gy of X-ray, which indicates activation of protective body functions under these conditions. At the same time pretreatment of animals with investigated agents did not restore the weight index of thymus after exposure to these rays compared to the group of untreated animals. Similar results are observed in the analysis of the spleen weight index. Thus, activation of the protection depends not only on the pretreatment quality, but also on the quality of revealing exposure.

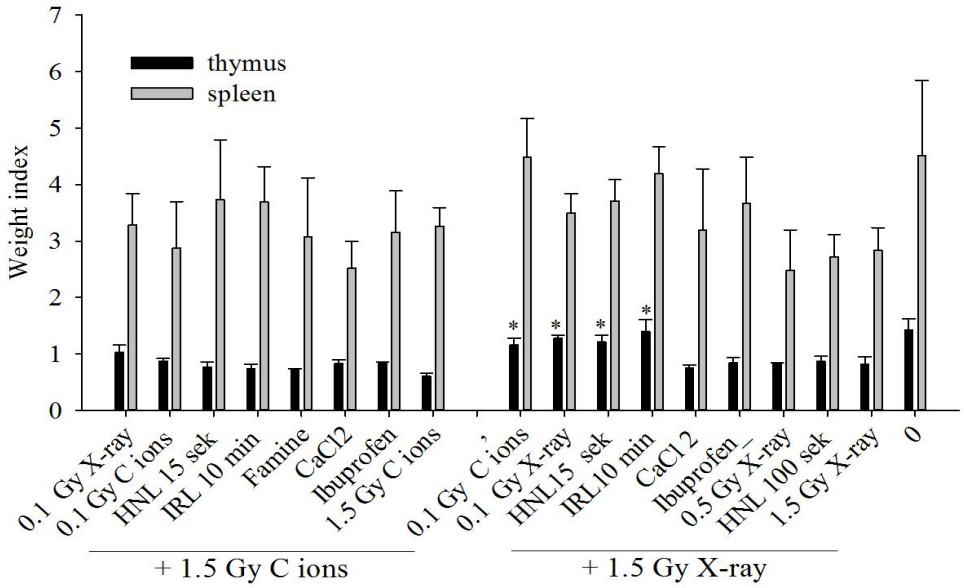

Figure 1. The weight index of thymus and spleen of mice treated to IRL, HNL, carbon ions, X-rays, $CaCl_2$ and ibuprofen, with subsequent exposures at a dose of 1.5 Gy of carbon ions or X-rays.
* Difference from a group of mice irradiated at a dose of 1.5 Gy, $p < 0.05$.

It is known that small doses of radiation stimulate weak oxidative stress with the formation of excessive amounts of different ROS. It has been shown in a number of works that the ROS concentration correlates with the activity of the organism [20–22], so the authors examined the level of ROS production in blood cells of the treated and then exposed to 1.5 Gy of X-rays or carbon ions mice. Data for assessing the level of ROS production by neutrophils in the whole blood of mice are

presented in figure 2. The amount of spontaneous ChL in neutrophils was measured, which characterizes the basal level of activation of these cells, and the stimulation of oxygen metabolism using opsonized zymosan was performed in order to determine the reserve activation capacity of neutrophils. The activation index was evaluated against the relation of area of ChL induced by zymosan to the area of spontaneous ChL.

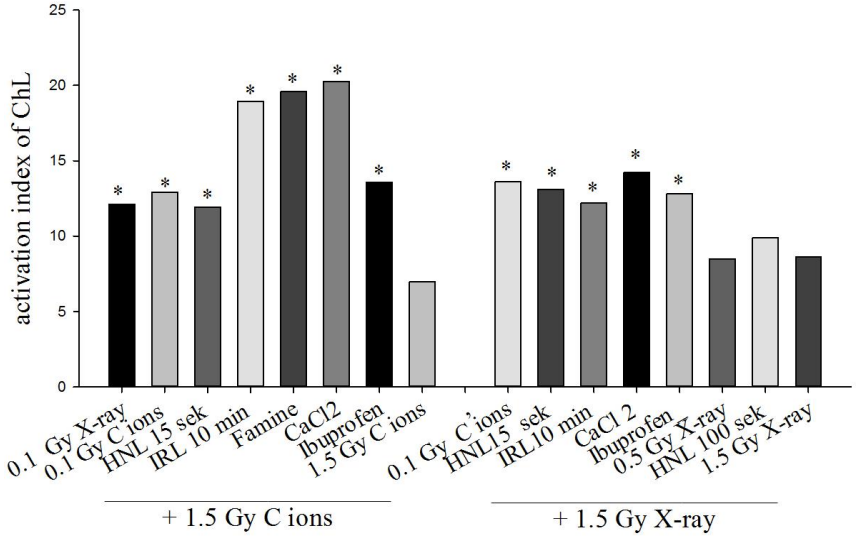

Figure 2. The index of chemiluminescence activation of mice treated to IRL, HNL, carbon ions, X-rays, $CaCl_2$ and ibuprofen, with subsequent exposures at a dose of 1.5 Gy of carbon ions or X-rays.
* Difference from the groups of mice irradiated at a dose of 1.5 Gy, $p < 0.05$.

Figure 2 presents the value of chemiluminescence AI for mice treated to all investigated agents, and subsequent exposure at a dose of 1.5 Gy of carbon ions or X-rays. It can be seen from figure 2 that the AI was significantly higher in all groups of pre-treated mice, compared to the group irradiated only at doses of 1.5 Gy of X-rays or carbon ions, indicating the activation of natural protection. At the same time, the preliminary irradiation of animals with X-rays at a dose of 0.5 Gy or HNL radiation for 100 sec and subsequent X-ray exposure at a dose of 1.5 Gy did not increase the AI compared with the group 1.5 Gy, i.e. there was no protection, same as during determination of the level of cytogenetic damage. The effects obtained can be explained by the inclusion of epigenetic mechanisms (signals) that are not associated with a change in the nucleotide sequence of DNA itself and can be indirectly encoded in the genome, which requires further research.

4. Conclusion

Thus, the results obtained on identification of methods for protection of mice treated with investigated physico-chemical agents, as well as in the case of induction of the AR, depend on the magnitude of the dose and the quality of the activating and revealing exposures, tissues and methods. These data demonstrate the relationship between primary signals and cellular responses in the hematopoietic and lymphoid organs, and confirm the assumption of activation of the body's natural defense by technology of induction AR.

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