Reduction of Radiation Damage in Mice after Acute and Prolonged Irradiation with Gamma Rays by Means of Laser Device

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Abstract: An opportunity to use laser radiation as a means to reduce negative aftermath of acute and prolonged exposure to ionizing radiation was checked. The mice were exposed to $\gamma$-rays of $^{60}$Co (whole body irradiation) in the dose of 7 Gy (the transitional clinical form of the acute radiation sickness). The dose rate at acute irradiation was 1.14 Gy/min, and at prolonged exposure, 0.027 Gy/min. Laser radiation in the dose 1 mJ/cm$^2$ was used to irradiate only the back of a mouse. First, the mice were exposed to $\gamma$-radiation, then to laser radiation. The time interval between two types of irradiation did not exceed 30 min. It was shown that the radiation protection of mice with laser radiation is possible at exposure to ionizing radiation in a wide dose interval and can reduce negative after-effects of both the acute and prolonged radiation exposure.

Key words: Radiation protection, prolonged irradiation, laser radiation.

1. Introduction

From the point of view of possible scenarios of excessive exposure to ionizing radiation on human beings the most relevant are the search resources for emergency use for the treatment of acute radiation injury, as well as preventive means to reduce the adverse consequences of prolonged radiation exposure to low dose [1].

As research in this field progresses, emphasis should be placed on developing radiation-protective agents that have low toxicity, a practical mode of administration, an extended shelf-life, and a wide window of protection [2].

The experiments we had conducted earlier on the action of 650 nm laser radiation, as well as the combined irradiation with laser radiation and $\gamma$-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 [3]. Research of the action of 5 Gy $\gamma$-radiation itself, as well as of the combined action of laser devices on survival, weight, skin and the general mitotic index of the bone marrow cells (mitotic index of all nucleus-containing cells of the bone marrow) of C57BL/6 experimental young mice was also carried out. The method of the laser radiation protection of biological objects contributes to an increase in the viability of mice, prevents the damages of skin, and also increases the mitotic activity of mice bone marrow cells [4].

The doses that lead to the bone-marrow clinical form of the acute radiation sickness (ARS) were used in the indicated experiments. In this connection, it is
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interesting to check the radioprotective action of the laser radiation on mice at the doses that lead to another form of ARS [5]. Besides, it is important to study an opportunity to use the laser radiation as a remedy that diminishes the unfavorable aftermath of the prolonged radiation action. For this purpose, we held experiments on the action of acute and prolonged gamma-irradiation in the dose of 7 Gy (a transitional form of ARS) and the combined action of gamma-rays and the laser radiation on mice of line C57BL/6.

2. Methods

The experiments were performed on young male C57BL/6 mice with the mass of 12-17 g. The conditions of managing and experiments complied with the rules of bioethical studies on animals [6].

The animals were fed with the standard briquetted feeding staff and drinking water from drinking cups. Before killing the mice were weighed. They were killed with the method of cervical dislocation; 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. 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 6ml 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 was calculated with the Goryaev chamber according to the conventional methods [7].

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 [7].

Each experimental group included 10 mice.

3. Irradiation

The mice were irradiated at the Medical-Technical Complex of the Laboratory of Nuclear Problems of the Joint Institute for Nuclear Research [8].

Gamma-therapy device ROCUS-M (“Ravenstvo” Co, St-Petersburg, Russia), with 60Co source and 7600 Ku activity was used for the γ-irradiation of mice.

To measure the dose rate of gamma radiation source the dosimeter PTW UNIDOS-E (of the company “PTW-Freiburg”, Germany) with an ionization chamber TM30013-03378 in the condition of the fully open collimator (the collimator dimensions 26 cm × 22 cm) was used.

Irradiation of mice was conducted at two points: point 1 is at the distance of 750 mm from the source (isocenter), point 2 is at the distance of 1,870 mm from the source.

To reduce the dose rate, an additional 30 mm-thick lead filter was installed in the beam route; it reduced the dose rate of the gamma radiation beam 6.95 times. At acute irradiation, the cage with the mice was placed in point 1 (dose rate, 1.14 Gy/min, the irradiation period, 6.14 min at the dose of 7 Gy); for prolonged irradiation, the cage was placed in point 2 (dose rate, 0.027 Gy/min, the irradiation period, 259.2 min at the dose of 7 Gy).

As a source of laser radiation we used a device that we developed for radiation protection of biological objects in the experiment (invention patent RU 2 428 228 C2) (Fig. 1). The wavelength of the radiation was 650 nm. The diameter of the laser beam in the irradiation point was 2.6 cm.

In case of combined irradiation of a mouse, the time interval between two types of irradiation did not exceed 30 min. First the mice were exposed to γ-radiation (whole body irradiation), then to laser radiation. When mice were irradiated with laser radiation
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they were placed, one by one, into a special bench. Only the furry back of each mouse and not the whole body was irradiated with laser radiation (dose 1 mJ/cm²). The area of the enlarged beam in the irradiation point was \( \approx 5.3 \text{ cm}^2 \) (approximately, 1/3 of the surface of the back).

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 acute gamma irradiation (7Gy) or exposure to combined irradiation (gamma-rays and laser radiation) in different periods after irradiation.

Data on the values of the peripheral blood parameters and bone marrow karyocytes of the mice that underwent prolonged gamma irradiation (7 Gy) and combined irradiation are presented in Table 2.

Table 3 presents the values of the mitoses number

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**Table 1** Values of the parameters of peripheral blood and of the number of bone marrow karyocytes of intact mice, as well as of the mice irradiated with 7 Gy gamma-rays (acute irradiation), and combined-irradiated with gamma-rays and laser radiation.

<table>
<thead>
<tr>
<th>Body mass (g)</th>
<th>Hemoglobin (g/L)</th>
<th>Leukocytes (10^3) mcl</th>
<th>Karyocytes (10^3) mcl</th>
</tr>
</thead>
<tbody>
<tr>
<td>15</td>
<td>34.3 ± 15</td>
<td>6.8 ± 2.3</td>
<td>152.5 ± 6</td>
</tr>
<tr>
<td>12</td>
<td>199 ± 7.7(^\circ)</td>
<td>2.3 ± 0.5(^\circ)</td>
<td>40.3 ± 7(^\circ)</td>
</tr>
<tr>
<td>14</td>
<td>123 ± 17.6(^\circ)</td>
<td>1.53 ± 0.3(^\circ)</td>
<td>43.4 ± 5.5(^\circ)</td>
</tr>
<tr>
<td>12</td>
<td>132.6 ± 12.6 (^\circ)</td>
<td>0.4 ± 0.01(^\circ)(^\circ)</td>
<td>23.5 ± 5.4(^\circ)(^\circ)</td>
</tr>
<tr>
<td>12</td>
<td>121 ± 17</td>
<td>0.44 ± 0.08(^\circ)</td>
<td>33 ± 4.7(^\circ)</td>
</tr>
</tbody>
</table>

\(^\circ\): The values truly differ from the values of parameters of intact mice.
\(^\circ\)^\(^\circ\): The values are truly lower than those of parameters in mice irradiated with 7Gy gamma-rays 24 h after irradiation.

**Table 2** Values of the parameters of peripheral blood and karyocytes of the mice exposed to gamma-rays in the dose of 7 Gy (prolonged irradiation) and combined-irradiated with gamma-rays and laser radiation.

<table>
<thead>
<tr>
<th>Body mass (g)</th>
<th>Hemoglobin (g/L)</th>
<th>Leukocytes (10^3) mcl</th>
<th>Karyocytes (10^3) mcl</th>
</tr>
</thead>
<tbody>
<tr>
<td>15</td>
<td>153 ± 32</td>
<td>1 ± 0.1(^\circ)</td>
<td>61.8 ± 8(^\circ)</td>
</tr>
<tr>
<td>15</td>
<td>122 ± 36</td>
<td>1.5 ± 0.9(^\circ)</td>
<td>61 ± 11(^\circ)</td>
</tr>
<tr>
<td>16</td>
<td>132 ± 30</td>
<td>0.62 ± 0.2(^\circ)</td>
<td>33 ± 4(^\circ)(^\circ)</td>
</tr>
<tr>
<td>16</td>
<td>113 ± 20</td>
<td>0.53 ± 0.1(^\circ)</td>
<td>39 ± 4.7(^\circ)</td>
</tr>
</tbody>
</table>

\(^\circ\): The values truly differ from the values of parameters of intact mice.
\(^\circ\)^\(^\circ\): The values are truly lower than those of parameters in mice irradiated with 7 Gy gamma-rays 24 h after irradiation.
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Table 3  The mitoses number per 1000 nucleated bone marrow cells in different periods after gamma-ray irradiation (acute and prolonged irradiation), and combined irradiation with gamma-rays and laser radiation.

<table>
<thead>
<tr>
<th>Type and dose of irradiation</th>
<th>Acute irradiation</th>
<th>Prolonged irradiation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>24 h</td>
<td>72 h</td>
</tr>
<tr>
<td>Intact mice</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gamma-rays, 7 Gy</td>
<td>10.5 ± 1.5</td>
<td>-</td>
</tr>
<tr>
<td>☼</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gamma-rays, 7 Gy + Laser radiation, 1mJ/cm²</td>
<td>10.1 ± 1.7</td>
<td>-</td>
</tr>
<tr>
<td>☼</td>
<td></td>
<td></td>
</tr>
<tr>
<td>☼: The values are truly lower than the value of the non-irradiated control.</td>
<td>☼: The values are truly lower than the value in irradiation with gamma-rays in the period of 24 h after prolonged irradiation.</td>
<td>☼: The values are absent, due to great exhaustion of the marrow.</td>
</tr>
</tbody>
</table>

In Fig. 1, a laser device for radiation protection of biological objects in the experiment is presented.

The dynamics of the mice’s death after acute irradiation with γ-rays is shown in Fig. 2, as well as after the combined action of ionizing and laser radiation.

Fig. 3 demonstrates the dynamics of the mice’s death after prolonged irradiation with γ-rays and after combined irradiation with gamma- and laser radiations.

5. Discussion

It can be seen (Table 1) that 24 h after irradiation with γ-rays leucopenia (a decrease of leucocytes in blood) and hyperchromemia (an increase in the hemoglobin content) are observed [9]. However, the hyperchromemia is not observed in mice that were combined-irradiated. 72 h after irradiation, the value of the number of blood leucocytes and bone marrow karyocytes is lower than that of 24 h after irradiation. In that period hemoglobin is on the level of the norm.

In the case of the prolonged irradiation of mice (Table 2) no changes in the hemoglobin values are observed. The level of leucocytes gradually reduced, and the number of karyocytes, like in the case of acute irradiation, decreases 72 h after irradiation.

On the ground of these data it can be concluded that in the values of the parameters given in Tables 1 and 2 the results of the irradiation with only ionizing radiation, in comparison with the combined irradiation, differ in the demonstration of hyperchromemia in the period of 24 h after the acute irradiation with γ-rays. It is well-known that hyperchromemia can be observed...
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in thickening of blood (as an aftermath of diarrhea, generation of exudates, transudates, uremia, cardio-pulmonary disease, intestinal obstruction, etc.).

Such situations in the conditions of our experiment are quite possible. As hyperchromemia is not observed in mice 72 h after the acute irradiation with gamma-rays it is probable that the organisms of mice eventually managed to overcome this problem without external interference.

It should be noted that in both variants of mice combined irradiation (acute and prolonged), at the time of 72 h after exposure, the values of the bone marrow karyocytes do not credibly differ from the values of karyocytes in irradiation only with gamma rays. However, they are accurately not lower than the values of the number of karyocytes in the period of 24 h after the irradiation with only ionizing radiation.

The results of the experiments given in Table 3 show that in case of acute irradiation of mice with γ-rays, 72 h after the irradiation there is a strong bone marrow devastation that does not allow the determination of mitoses number. At prolonged irradiation with ionizing radiation, the number of mitotic cells decreases over time and, in the case of combined exposure remains at the same level, due to the effect of laser irradiation.

Death dynamics of mice after acute irradiation with gamma-rays and combined irradiation with ionizing and laser radiations shows that in both groups were radiosensitive mice that died during the first two days after exposure (Fig. 2). Then, the mice that were irradiated with only ionizing radiation demonstrated a sharp decline in survival which led to 100% lethality in 5 days after irradiation. The mice that were irradiated also with laser died gradually, the 100% lethality occurred on the 11th day after irradiation.

Unfortunately, as we know, life span is often the only result of observations that can be used to characterize the overall picture of the clinical response to radiation and forms of death. Usually, in experiments with small laboratory animals, the cause of death is considered to be digestive or marrow syndrome only on the basis of the time of death [10].

In the transition region of the irradiation doses the ultimate time is chosen that corresponds to 5 or 8 days and reflects the lethality tendency around two modes, which reflects the trend of mortality around two, sometimes overlapping, modes, one of which is less than the above mentioned periods. These data correspond to the clinical course of the disease with symptoms of intestinal syndrome prevailing in small periods of survival and bone marrow syndrome with longer survival periods. In the case of following this approach, it is possible to say that (in terms of our experiment) acute irradiation with gamma-rays led to the death of the intestinal syndrome, because 0% survival was observed in 5 days after exposure. During this period the survival of the combined-irradiated mice is 40%, and the 100% death is observed on the 11th day after irradiation. It is very difficult to say why the mice die after the 5th day of irradiation. It can be the result both of an incipient bone marrow syndrome and delayed intestinal syndrome at the combination of the two syndromes, or as a result of absolutely different causes. From the point of view of our task in the experiment the main result is that at the dose of acute irradiation of 7 Gy laser radiation can reduce radiation damage in mice.

After prolonged irradiation with γ-rays, as well as after the combined action another picture of the mice death is observed, in both variants of irradiation the curves are benched, with a considerable time shift, however (Fig. 3). As a result of the latter, 30% of the mice survival was observed on the 19th day after irradiation with gamma-rays and on the 38th day after the combined irradiation. It is obvious that the prolonged irradiation led to a considerable reduction in radiation damage of mice, in comparison to the acute irradiation.

It is well-known that the time factor in the prognosis of possible aftermath of irradiation occupies an important place, due to the recovery processes in
tissues and organs that start to develop after radiation damage. According to the data of the majority of radiobiological studies, the reduction of the radiation dose rate (at one and the same absorbed dose) decreases the biological effect, and for each of the criteria that lay the basis of this regularity evaluation there are their own strictly definite ranges of the dose rates when the above mentioned regularity is correct [11]. As a result of the action of low-rate ionizing radiation on the organism and, simultaneously, the presence of processes of the post-irradiation recovery of tissue the clinical picture has a number of different features, in comparison to the short-term exposure. In prolonged irradiation the same forms of the radiation sickness occur as in the short-term exposures; dependence of the severity of the dose is retained, however, the initial reaction may be postponed. The results we obtained, given in Fig. 2 and 3, clearly demonstrate this effect.

However, in this paper we are interested in the aspect of possible reduction of radiation damage in mice with laser radiation. The curve of the death of combined irradiated mice (Fig. 2) testifies to the fact that laser irradiation leads to considerable change in the survival terms in the case of prolonged irradiation of mice.

6. Conclusions

On the basis of all the above mentioned results and those that the authors obtained earlier [3, 4], it can be concluded that the method of the laser radioprotection of biological objects is possible at exposure to ionizing radiation in a wide dose interval.

In addition, laser irradiation reduces the adverse effects of both acute and prolonged radiation exposure. The obtained result is very important for radiation protection as it is known [12], in case of prolonged irradiation of the organism with ionizing radiation short-action type protective agents are not effective; in some cases even a negative effect is possible.

We would also like to draw attention to the fact that the laser devices we developed for radiation protection of biological objects in the experiment have a practical control mode and allow quick and errorless irradiation of biological objects in the dose necessary for radio-protection.

References