

# Complex Phototherapy for Compensation of Damages Induced by High-Intensity Laser Radiation in Experiment

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**The aim of the investigation** was to develop a new technology of complex phototherapy based on consecutive biological tissue exposure to high-intensity laser radiation and low-intensity broadband red light and to evaluate its possibilities in experiment.

**Materials and Methods.** There were studied the effects of consecutive exposure of white rat tissues to high-intensity red and infrared radiation and low-intensity broadband red light on spontaneous and metal-catalyzed oxidative modification of proteins. The study was performed on white outbred rats with a body mass of 180 to 250 g, which were divided into 5 groups. Control group 1 included animals whose inner surface of the thigh was exposed locally to laser light radiation with a wavelength of 671 nm (red laser) and the power of 50 mW. Test group 2 underwent locally red laser radiation of the inner femoral surface and three consecutive sessions of low-intensity broadband red light exposure (a wavelength of 630 nm, light spot intensity of 5 mW/cm<sup>2</sup>). Control group 3 was exposed locally to 980 nm laser radiation of the inner femoral surface (infrared laser with the power of 50 mW). Test group 4 was exposed locally to infrared laser radiation of the inner femoral surface and three consecutive sessions of low-intensity broadband red light. Group 5 (intact) was not exposed to radiation. Samples of femoral tissue and blood serum were taken on day 3 in all groups of animals.

**Results.** The content of neutral and basic aliphatic dinitrophenylhydrazons in the tissues exposed to red and infrared laser radiation was found to be increasing. In subsequent sessions of low-intensity red light phototherapy there occurred sharp decrease in the levels of protein oxidative modification products to normal values.

**Conclusion.** The developed technology of complex phototherapy including sessions of consecutive exposure of biological tissues to laser and broadband red light provides radiation safety of procedures.

**Key words:** red light; protein oxidative modification; high-intensity laser radiation.

Lasers generating intensities around a few watts and even dozens of watts per a square centimeter are widely used in medicine, for example, for photodynamic therapy of oncological patients. Such high-intensity light may cause serious side effects in human and animal organism, including burns, eye lesions, CNS disorders, cardio-vascular and endocrine abnormalities, genetic alterations [1]. When radiation doses are high it is possible to observe local rise of temperature, inhibition of tissue and organ functions, when they are super-high there may occur tissue ablation and a burn in the light spot area [2, 3].

Numerous investigations [4–6] show that low-intensity red light can also influence the functional condition of live cells, tissues, organs, an organism as a whole and act as a factor fully or partially compensating oxidative stress consequences. Hence, it seems urgent to explore phototherapeutic techniques that allow to compensate fully or partially oxidative stress sequelae of organism tissue exposure to high-intensity light pulses and to provide radiation safety of photodynamic therapy and

treatment of inappropriate light effects. The content of free-radical protein oxidation products in the light spot area of the irradiated object may serve an objective index of the organism alteration level by a physical agent and sequelae compensation processes.

**The aim of the investigation** was to develop a new technology of complex phototherapy based on consecutive exposure of biological tissues to high-intensity laser radiation and low-intensity broadband red light and to estimate such possibilities in experiment.

**Materials and Methods.** The study was performed on white outbred rats with a body mass of 180 to 250 g, which were divided into 5 groups. Control group 1 included 10 animals whose inner surface of the thigh was locally exposed to laser light radiation with a wavelength of 671 nm (red laser) and the power of 50 mW. Laser radiation intensity in the light spot area was 0.55 W/cm<sup>2</sup> with 5 min exposure time for each field (the radiation area was divided into 9 fields each 1 mm<sup>2</sup> in size).

Test group 2 included 10 rats which underwent laser radiation of the inner femoral surface locally according

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to the same scheme as the control group and three subsequent sessions of low-intensity broadband red light exposure afterwards (once a day for 20 min). Laser radiation intensity in the light spot area was 5 mW/cm<sup>2</sup>. The light of a super-bright LED with the maximum radiation spectrum of 630 nm and a half-height width of 20 nm was used in the experiment.

Control group 3 and test group 4 each included 10 animals whose inner femoral surfaces were locally exposed to high-intensity infrared (IR) laser radiation. The experiment, aimed to study the effects of IR laser radiation on free-radical protein oxidation, was carried out according to the schemes of groups 1 and 2, respectively. The intensity of laser radiation and the source of broadband light in the light spot area also amounted to 0.55 W/cm<sup>2</sup> and 5 mW/cm<sup>2</sup>, respectively, IR radiation wavelength was 980 nm.

Group 5 (intact) included 10 rats, which were not exposed to radiation.

The ethical principles established by the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes (adopted in Strasburg on 18.03.1986 and confirmed in Strasburg on 15.06.2006) and approved by the Ethics Committee of Nizhny Novgorod State Medical Academy were strictly followed in the study.

Samples of the femoral tissue and blood serum were collected on day 3 in all groups of animals. Protein oxidative modification (POM) products, neutral and basic aldehyde- and cetone-dinitrophenylhydrazons, were determined by the level of carbonyl derivatives [7].

Spontaneous POM characterizes constitutive activity of POM; induced and metal-catalyzed POM characterizing POM increment after stimulation indicates the quantity of the substrate for POM and the possibility of its involvement in these processes [7]. According to the authors, metal-catalyzed POM technique makes it possible to determine intermediate oxidation products by stimulating them to generate end products, i.e. carbonyl derivatives. Neutral aliphatic aldehyde-dinitrophenylhydrazons were registered at a wavelength of 356 and 363 nm, neutral aliphatic cetone-dinitrophenylhydrazons at 370 nm and basic aliphatic aldehyde- and cetone-dinitrophenylhydrazons at 430 and 530 nm.

The results were statistically processed using application software package Microsoft Excel and SPSS Statistics (Version 21). Index reliability in the groups was evaluated using Student's t-test. Experimental data compliance with normal distribution was tested with Kolmogorov–Smirnov's test. The data were presented as mean and standard error of mean (M±m).

**Results and Discussion.** Analysis of spontaneous POM product content in the muscle tissue of laboratory animals (Table 1) shows that high-intensity laser radiation exposure is followed by statistically significant increase in the content of oxidation products in the biomaterial (control groups) (p≤0.01), while subsequent low-intensity broadband red light radiation (test groups) causes decrease in these products. Also it should be underlined that red light laser radiation provides considerable growth of final POM products in the muscle

Table 1  
The content of spontaneous protein oxidative modification products in the biomaterial of rats (unit of optical density/g of protein)

Laser wavelength	Intact group	Control groups (1 and 3)		Test groups (2 and 4)	
		Red light	IR radiation	Red light	IR radiation
<i>In the muscular femoral tissue</i>					
356 nm	0.314±0.003	1.037±0.054**	0.795±0.025**	0.691±0.023**	0.495±0.002**
363 nm	0.324±0.004	0.994±0.054**	0.731±0.019**	0.387±0.011**	0.523±0.002**
370 nm	0.204±0.004	2.190±0.045**	0.877±0.027**	1.110±0.059**	0.325±0.010**
430 nm	0.076±0.002	0.818±0.037**	0.429±0.043**	0.480±0.011**	0.180±0.010
530 nm	0.009±7.4·10 <sup>-6</sup>	0.059±0.008**	0.049±0.001**	0.015±0.003**	0.014±0.002**
<i>In the blood serum</i>					
356 nm	0.40±0.006	1.096±0.085*	0.939±0.001**	1.061±0.081**	0.648±0.015**
363 nm	0.404±0.006	0.976±0.087*	0.921±0.001**	0.934±0.067**	0.660±0.016**
370 nm	0.452±0.005	0.808±0.081*	0.864±0.001**	0.780±0.055**	0.617±0.016**
430 nm	0.198±0.001	0.434±0.030**	0.562±0.002**	0.395±0.015**	0.428±0.006**
530 nm	0.008±0.001	0.164±0.012**	0.250±0.001**	0.051±0.009**	0.048±0.001**

Note. Statistically significant differences (p≤0.05): \* between intact and control groups; \*\* between intact and test groups; + between control and test groups.

tissue compared to the data obtained from the group exposed to IR (the exception being only neutral aliphatic aldehyde-dinitrophenylhydrazons registered at 363 nm). Therewith, oxidation products of neutral character prevailed in the samples irradiated by red light, and basic POM products prevailed after IR radiation exposure. The reverse tendency was observed in the blood serum (high-intensity IR radiation led to a greater content of POM products than red laser light).

Subsequent exposure of the same femoral area of a rat to low-intensity red light caused considerable accumulation of final protein oxidation products in the muscle tissue homogenate. This effect was observed in both test groups which underwent prior exposure to high-intensity laser light (both red and IR spectrum) and subsequent low-intensity red light exposure. Despite the fact that the absolute value of POM product content in the test groups was significantly higher than the respective content in the intact group the ratio of POM content in the test and control groups exposed to high-intensity red light amounted to 2.32, and the ratio in the groups exposed to IR laser radiation was nearly 2.36. Thus, a session of exposure to low-intensity red light provided more than twofold decrease in the level of POM products.

Statistically significant decrease in the level of spontaneous POM products in the muscular femoral tissue and the blood serum of both test groups of animals suggests activation of the anti-oxidant system of the organism. In this case the effect of final POM product reduction was more significant in rats' muscular tissue than in their blood serum.

The performed study of induced POM process activity under exposure of laboratory animals' muscular femoral tissue to high-intensity laser light also demonstrated sharp increase in the level of POM products and equally significant decrease in intermediate POM products in the test groups exposed to consecutive low-intensity red light (Table 2).

Considering the effects of red and IR radiation on induced POM it should be noted that the studied products have reverse content dynamics in the blood serum compared to their levels in the muscular tissue. Thus, basic aldehyde- and cetone-dinitrophenylhydrazons prevail in the blood serum of rats exposed to high-intensity red light while after IR laser exposure products of neutral character prevail. With subsequent exposure of the irradiated area to low-intensity red light the level of above-listed protein oxidation products decreases.

Similar effects of normalization of POM processes and antioxidant enzyme activation in phototherapy of organs and tissues exposed to ionizing radiation were also observed by us [8, 9]. The reported effects of decreased POM product levels in the tissues of animals exposed to high-intensity laser radiation and subsequent low-intensity red light result from a cascade of photochemical reactions. They involve decomposition of molecular nitrosyl complexes (including cytochrome C oxidase); subsequent stimulation of mitochondrial respiratory chain in the cells of tissues irradiated by low-intensity red light [10]; vasolytic effect due to the release of free nitric oxide in the blood flow; ATP synthesis stimulation and energy deficit elimination in the damaged cells [11];

Table 2

**The content of induced protein oxidative modification products in the biomaterial of rats (unit of optical density/g of protein)**

Laser wavelength	Intact group	Control groups (1 and 3)		Test groups (2 and 4)	
		Red light	IR radiation	Red light	IR radiation
<i>In the muscular femoral tissue</i>					
356 nm	0.049±0.001	0.184±0.007**	0.085±0.001**	0.072±0.001**	0.059±0.002**
363 nm	0.048±0.001	0.160±0.008**	0.085±0.001**	0.073±0.001	0.062±0.001
370 nm	0.029±0.001	0.163±0.006**	0.074±0.001**	0.060±0.001	0.060±0.007
430 nm	0.017±0.001	0.058±0.001**	0.077±0.012**	0.045±0.001	0.023±0.001
530 nm	0.005±0.001	0.050±0.001**	0.029±0.001**	0.031±0.001	0.012±0.001**
<i>In the blood serum</i>					
356 nm	0.040±0.001	0.125±0.001**	0.153±0.050	0.072±0.001**	0.099±0.020
363 nm	0.038±0.001	0.106±0.002*	0.147±0.040**	0.087±0.001**	0.054±0.001**
370 nm	0.044±0.001	0.111±0.001**	0.139±0.043	0.079±0.001**	0.044±0.001
430 nm	0.018±0.001	0.053±0.001**	0.044±0.001**	0.041±0.001**	0.023±0.001**
530 nm	0.002±0.001	0.050±0.001***	0.110±0.047**	0.009±0.001	0.032±0.001**

Note. Statistically significant differences ( $p \leq 0.05$ ): \* between intact and control groups; \*\* between intact and test groups; + between control and test groups.

photo-activation of antioxidant enzymes, such as superoxide dismutase, catalase, NO-synthase [12], glutathione peroxidase and myeloperoxidase [13]. All these reactions directly or indirectly modify chain processes of protein oxidation making it possible to consider POM product level to be a marker characterizing the efficacy of light radiation sessions. The obtained data enable us to offer a new method of complex phototherapy for the tissues damaged by high-intensity laser radiation.

**Conclusion.** The performed study allowed us to prove the application efficiency of methods of determining POM product content and evaluating the consequences of muscular tissue exposure to high-intensity laser radiation. The obtained data make it possible to offer new technology of complex phototherapy involving sessions of consecutive exposure of live tissues to laser and broadband red light, which provides radiation safety of procedures.

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