RAD Conference Proceedings, vol. 2, pp. 15–18, 2017
www.rad-proceedings.org

# THE STUDY OF BIOLOGICAL EFFECTS INDUCED BY ACCELERATED $^{12}C$ IONS WITH AN ENERGY OF 450 MeV/N ON MICE *IN VIVO*

**Svetlana Sorokina[1*], Svetlana Zaichkina[1], Olga Rozanova[1], Alexander Shemyakov[1], Helena Smirnova[1], Sergey Romanchenko[1], Alsu Dyukina[1], Olga Vakhrusheva[1], Vladimir Pikalov[2]**

[1]Institute of Theoretical and Experimental Biophysics, Russian Academy of Sciences, Pushchino, Russia
[2]Institute of High Energy Physics, National Research Center "Kurchatov Institute", Protvino, Russia

***Abstract.*** *In connection with the active space exploration and the search for new sources for tumor radiotherapy, studies of the effects of low doses of radiation, which are characterized by a high LET, are currently of particular interest. The therapy with heavy charged particles becomes of more and more interest all over the world, and many medical centers tend to use heavy ion beams in radiotherapy. We investigated the biological effects induced by accelerated $^{12}C$ ions with an energy of 450 MeV/n in the Bragg peak in a dose range of 0.1–1.5 Gy in mice in vivo. It was found that: (1) the dose dependence of the level of cytogenetic damage in the bone marrow is nonlinear; (2) changes of the thymus and spleen weight index depend on the dose and the quality of radiation, and this index is considerably reduced as compared to that of unirradiated mice; and (3) the level of spontaneous ROS production in blood cells increases in comparison with irradiation at the same doses of X-rays. We calculated the ratio of biological effects under the action of accelerated carbon ions to the effects of same doses of X-ray radiation. The obtained coefficient served as an index of the radiation efficiency (IRE). Thus, it was found that the average IRE value for accelerated carbon ions with an energy of 450 MeV/n in the Bragg peak in the dose range examined varied from 1.1 to 2.4 and was independent of selected biological endpoints in mice in vivo.*

***Key words*****:** *Carbon ion, dose dependence, micronucleus test, weight index, ROS, chemiluminescence, cancer therapy, Bragg peak, mice*

**DOI:** 10.21175/RadProc.2017.04

## 1. INTRODUCTION

The world clinical practice shows that radiation therapy is the primary method of therapy for cancer. Up to 70% of cancer patients need one or another kind of radiation therapy, with advisable exposure to proton or heavy ion beams for approximately 20% of patients. The capacity of hospital centers for hadron therapy is about 1000 patients/year [1]. Now in the world, there are a total of 9 centers where beams of carbon ions are used for radiotherapy. Currently, a new carbon beam therapy with accelerated $^{12}C$ ions with an energy of 450 MeV/n is being developed on the basis of the U-70 particle accelerator at the Institute of High Energy Physics (Protvino, Russia). The studies of the biological effects induced by accelerated $^{12}C$ ions on mice in vivo are not only of fundamental importance but also are necessary for understanding the mechanisms underlying mutagenesis and carcinogenesis [2]. The results of these investigations can be used in practice for solving important problems of modern radiobiology, such as the assessment of the risk of exposure to low doses as well as a search for the possibility of protection against the damaging effects of low doses of radiation and the most efficient sources of radiation to treat cancer. Conventional radiation therapy uses photons (gamma- and X-rays) to attack cancerous and noncancerous tumors. Photon beams carry a low radiation charge and have no mass than proton beams. As a consequence, much of the photon beam energy is deposited in the healthy tissue surrounding a tumor, causing side effects and unnecessary tissue damage while sometimes not even reaching the tumor with an adequate dose of radiation. It is well known that high-density radiation is more effective in inducing biological damage than X-rays or γ-radiation [3, 4]. It is characterized by a large value of relative biological effectiveness of cell damage, reduced oxygen effect, and weak dependence on the cell cycle, which makes it potentially more suitable for tumor radiotherapy than the low-density radiation [5]. Charged particle beams are characterized by a better dose distribution in the target than photon beams and dose sparing of normal tissue near the tumor.

This is due to a special mechanism of transferring the energy of accelerated particles in biological tissues in the Bragg peak, which consists of maximum energy production at the end of the path when the ion stops in the tumor. Thus, therapy with heavy charged particles becomes of more and more interest all over the world, and many medical centers tend to use charged particles

[*]sv0723@yandex.ru

in radiotherapy. However, when irradiated before and after the Bragg peak, healthy tissues also experience a dose load that should also be taken into account [6, 7, 8].

The second important aspect of the study of the biological effect of accelerated carbon ions is related to the current stage of development of space radiobiology, because in the near future some countries plan long interplanetary manned flights to the Moon and Mars during which the crew will be exposed to galactic cosmic radiation in the spectrum of which protons and high energy ions (carbon and iron) dominate. The research of the biological effect of accelerated heavy particles in animals is insufficient because of the limited sources of high-energy radiation and the complexity of these experiments [9]. A study of low doses of accelerated carbon ions, which are comparable with predicted doses during space flights, will assess the contribution of the genetic risk to the overall spectrum of adverse effects of flight factors. In addition, it is very important to solve the issues related to the evaluation of radiation effects on persons working in mixed radiation fields and their offspring.

The purpose of this study was to investigate the biological effects induced by accelerated carbon ions with an energy of 450 MeV/n in the Bragg peak in mice: the dose dependence of cytogenetic damage to the bone marrow, the weight index for the thymus and the spleen, the induction of reactive oxygen species (ROS) in whole blood and to calculate the IRE.

## 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.

Mice were irradiated with accelerated carbon ions with an energy of 450 MeV/n on a U-70 particle accelerator at the Institute of High Energy Physics (Protvino, Russia) in a dose range from 0.1 to 1.5 Gy in the spread-out Bragg peak. The Bragg peak was modified with the use of the ridge filter. We used the pulsed radiation mode (one pulse per 8 s). For comparison, another group of mice was irradiated with X-rays in the same dose range. Mice were exposed to X-ray radiation using a RUT setup (Mosrentgen, Russia; 1 Gy/min, 200 kV, 8 mA; Puschino). Sham-irradiated animals were used as controls. The gafchromic EBT3 films (USA) and a neutron monitor were used for carbon beam profile verification and dose control.

The level of cytogenetic damage to the bone marrow was assessed using the frequency of occurrence of polychromatic erythrocytes (PCEs) with micronuclei (MN) (the micronucleus test). To determine the level of cytological damage, animals were euthanized by cervical dislocation 28 h after revealing irradiation, and cytological preparations of the bone marrow were prepared using the standard technique [10]. At least five animals were used for one point (15 000–30 000 PCEs were counted). Relative weights of the thymus and spleen (weight index) were calculated as the ratio of the mean absolute organ weight to the mean animal weight in the group. The level of spontaneous ROS production was assessed in whole blood using spontaneous luminal-dependent chemiluminescence (CL) on a 12-channel CHEMILUM-12 device. Although, along with neutrophils, the whole blood contains erythrocytes, monocytes, and platelets, they do not significantly contribute to the final chemiluminescence. Luminol interacts with ROS (HO, $O_2$-, and $H_2O_2$), which makes it possible to measure the entire ROS pool in the whole blood, and acts as a nonspecific luminescent probe.

The significance of differences between groups was estimated using the Student's *t-test*.

## 3. Results

### *3.1. Dose dependence of cytogenetic damage*

To study the dose dependence, one group of mice was irradiated with accelerated carbon ions in a dose range of 0.1–1.5 Gy, and another group was irradiated with X-rays in the same dose range. The dose dependences obtained were compared, and these data were used to calculate the IRE of accelerated carbon ions with an energy of 450 MeV/n in the Bragg peak. The results of the experiment are shown in Fig. 1. It can be seen that the segments of curves at low doses from 0.1 to 1 Gy statistically significantly diverge ($p \leq 0.05$) as the dose increases and practically coincide at the dose of 1.5 Gy. Thus, the IRE reached the maximum value of 2.4 at low doses and was equal to 1 at 1.5 Gy.

These data differ from the results obtained in the our study concerning the effect of accelerated $^{12}C$ ions, with energies of 200 MeV/n to the Bragg peak, in the Nuclotron appliance of the Joint Institute for Nuclear Research (Dubna, Russia) on the level of cytogenetic damage to the mice bone marrow, and demonstrate that segments of curves obtained at low, 0 to 0.5 Gy, doses were almost identical and diverged only with the dosage increase [11]. At doses from 0 to 0.5 Gy, statistically non-significant differences between the groups were observed, and at higher doses the differences between the groups became statistically significant ($p \leq 0.05$). Thus, the IRE was equal to 1 at low doses and reached the maximum value of 1.4 as the dose increased.

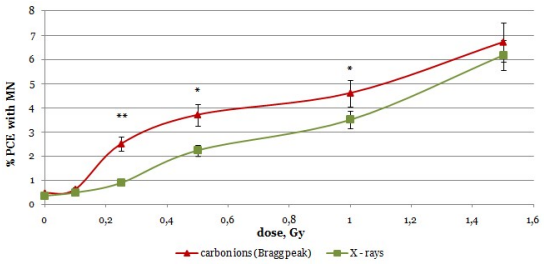


Figure 1. The number of PCE with MN in the bone marrow of mice irradiated with accelerated carbon ions and X-rays (each point represents the data from 6 mice per treatment group)

### *3.2. Weight index for lymphoid organs*

In the next series of experiments, we studied the influence of accelerated carbon ions and X-ray on the weight index for the thymus and the spleen in mice.

As shown in Fig. 2 (a), the weight index for the thymus in mice irradiated with carbon ions at doses from 0.1 to 0.5 Gy statistically significantly increased compared to that of mice irradiated with X-rays at the same doses ($p \leq 0.05$).

The average IRE of with accelerated carbon ions with an energy of 450 MeV/n in the dose range from 0.1 to 0.5 Gy determined by the weight index for lymphoid organs in mice *in vivo* was about 1.5.

As it can be seen in Fig. 2 (b), the weight index for the spleen of mice irradiated with carbon ions at the doses of 0.1, 1 and 1.5 Gy was significantly lower compared to mice irradiated with X-rays at the same doses ($p \leq 0.05$). Moreover, the dose-dependent decrease in the weight index for the spleen of mice irradiated with carbon ions and X-rays relative to the control was registered throughout the examined dose range.

Kallman and Kohn have shown that thymus and spleen weight loss is a highly variable endpoint after irradiation, which depends on the time of sampling [12, 13]. Presumably, it is for this reason that we observe different dynamics of changes in the weight index for lymphoid organs at the same doses.

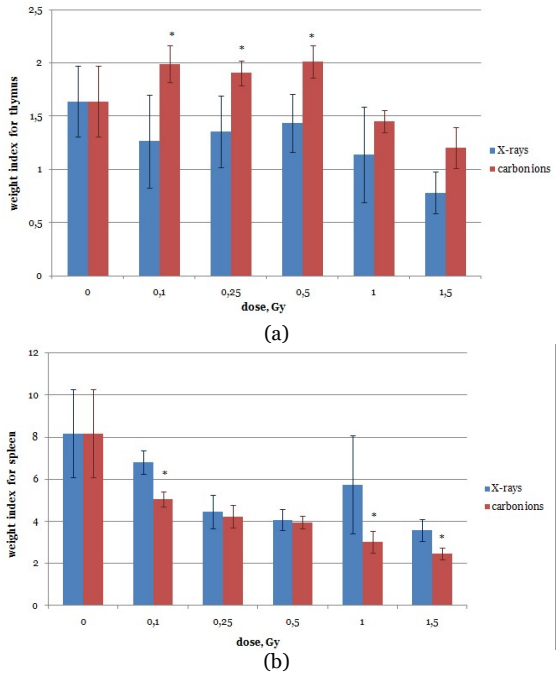


Figure 2. Weight index for the thymus (a) and the spleen (b) of mice irradiated with accelerated carbon ions and X-rays (each point represents the data from 6 mice per treatment group)

### *3.3. ROS production*

The data on the level of ROS production in blood cells of mice irradiated with accelerated carbon ions with an energy of 450 MeV/n and X-rays in a dose range of 0.1–1.5 Gy are shown in Fig. 3.

As expected, the irradiation throughout the dose range significantly intensified ROS production in comparison with intact animals. At the same time, the level of ROS production in mice irradiated with accelerated carbon ions at doses from 0.1 to 1 Gy significantly increased compared with irradiation at the same doses of X-rays. The average IRE evaluated by spontaneous ROS production for the accelerated carbon ions with an energy of 450 MeV/n in the dose range from 0.1 to 1 Gy in mice *in vivo* was about 2.

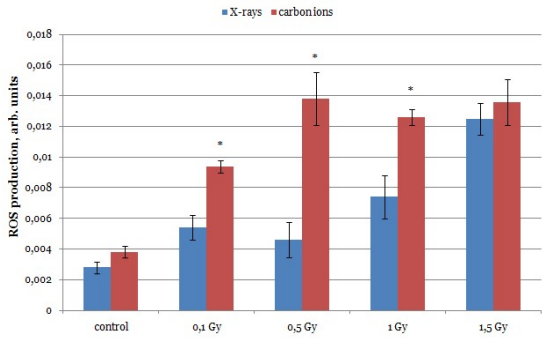


Figure 3. The level of ROS production in blood cells in mice irradiated with accelerated carbon ions and X-rays (each point represents the data from 6 mice per treatment group)

It is well known that the physiological levels of reactive oxygen and nitrogen species determine the roles they play in many cellular functions. In irradiated cells, the levels of these reactive species may increase due to perturbations in oxidative metabolism and chronic inflammatory responses, thereby contributing to acute and long-term effects of exposure to ionizing radiation on genomic stability. Delayed effects of radiation exposure have been attributed to persistent oxidative stress in tissues, which results from functional changes in mitochondria, a major source of ROS in cells. Although ROS generation in mitochondria is a part of normal oxidative metabolism, enhanced ROS production could occur following damage to the mitochondrial membrane and loss of the mitochondrial membrane potential (MMP) due to exposure to stressors like radiation and chemicals. Compared with low-LET radiation, high-LET radiation has been shown to induce higher oxidative stress and greater DNA damage response determined by 53BP1 and γH2AX accumulation in cells [14, 15].

## 4. Conclusion

In summary, the study of the effect of irradiation of mice with carbon ions with an energy of 450MeV/n in the Bragg peak in a dose range of 0.1–1.5 Gy *in vivo* showed that (1) the dose dependence of the level of cytogenetic damage in the bone marrow is nonlinear; (2) the changes of thymus and spleen weight index depend on dose and quality of radiation and are considerably reduced as compared to unirradiated mice; and (3) the level of ROS production in blood cells increases in comparison with irradiation in the same doses of X-rays.

Thus, it was found that the average value of IRE for accelerated carbon ions with an energy of 450 MeV/n in the Bragg peak in the examined dose range varied

from 1.1 to 2.4 and was independent of selected biological endpoints in mice *in vivo*.

## References


1. A. H. Aitkenhead et al., "Modelling the throughput capacity of a single-accelerator multitreatment room proton therapy centre," *The British Journal of Radiology*, vol. 85, no. 1020, pp. e1263–e1272, Dec. 2012.
   DOI: 10.1259/bjr/27428078
   PMid: 23175492
   PMCid: PMC3611733
2. M. H. Barcellos-Hoff et al., "Concepts and challenges in cancer risk prediction for the space radiation environment," Life Sciences in Space Research, vol. 6, pp. 92-103, Jul. 2015.
   DOI: 10.1016/j.lssr.2015.07.006
   PMid: 26256633
3. F. A. Cucinotta, "A New Approach to Reduce Uncertainties in Space Radiation Cancer Risk Predictions," *PLoS ONE*, vol. 10, no. 3, p. e0120717, Mar. 2015.
   DOI: 10.1371/journal.pone.0120717
   PMid: 25789764
   PMCid: PMC4366386
4. M. E. Gaulden, "Biological Dosimetry of Radionuclide and Radiation Hazards," *Journal of Nuclear Medicine*, vol. 24, no. 2, pp. 160-164, Feb. 1983.
   PMid: 6822879
5. J. S. Loeffler, M. Durante, "Charged particle therapy-optimization, challenges and future directions," *Nat. Rev. Clin. Oncol.*, vol. 10, no. 7, pp. 411-424, Jul. 2013.
   DOI: 10.1038/nrclinonc.2013.79
   PMid: 23689752
6. S. Brita et al., "Relative biological effectiveness of carbon ions for tumor control, acute skin damage and late radiation-induced fibrosis in a mouse model," *Acta Oncologica*, vol. 54, no. 9, pp. 1623–1630, Aug. 2015.
   DOI: 10.3109/0284186X.2015.1069890
   PMid: 26271798
7. V. Balakin et al., "Hypofractionated irradiation of the solid form of Ehrlich ascites carcinoma in mice by a thin scanning proton beam," *Biophysics*, vol. 61, no. 4, pp. 682–686, Jul. 2016.
   DOI: 10.1134/S0006350916040047
8. M. Durante, "New challenges in high-energy particle radiobiology," *Br. J. Radiol.*, vol. 87, no. 1035, Mar. 2014.
   DOI: 10.1259/bjr.20130626
   PMid: 24198199
   PMCid: PMC4064605
9. S. Zaichkina, "Peculiarities of the effect of low-dose-rate radiation simulating high-altitude flight conditions on mice in vivo," *Radiat. Environ. Biophys.*, vol. 46, no. 2, pp. 131–135, Apr. 2007.
   DOI: 10.1007/s00411-007-0107-2
   PMid: 17415582
10. W. Schmid, "The micronucleus test," *Mutat. Res.*, vol. 31, no. 1, pp. 9–15, Feb. 1975.
    DOI: 10.1016/0165-1161(75)90058-8
11. V. Balakin et al., "Study of cytogenetic effects induced by accelerated (12)C ions with energy of 200 MeV/nucleon in mice," *Dokl. Biochem. Biophys.*, vol. 439, pp. 192-194, Jul-Aug. 2011.
    DOI: 10.1134/S1607672911040120
    PMid: 21928143
12. R. Kallman, H. Kohn, "The reaction of the mouse spleen to X-rays measured by changes in organ weight," *Radiat. Res.*, vol. 3, no. 1, pp. 77-87, Sep. 1955.
    DOI: 10.2307/3570274
    PMid: 13255011
13. R. Kallman, H. Kohn, "The reaction of the mouse thymus to X-rays measured by changes in organ weight," *Radiat. Res.*, vol. 2, no. 3, pp. 280-293, May 1955.
    DOI: 10.2307/3570256
    PMid: 14372047
14. K. Datta et al., "Exposure to Heavy Ion Radiation Induces Persistent Oxidative Stress in Mouse Intestine," *PLoS ONE*, vol. 7, no. 8, p. e42224, Aug. 2012.
    DOI: 10.1371/journal.pone.0042224
    PMid: 22936983
    PMCid: PMC3427298
15. S. Sorokina et al., "Relative biological efficiency of protons at low and therapeutic doses in induction of 53BP1/γH2AX foci in lymphocytes from umbilical cord blood," *Int. J. Radiat. Biol.*, vol. 89, no. 9, pp. 716-723, Sep. 2013.
    DOI: 10.3109/09553002.2013.797619
    PMid: 23607485