NORMALIZATION OF FREE-RADICAL OXIDATION PROCESSES IN MUSCULAR TISSUE IN RADIATION DISEASE BY LOW-INTENSITY RED LIGHT EXPOSURE IN EXPERIMENT

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The aim of the investigation was to assess the level of protein oxidative modification (POM) and lipid peroxidation (LP) products in muscular tissue of rats with radiation disease when exposed to low-intensity red light.

Materials and Methods. We studied the level of direct and induced POM, as well as LP parameters in rat femoral muscular tissue after ionizing radiation exposure and correction of radiation sequellae by low-intensity red light. Free-radical oxidation intensity was estimated by the content of POL products — neutral and basic aliphatic aldehyde- and cetone-dinitrophenylhydrazones; the level of LP products — diene conjugates, triene conjugates and Schiff's bases. SPSS software application package was used for statistical processing.

Results. Rat muscular tissue showed the decreased intensity of oxidative processes — POM and LP after the ionizing radiation area had been exposed to low-intensity incoherent red light, i.e., there was the intensification of oxidative processes. The following exposure of the same area to low-intensity red light resulted in decreased level of oxidation products.

Conclusion. The exposure of biological tissues to low-intensity incoherent red light after ionizing radiation contributes to the decrease of accumulation of POM and LP products and prevents from ozidative stress development.

Key words: free-radical oxidation; ionizing radiation; low-intensity red light.

At present the problem of searching for effective radioprotector remains burning in connection with a widespread application of ionizing radiation in medicine. Exposure to ionizing radiation results in the generation of ions, excited molecules and free radicals in the tissues, the latter leading to the damage of proteins, lipids , and DNA molecules [1]. Low-intensity red light is known to contribute to activation of antioxidant enzymes, and, consequently, can influence the development of lipid peroxidation reactions (LP) and protein oxidative modification (POM) [2].

The aim of the investigation was to assess the level of protein oxidative modification (POM) and lipid peroxidation (LP) products in the muscular tissue of rats in the process of radiation disease development acting upon the irradiated focus by the low-intensity red light.

Materials and Methods. The work was carried out on the outbred white rats with a body mass of 180–250 g, which were divided into 3 groups. The control group included 10 rats exposed locally to ionizing radiation with the dose of 9 Gy. 1 cm^2 area on the inner side of the femor was irradiated. The unit "Luch-1" (Russia) was used for this purpose.

The second (test group) consisted of 10 rats, whose inner surface of the femor was also irradiated according to the same scheme and undergone three consecutive sessions of exposure to low-intensity wide-band red light (during 20 min daily). The intensity of wide-band light was 5 mW/cm^2. In the experiment the light of superbright LED with a maximum of spectral range of 630 nm and width at a half-hight of 20 nm was used. Samples of the femoral tissue in the control and test groups were taken on the fourth day.

The third group (intact) comprised 10 animals, not exposed to either gamma-radiation or wide-band red light.

When carrying on the experiment, ethical principles adopted by the European Convention for the Protection of Vertebrate Animals Used for Experimental or Other Scientific Purposes (adopted in Strasbourg on 18 Mach 1986 and reaffirmed in Strasbourg on 15 June 2006) have been rigorously followed.

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The intensity of free-radical oxidation was estimated by the content of POM products — neutral and basic aliphatic aldehyde- and cetone-dinitrophenylhydrazones, and by the level of LP products — diene conjugates (DC), triene conjugates (TC), and Schiff's bases (SB).

Investigations of POM were conducted by the quantity of carbonyl derivatives (according to A.A. Dubinina's method) [3]: neutral aliphatic aldehyde-dinitrophenylhydrazones were registered at 356 and 363 nm, neutral aliphatic cetone-dinitrophenylhydrazones — at 370 nm, and basic aliphatic aldehyde- and cetone-dinitrophenylhydrozones at 430 and 530 nm.

Spontaneous POM and POM induced by hydrogen peroxide and iron sulfate were studied. If the first parameter characterizes a constituent activity of POM, the second one demonstrates the increment of protein oxidative modification after stimulation, indicates to the quantity of the substrate for POM, and the possibility of its involvement into these processes. On the whole, induced POM may be considered as a marker of the system oxidation stability, an indicator of stress tolerance of the tissue studied. The method of induced POM makes it possible to determine the intermediate oxidation products, stimulating them to generate the end products, i.e. carbonyl derivatives.

The level of POM products was evaluated using I.A. Volchegorsky's method in heptane-isopropanol fractions [4]. According to the author's data neutral lipids are mainly extracted in heptanes, while phospholipids — in isopropanol; thus, heptane fraction shows the activity of POM in neutral lipids, and isopropanol one — in phospholipids.

Data were statistically processed using an application program package SPSS. The distribution of the majority of the investigated parameters was normal or near to normal, which allowed to use the parametric Student t-criterion to determine the difference between the two groups. The distribution of POM products did not turn out to be normal, therefore nonparametric criterion was used. Differences at $p \leqslant 0.05$ were considered to be statistically significant. The results are presented as $M \pm \delta$, where M is a mean value, and δ is a mean square deviation.

Results and Discussion. At the first stage of the work the content of spontaneous POM products (aliphatic aldehyde- and cetone-dinitrophenylhydrazones of neutral and basic character) extracted from the muscular tissue of the rat femor was determined, as well as the total protein by the biuret method in the same samples, presenting the results in units optical density/g of protein (Table 1).

The data obtained speak of the statistically significant changes of POM level in the test group compared to the control one (except aliphatic cetone-dinitrophenylhydrazones of the basic character, being determined at λ=530 nm, which may be connected with a small quantity of these products in the rat muscular tissue). Thus, after the exposure of the gamma-irradiated animal zone to a wide-band red light a reliable reduction of POM product occurred. Besides, as it is seen from Table 1, differences between the intact and test groups are absent, testifying to normalization of the POM processes after the action of the intensive red light on the sample.

The next part of investigation dealt with determining intermediate POM product content in the femoral muscular tissue of the rats (Table 2).

The data presented show that the process of intermediate POM products generation in the muscular tissue of the rats from the control group goes more intensively. Statistically significant differences were obtained in determining the content of all intermediate POM products, except aliphatic cetone-dinitrophenyl hydrazoes of the neutral character. Thus, in the

Table 1

Content of aliphatic aldehyde- and cetone-dinitrophenylhydrazones of neutral and basic character in the muscular femoral tissue of the rats in spontaneous protein oxidative modification, units optical density/g of protein

Wavelength, nm	Intact group	Control group	Test group	p
356	0.320±0.039	0.530±0.061*	0.360±0.082**	0.021* 0.014**
363	0.340±0.044	0.540±0.058*	0.380±0.094**	0.034* 0.030**
370	0.380±0.060	0.550±0.070*	0.400±0.089**	0.030* 0.023**
430	0.190±0.023	0.340±0.034*	0.210±0.041**	0.004* 0.002**
530	0.029±0.011	0.048±0.016*	0.033±0.010	0.050* 0.445**

Note: here and further content of POM products — aliphatic aldehyde and cetone-dinitrophenylhydrazones of neutral and basic character — is indicated in accordance with the wavelength, at which maximal absorption is observed in these products. * — statistically significant differences between intact and control groups; ** — between control and test groups.

Table 2

Content of aliphatic aldehyde- and cetone-dinitrophenylhydrazones of neutral and basic character in the muscular femoral tissue of the rats in induced protein oxidative modification, units optical density/g of protein

Wavelength, nm	Intact group	Control group	Test group	p
356	0.200±0.025	0.240±0.077*	0.210±0.029**	0.038* 0.046**
363	0.240±0.037	0.270±0.075*	0.250±0.044**	0.033* 0.050**
370	0.230±0.034	0.244±0.090	0.242±0.046	0.070* 0.060**
430	0.140±0.022	0.190±0.046*	0.150±0.025**	0.043* 0.050**
530	0.023±0.001	0.031±0.080*	0.025±0.007**	0.040* 0.050**

* — statistically significant differences between the intact and control groups; ** — between the control and test groups

Table 3

Content of lipid peroxide products in the muscular femoral tissue of the rats, relative units

LP products	Intact group	Control group	Test group	p
DC	0.210±0.020	0.240±0.049*	0.220±0.014**	0.045* 0.050**
TC	0.200±0.035	0.220±0.043*	0.200±0.078**	0.050* 0.050**
SB	14.9±2.9	25.6±4.9*	16.6±4.4**	0.010* 0.020**

* — statistically significant differences between the intact and control groups; ** — between the control and test groups

biomaterial of the animals from the control group more substrate quantity for POM was observed compared to the test group. When findings of the intact and test groups were compared, statistically significant differences were not revealed, which allows to make a conclusion that wide-band red light produces a corrective action on the tissues irradiated by ionizing radiation.

The last step of the investigation was to determine the products of lipid peroxidation in the irradiated femoral muscular tissue of the rats before and following the red light exposure. At this stage statistically significant differences were also obtained in the content of the primary LP products and final products (SB) in the tissue homogenate of the test and control animal groups (Table 3).

The data obtained speak of the statistically significant reduction of the content of the intermediate (DC and TC) and final (SB) LP products in case of the exposure of the irradiated muscular tissue to wide-band red light. When analyzing LP products, no statistically significant differences between the intact and test groups were revealed just as in investigations of POM products, testifying to the approximation of the values in the test group to the norm.

These findings are completely in line with the data reported in the literature, showing the activation of free-radical protein and lipid oxidation process in different pathological conditions [5, 6]. Besides, the present understanding of the mechanisms of photobiological red light effect can explain the observed normalization of the free-radical oxidation level. The active forms of oxygen, generating in the process of radiolysis, are known to cause accumulation of POM and LP products in the tissues. Similar effects were observed not only under the action of ionizing radiation, but also in experimentally induced ischemia and reperfusion [7, 8], and also in the development of asphyxia [9]. The same effects of reducing the content of free-radical oxidation products and activation of antioxidant enzymes were obtained experimentally in the works [10, 11]. Several photochemical reactions lie in the basis of these processes. Firstly, under the action of the red light a release of cytochrome-c-oxidase-bound nitric oxide is observed [12]; secondly, activation of superoxide dismutase and NO-synthase occurs [13]; thirdly, stimulation of ATP synthesis takes place [14]. Each of these reactions, especially activation of antioxidant enzymes, is capable of influencing significantly the normalization of the processes of free-radical oxidation in the damaged tissues. For example, nitric oxide

synthetized by mitochondrial NO-synthase, inhibits respiration due to generation of cytochrome-c-oxidase bond [15]. Photolysis of these molecular complexes results in the release of free nitric oxide and reactivation of electron carriers in the respiration chain. Ultimately, restoration of the respiration processes and activation of ATP molecule synthesis occurs.

Conclusion. The effect of ionizing radiation on the biological tissues leads to the reduction of antioxidant protection and to activation of free-radical processes, revealing themselves in the increase of the level of protein oxidative modification and lipid peroxide products. The following exposure to low-intensity incoherent red light contributes to the reduction of the product accumulation level and prevents the development of the oxidative stress in the biological tissues, affected by ionizing radiation. The effect observed is likely to be associated with the increase of antioxidant enzyme activity under the action of the wide-band red light.

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