

Radioprotective Effect of Laser Radiation With a Wavelength of 532 nm on Fibroblast Cells

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Abstract: Studies have been carried out to test the assumption that the primary photoreceptors for the radioprotective action of 633 nm laser radiation are the cytochrome-c-oxidases. To do this, the device was created for radiation protection of biological objects based on laser module with a wavelength of 532 nm. Experiments conducted on murine fibroblast cells showed that the radioprotection effect of laser irradiation was observed in the dose interval about 0.4-0.85 mJ/cm². Maximum radioprotection effect is observed at laser radiation energy density ~ 0.56 mJ/cm². The determination of the cell survival with the automatic counter CT20 after the action of the ionizing and combined irradiation showed that radioprotective action of the laser radiation with the wavelength of 533 nm, as well as the radiation with the wavelength of 633 nm, is transferred by the mechanism of the “bystander effect”. In addition, it was found that radioprotection effect of laser irradiation observed on the criterion of number of surviving single cells, compared with cells exposed to γ -radiation Laser irradiation, produces effective radioprotecting action also on the criterion of grown cell colonies. The value of the dose modification factor (DMF) calculated on 50% cell survival (LD₅₀) is equal to 1.4. The results suggest that in the case of radioprotective action of small doses of laser radiation with a wavelength of 633 nm, as well as 532 nm primary photoreceptors is the cytochrome-c-oxidases.

Key words: Gamma-radiation, laser radiation, radioprotective effect.

1. Introduction

Previously we have shown that both the preliminary and subsequent, as well as the simultaneous with the laser radiation (633 nm), irradiation of mice fibroblasts, leads to the survival increase of cells that were exposed to γ -radiation or protons. The maximal radioprotecting effect was observed at energy density of the laser radiation of ~ 1 mJ/cm² [1].

The results were also received indicating the possibility of transmission of effect of laser radiation on the “bystander” effect [2].

To ascertain the mechanism of the radioprotective action of the laser radiation, it is of great interest to check out the assumption that in the radioprotective action of low-dose laser radiation with the wavelength of 633 nm the primary photoreceptor is the cytochrome-c-oxidase—a component of the

respiratory chain located in cell mitochondria [3].

To this end, we have established the device based on the laser module with a wavelength of 532 nm (Fig. 1), since this wavelength is also absorption spectrum of cytochrome-c-oxidase.

2. Methods

The cells of mice fibroblasts C3H10T1/2 were obtained by Reznikoff et al. [4]. We used in our experiments a cell line from the collection of the Institute of Cytology Russian Academy of Science (St. Petersburg). Cells C3H10T1/2 that underwent not more than 18 passages was used in the experiments.

The cells were grown in the Eagle medium with Earle salts, plus fetal bovine serum (10%), penicillin (50 units/mL) and streptomycin (50 mcg/mL).

To synchronize the cultures, fibroblasts with density of $2.7 \cdot 10^3$ cells/cm² were sown in plastic flasks with the surface area of 25 cm² and were grown during 11 days in thermostat at the temperature of

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37 °C. The medium was freshened 6 days after the plating. This 11-daily culture contains 94% of the cells in the G1 phase [5].

2.1 Definition of Survival

To determine the survival rate, such amount of cells was sown into five flasks that would make it possible to get as a result about 100-200 cell colonies per flask. After 10 days of incubation at 37 °C the colonies were fixed with methanol and stained with Giemsa dye. The colonies that contained over 50 cells were considered as survivors. The survival rate was determined by comparison of the quantity of colonies that grew in irradiated and not irradiated (control) samples. The efficiency of the plating for control cells was about 10%.

In the experiments with the device “Cell counter” CT20 (Bio-Rad Laboratories, USA) a monolayer of fibroblast cells grown on the surface of the plastic flask’s wall was exposed to ionizing radiation. One hour after irradiation with γ -rays, the cell monolayer was irradiated with the maximally effective dose of laser radiation $\sim 0.56 \text{ mJ}/\text{cm}^2$ (Fig. 3). It should be mentioned that not all the surface of the flask wall was irradiated but only its central part with the area of 5.3 cm^2 , as we had shown earlier that the radioprotecting action of the laser radiation is transferred by the mechanism of the “bystander” effect [2]. Four hours after irradiation with γ -rays the cells that were located on the surface both of the control and all irradiated flasks were suspended in the 0.25% solution of trypsin. Then the suspension was centrifuged, the trypsin was drained and nutritive medium was added. After that the cell suspension and the trypan blue colorant were mixed in equal volumes and were filled into slides to determine the cell survival with the automatic counter CT20.

3. Irradiation

The irradiation of cells was conducted at the Medico-Technical Complex of the Laboratory of

Nuclear Problems at the Joint Institute for Nuclear Research where oncological diseases therapy treatment is held [6].

The gamma-therapy device “Rokus—M” of the firm “Ravenstvo” was used for irradiation of cells with γ -rays, with the source ^{60}Co and dose rate $\sim 0.67 \text{ Gy}/\text{min}$. The absorbed dose was measured with the clinical dosimeter CD 27012 (Germany). The error in the estimation of the absorbed dose did not exceed 5%.

In the experiments to determine the dose interval and the value of the energy density of the laser radiation that produces maximal effective radioprotecting effect, we used cell suspension in the nutritive medium. First, the cells were exposed to γ -rays, then to laser radiation for 20 minutes.

For cell irradiation we used laser device based on laser module with a wavelength of 532 nm, which operates in continuous mode. This device differs from the one we designed earlier—“The device for radiation protection of biological objects in the experiment” [7] that laser module with a wavelength of 633 nm replaced by module with a wavelength of 532 nm.

In the experiments to determine the dose interval and the value of the energy density of the laser radiation that produces maximal effective radioprotecting effect, we used cell suspension in the nutritive medium. First, the cells were exposed to γ -rays, then to laser.

A packet of “Microsoft Excel 2010” computer program was used for the data statistical processing.

Laser power measured using a Laser Power Meter LP1 (Sanva Electronic Instrument Co. Ltd., Japan) was equal to 0.3 mW, size surface irradiated by a monolayer of cells $\sim 5.3 \text{ cm}^2$.

Time of cells exposure by laser varied from 4 to 16 seconds at doses shown on Fig. 2.

4. Results

Fig. 1 shows the device that we designed for radiation protection of biological objects with the use

of the laser module with the wave length of 532 nm.

Fig. 2 shows the results of the experiments to determine the dose interval and the values of the energy density of the laser irradiation that produces maximal effective radioprotecting effect.

Fig. 3 shows the cell survival that was determined with the automatic counter CT20 after the exposure to ionizing and combined irradiation.

5. Discussion

It can be seen in Fig. 2 that the radioprotecting action of the laser radiation is observed in the dose interval of the irradiation energy density from 0.4 to 0.85 mJ/cm², with the maximal effectiveness at the density of the laser irradiation energy of ~0.56 mJ/cm².

The determination of the cell survival rate with the



Fig. 1 A device for radiation protection of biological objects with the use of a laser module with the wave length of 532 nm.

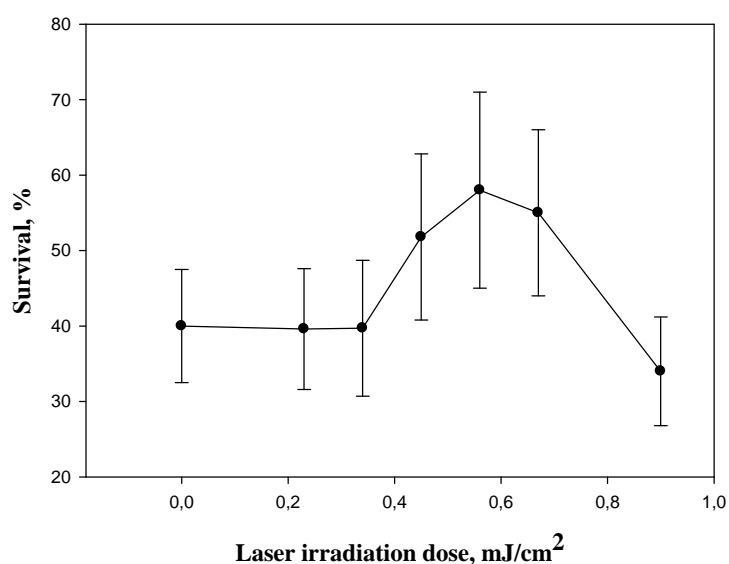


Fig. 2 Dependence of the effectiveness of radioprotecting action of the laser radiation on the fibroblast cells after the exposure to γ -rays in the dose 4 Gy on the density of the laser irradiation energy.

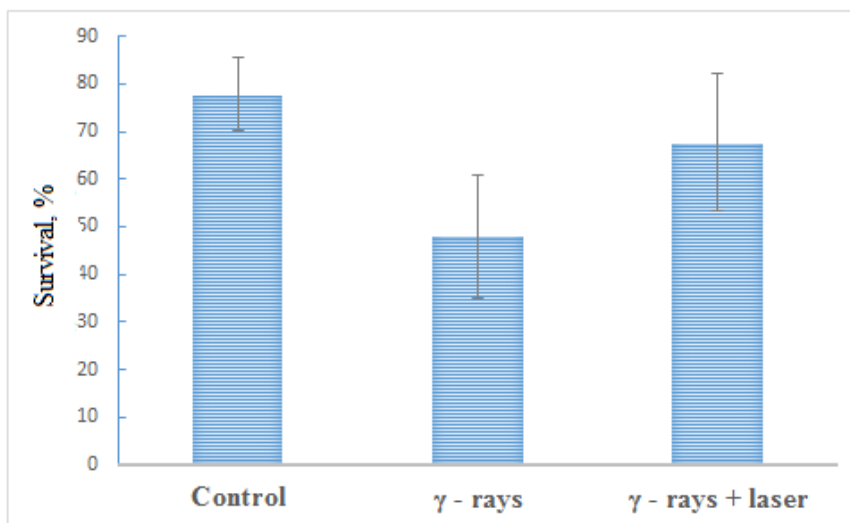


Fig. 3 Survival rate of fibroblast cells.

Control - non-irradiated cells;

γ-rays in the 4 Gy dose;

γ + laser - - γ-rays in the 4Gy dose + laser in the dose 0.56 mJ/cm².

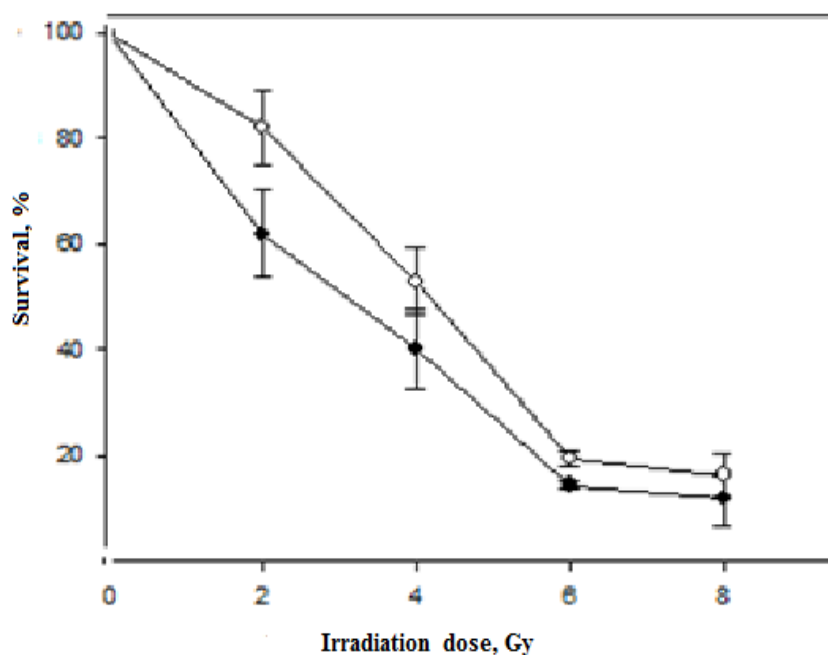


Fig. 4 Survival curves of the fibroblast cells.

● - γ-rays;

○ - γ-rays + laser in the dose of 0.56 mJ/cm².

automatic counter CT20 after the exposure to ionizing and combined irradiation showed that the radioprotecting action of the laser radiation with the wave length of 533 nm, as well as radiation with the wave length of 633 nm is transferred according to the

mechanism of the “bystander” effect [6]. Besides, it turned out that the radioprotecting effect of the laser irradiation is observed on the criterion of the quantity of the survived single cells, in comparison with the cells that underwent irradiation with γ-rays (Fig. 3).

The survival curves of the fibroblast cells irradiated with γ -rays, as well as with combined irradiation of γ -rays and laser (0.56 mJ/cm^2), shown in Fig. 4 indicate that the laser irradiation produces effective radioprotecting action on the criterion of grown cell colonies. The value of the dose modification factor (DMF) calculated on LD_{50} is 1.4.

6. Conclusions

The obtained results are in favor of the supposition that at radioprotecting action of low doses of the laser radiation with the wavelengths of 633 nm and 532 nm the primary photoreceptor is the cytochrome-c-oxidase which is present in inner membrane of mitochondria of all eukaryotes.

Speaking about the possible mechanism of radioprotective action of cytochrome *c* it should be noted that Cytochrome *c* is primarily known for its function in the mitochondria as a key participant in the life-supporting function of ATP synthesis. However, when a cell receives an apoptotic stimulus, cytochrome *c* is released into the cytosol and triggers programmed cell death through apoptosis [8].

In this regard, we expect that the study of the ratio forms of death (apoptosis and necrosis) of fibroblast cells after exposure to γ -rays and combined radiation by γ -rays and laser radiation with wavelengths of 633 nm and 532 nm will help us understand the mechanism of this effect.

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