The Effectiveness of Radiation Damage Reduction in Mice by Laser Light in Dependence of the Time Interval between Exposures

Karine Voskanyan^{1*}, Svetlana Vorozhtsova², Alla Abrosimova², Gennady Mitsyn¹ and Victor Gaevskiy¹

1. Joint Institute for Nuclear Research, Dubna 141980, Russia

2. Institute of Medico-Biological Problems of the Russian Academy of Sciences, Moscow 123007, Russia

Abstract: A research was carried out to determine the period of time during which it is possible to reduce the radiation damage in mice by means of laser radiation (650 nm) after gamma irradiation. First, the mice were exposed to γ - radiation (whole body irradiation), then after 2 h or 24 h they were irradiated with laser radiation. The results of these studies have shown that the use of laser irradiation to reduce radiation damage in mice is effective 24 h after the exposure to 5 Gy ionizing radiation which leads to the bone-marrow clinical form of the ARS (Acute radiation sickness). In case of the lethal dose of ionizing radiation 7 Gy (the transitional clinical form of the ARS), the increase in life expectancy of mice is observed using laser radiation both 2 and 24 h after the exposure to γ - radiation, but the effectiveness of the laser used 2 h after the ionizing radiation is significantly more efficient.

Key words: Gamma radiation, laser radiation, reduction of the radiation damage.

1. Introduction

The experiments we conducted earlier on the action of 650 nm laser radiation, as well as the combined irradiation with laser radiation and γ - rays ^{60}Co of C57BL/6 mice showed that the red spectral region laser radiation can be used to improve the recovery of hematogenesis after their exposure to ionizing radiation [1]. The method of the laser radiation protection of biological objects also contributes to an increase in the viability of mice, prevents the damages of skin, and increases the mitotic activity of mice bone marrow cells [2].

In the above experiments, the time interval between exposures to ionizing and laser radiation should not exceed 30 min. From a practical point of view, the definition of the time interval during which the laser irradiation has the potential to reduce the radiation damages in mice is very important.

2. Methods

The experiments were performed on CD1 mice with the mass of 21-26 g. The conditions of managing and experiments complied with the rules of bioethical studies on animals [3]. The animals were fed with the standard briquetted feeding staff and drinking water from drinking cups. Before killing the mice were weighed then some blood was taken from the tails of the experimental animals to count the amount of leukocytes of peripheral blood and to determine the amount of hemoglobin in blood. They were killed with the method of cervical dislocation. To analyze the number of karyocytes, a thigh of the animal, with muscles cleaned off, was put into a chemical cup of 50 mL and ground up in 6 ml of the 3% solution of acetic acid. To determine the content of hemoglobin in blood, we used the measuring device “Gemoglobinometr Mini-Gem 540” (CJSC scientific-industrial enterprise TEKHNOMEDIKA, Russia). The number of leukocytes and karyocytes were calculated with the Goryaev chamber according

*Corresponding author: Karine Voskanyan, doctor, leading scientist, research fields: radiation biology, photobiology and radiation therapy. E-mail: voskan@jinr.ru.

to the conventional methods [4]. To define the mitotic index, bone marrow was extracted from the naked thighbone with a syringe, and then it was turned in a suspension in the hypotonic solution of sodium citrate with further preparation of smears on the slide plate. A smear of bone marrow was fixed in the absolute methyl alcohol and then dyed according to the method of Romanovsky-Gymza [4].

3. Irradiation

The mice were irradiated with ionizing (whole body irradiation) and laser radiation, separately one by one in a special bench at the Medical-Technical Complex of the Laboratory of Nuclear Problems of the Joint Institute for Nuclear Research [5]. Gamma-therapy device ROCUS-M ("Ravenstvo" Co, St-Petersburg, Russia), with Co^{60} source and 7600 Ku activity was used for the gamma-irradiation of mice. Dose power was 1 Gy/min at the place of irradiation. As a source of laser radiation we used a device that we developed for radiation protection of biological objects in the experiment [6]. Laser radiation in the dose $1\text{mJ}/\text{cm}^2$

irradiated only the furry back of a mouse. The area of the enlarged beam in the irradiation point was almost 5.3 cm^2 (approximately, 1/3 of the surface of the back).

First, mice were exposed to γ -radiation (5 or 7 Gy) and then to laser radiation at different time - 2 or 24 h after exposure to γ -rays.

A packet of conventional methods included into the "Microsoft Excel 2010" computer program was used for the data statistical processing.

4. Results

Table 1 contains the values of the peripheral blood parameters and bone marrow karyocytes of intact mice, as well as the values of the same parameters of the mice that underwent gamma irradiation (5 Gy) or exposure to combined irradiation gamma-rays and laser radiation (at different periods after exposure to γ -rays) 15 days after exposure.

Table 2 presents the values of the mitoses number per 1,000 nucleated bone marrow cells in different periods after gamma-ray irradiation of mice through

Table 1 Values of the parameters of peripheral blood and of the number of bone marrow karyocytes of intact mice (females), as well as of the mice irradiated with 5 Gy gamma-rays and combined-irradiated with gamma-rays and laser radiation at different time after exposure to γ -rays (15 days after their irradiation).

The number of mice	Body mass (g)	Hemoglobin (g/L)	Leukocytes (10^3 mcl)	Karyocytes (10^3 mcl)
Intact mice				
6	22.85 ± 3.2	135 ± 27	3.2 ± 0.3	75.1 ± 5.9
gamma-ray irradiation				
6	22 ± 2.8	123 ± 29.6	2.11 ± 0.27	$28.5 \pm 2.8^{**}$
gamma-ray and laser irradiation (2 h later)				
6	24 ± 3.7	117.8 ± 30.6	2.24 ± 0.5	$21.1 \pm 3.3^{**}$
gamma-ray and laser irradiation (24 h later)				
6	21.6 ± 2.07	122.4 ± 18.7	1.96 ± 0.3	$39 \pm 7.4^{*..**}$

****** - The number of karyocytes truly lowers values of karyocytes of intact mice.

***** - The values are truly higher than those of parameters in mice irradiated with laser 2 hours later.

Table 2 The mitoses number per 1,000 nucleated bone marrow cells of mice (females) after gamma-ray irradiation in a dose of 5 Gy as well as combined irradiated with gamma-rays and laser radiation 2 and 24 h after exposure to γ -rays (15 days after irradiation).

Intact mice	gamma-ray	gamma-ray + laser (2 h later)	gamma-ray + laser (24 h later)
18 ± 5.2	$6.37 \pm 1.6^{**}$	$6 \pm 0.2^{**}$	$20.6 \pm 6.9^*$

***** - The value is truly higher than those of parameters in mice irradiated with gamma-ray

****** - The value is truly lower than values of karyocytes of intact mice.

Fig. 1 Death dynamics of mice (males) after irradiation with 7 Gy gamma-rays and combined gamma-rays and laser irradiation at different terms after the gamma-rays irradiation. 1 - γ -rays irradiation; 2 - γ -rays + laser (24 h later) irradiation; 3 - γ -rays + laser (2 h later) irradiation.

15 days after gamma-ray irradiation in a dose of 5 Gy as well as combined irradiated with gamma-rays and laser radiation 2 and 24 h after gamma-ray irradiation.

The dynamics of the mice death after irradiation with 7 Gy gamma-rays, as well as after the combined gamma-rays and laser irradiation at different terms after, the gamma-rays irradiation is shown in Fig. 1.

The survival in the control group of mice was 100% during the whole period of observation. In all groups of the studies there were 10 mice.

5. Discussion

It can be seen (Table 1) that blood parameters after exposure only to γ -rays do not truly differ from the results of combined irradiation. However, the number of karyocytes in mice irradiation with laser 24 h after, γ -irradiation is truly higher than in irradiation with laser 2 h later.

The results given in Table 2 show that the mitotic index of the bone marrow cells is on the control level in the case of laser irradiation 24 h after the irradiation with ionizing radiation. The above shown results give ground for a conclusion that the application of laser to stimulate post-radiation hematosis recovery 24 h after radiation damage is effective.

In these experiments, dose of ionizing radiation was used which causes the bone marrow form of ARS (Acute radiation syndrome) in mice, therefore it is possible that the ineffectiveness of laser irradiation 2 h after the action of ionizing radiation is related to the regularities of cell devastation – the most important symptom of the hematopoietic organs' damage [7]. It is well-known that doses of 2-10 Gy cause bone marrow cells' death immediately at the moment of irradiation or in mitoses, and cells lose their ability to divide. Hypoplasia and aplasia of bone marrow are observed during the first day after irradiation that is

connected to the mass mortality of cells [8]. It has been found that the post-irradiation recovery of hematopoietic cells of bone marrow is defined by the processes of proliferation and differentiation of not much damaged or not damaged stem cells. It is probable that laser radiation applied 24 h later stimulates these processes.

The dynamics of mice mortality after 7 Gy gamma-ray radiation and after irradiation to γ -rays and subsequent exposure to laser radiation (Fig. 1) show that laser irradiation of mice 2 and 24 h after the action of gamma-radiation leads to delay of the mortality start; however, laser irradiation 2 h after radiation damage is more effective.

In this experiment the lethal dose of ionizing radiation (7 Gy) was used that causes the intermediary form of ARS (Acute radiation sickness) which is characterized by severe damage of intestines and hematopoietic organs. The dose of 7 Gy is considered minimal that leads to 100% mortality, wherein the cellular devastation of intestines is the main reason of death during the second week after irradiation [9]. The presented dynamics in Fig. 1 of mice mortality after gamma-ray irradiation in the dose of 7 Gy fully corresponds to the above mentioned.

The results of our experiments given in Table 1 shows that the mitotic index of bone marrow cells is on the control level at laser irradiation 24 h after irradiation with ionizing radiation. On the assumption of this and with an account of the fact that the dose of ionizing radiation in the experiments on the mice survival was of the value that led to the intermediary form of ARS it can be presupposed that in irradiation of mice with laser 24 h after gamma-irradiation the survival period increases for those mice who had the bone marrow syndrome of ARS prevailing. Then, the effectiveness of laser application 2 h later can be the result of the increasing number of survived mice with intestines damage. Judging by the results given in Fig. 1, it can be presupposed that the number of such mice was bigger.

6. Conclusions

On the basis of the results given above and obtained earlier, it can be concluded that the decrease of radiation damage in mice with 650 nm laser radiation (energy density of 1 mJ/cm²) is observed when laser is applied during 30 min after the action of acute and prolonged irradiation with ionizing radiation [1, 10], independent of the irradiation dose (in the dose interval up to 7 Gy), as well as 24 h after the action of ionizing radiation in the dose 5 Gy that leads to the bone marrow form of ARS. At a lethal dose of ionizing radiation 7 Gy that leads to the intermediary form of ARS an increase in life span of mice was observed when laser radiation was applied both 2 and 24 h after the action of ionizing radiation. However, the effectiveness of laser applied 2 h after the damage caused by ionizing radiation is considerably higher.

References

- [1] Voskanyan, K., Vorozhtsova, S., Abrosimova, A., Mitsyn G. and Gaevsky, V. 2012. "Laser Light Induced Modification of the Mice Peripheral Blood Parameters and the Number of Bone Marrow Karyocytes after the Action of Ionizing Radiation." *Journal of Physical Science and Application* 2 (2): 7-10.
- [2] Voskanyan, K., Vorozhtsova, S., Abrosimova, A., Mitsyn G. and Gaevsky, V. 2012. "Laser Device for the Protection of Biological Objects from the Damaging Action of Ionizing Radiation." *Journal of Physical Science and Application* 2 (6): 152-7.
- [3] Genin, A., Iljin, E., Kaplansky, A. and Kasatkina, K. 2001. "Bioethical Regulations of Conducting Research on Man and Animals in Aviation, Space and Naval Medicine." *Aviokosmicheskaya I Ekologicheskaya Meditsina* 35 (4): 14-20.
- [4] Koblov, F. 1979. *Methods and Devices for Clinical and Laboratory Research*. Meditsina, Moscow.
- [5] Savchenko, O. V. 1996. "Status and Prospects of New Clinical Methods of Cancer Diagnostics and Treatment Based on Particle and Ion Beams Available at JINR." In *Communication of the Joint Institute for Nuclear Research, Dubna*.
- [6] Voskanyan, K., Vorozhtsova, S., Abrosimova, A., Mitsynand, G. and Gaevsky, V. 2013. "Laser Device for the Protection of Biological Objects from the Damaging Action of Ionizing Radiation." In *Proceedings of the 2nd International Conference on Nanotechnologies*

and Biomedical Engineering, Chisinau, Republic of Moldova.

- [7] Kudrešov, Y. B. and Berenfel'd, B. S. 1982. *Basics of radiation Biophysics*. Moscow.
- [8] Alexandrov, Y. A. 2007. *Basics of Radiation Ecology: Tutorial*. Yoshkar-Ola.
- [9] Yarmonenko, S. P. 1988. *Radiobiology of Humans and*

Animals. Moscow.

- [10] Voskanyan, K., Vorozhtsova, S., Abrosimova, A., Mitsyn, G., Gaevsky, V. and Molokanov, A. 2014. "Reduction of Radiation Damage in Mice after Acute and Prolonged Irradiation with Gamma Rays by Means of Laser Device." *Journal of Physical Science and Application* 4 (8): 501-6.