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PROTEINS OXIDATIVE MODIFICATION AND ANTIOXIDANT ENZYMES ACTIVITY IN THE LIVER MITOCHONDRIA OF RATS UNDER LASER IRRADIATION AND ADMINISTRATION OF ω-3 POLYUNSATURATED FATTY ACIDS

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The effect of laser irradiation of rats combined with omega-3 polyunsaturated fatty acids (ω-3 PUFA) administration on the proteins oxidative modification and superoxide dismutase and catalase activity in the mitochondrial fraction of the liver was investigated. Animals were irradiated with a 650 nm laser diode in the abdomen daily for 4 min at the distance of 10 cm from the skin surface. ω-3 PUFA were administered per os at a daily dose of 120 mg/kg of the body weight. Fatty acids in the fish oil were identified by gas chromatography. Animals were divided into five groups (12 animals in each group): I – intact rats (control); II – rats exposed to the daily laser irradiation for 7 or 14 days ; III – rats that received ω-3 PUFA two hours after irradiation; IV – rats that received ω-3 PUFA two hours before irradiation; V – rats that received ω-3 PUFA for 7 days before irradiation. The mitochondrial fraction of rat liver was obtained by differential centrifugation. The increase in the content of protein carbonyl derivatives and a decrease of protein thiol groups in the liver mitochondrial fraction were detected after seven-day laser irradiation of rats. As the duration of irradiation increases, superoxide dismutase and catalase activity was decreased, indicating a depletion of mitochondrial antioxidant reserves. No antioxidant effect was observed when ω-3 PUFA were administrated after laser irradiation and a slight antioxidant effect was shown when ω-3 PUFA were administrated two hours before irradiation. Preliminary seven-day administration ω-3 PUFA before laser irradiation was the most effective, as it reduced the level of protein carbonyl derivatives and $O_2^{\bullet-}$-generation, increased proteins SH-groups content and antioxidant enzymes activity.

K e y w o r d s: SH-groups, carbonyl derivatives, superoxide radical, laser irradiation, ω-3 polyunsaturated fatty acids, liver mitochondria, superoxide dismutase and catalase activity.

The use of laser irradiation is becoming increasingly common today, as this type of irradiation can have biostimulating and physiotherapeutic effects [1]. The efficiency or negative effects of laser radiation depend on the power, wavelength, pulse duration, repetition rate, interaction time, biochemical and physicochemical characteristics of the tissue exposed to irradiation [2, 3]. With prolonged exposure to laser radiation on biological tissues in the body are complex biochemical processes, which can promote the emergence and the accumulation of free radicals, which will lead to the intensification of oxidative modification of proteins and the accumulation in cells [4, 5]. These changes may underlie the anti-carcinogenic effect of the laser, during the action on the transformed tissues [6], which will reduce tumor growth in the body. However, the intensification of free radical processes during the direct impact of the laser on normal body tissues can adversely affect the functioning of organs, including the liver. However, along with the use of laser irradiation, it would be appropriate to use essential nutrients with antioxidant properties that would reduce the negative effects of the laser. Such nutrients include ω-3 polyunsaturated fatty acids (PUFA) [7].

ω-3 PUFA provides stability of cell membranes, maintains energy balance in the cell, synthesizes eicosanoids. Due to ω-3 PUFA reduces the activity of the inflammatory process, as they stimulate the production of specific anti-inflammatory mediators, and also have an inhibitory effect on the synthesis of pro-inflammatory substances (COX 2, nitric oxide synthase, tumor necrosis factor, interleukins 1, 6 and 12 in endothelial cells) [8-10]. Due to these properties, ω-3 PUFA promote an active immune response of the body and show a protective effect in response to external factors [11].

The issues of the combined action of laser irradiation and ω-3 PUFA on the biochemical processes of liver cells remain open. However, the scheme of their combined application is not clear, because ω-3 PUFA can be substrates of laser irradiation, thus triggering free radical processes in the body.

Given the above, the aim of this study was to evaluate the intensity of oxidative modification of proteins and the activity of antioxidant enzymes in the mitochondrial fraction of rat liver under the action of laser irradiation and additional administration of ω-3 PUFA.

Materials and Methods

The experiment were conducted on white outbred rats weighing 130-150 g. Animal keeping, handling and manipulating were in accordance with the article 26 of the Law of Ukraine No 3447-IV 21.02.2006 "On the Protection of Animals from Cruelty", "European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes" (Strasbourg, 1986) and with regard to NIH Guide for the Care and Use of Laboratory Animals, approved 20. 09. 2001 by the First Ukrainian National Congress on Bioethics, and taking into account the provisions set out in [12].

All animals were divided into five groups (12 animals in each group): I – intact animals (control); II – rats were exposed to the laser diode; III – rats, which ω-3 PUFA was injected after laser irradiation; IV – rats, which ω-3 PUFA was injected daily two hours before laser irradiation; V – rats, which ω-3 PUFA was injected 7 days before the application of laser irradiation.

Irradiation of the animals was carried out daily for 4 min with a laser diode with a wavelength of 650 nm, a power of 50 mw in the abdomen at a distance of 10 cm from the skin surface. The source of ω-3 PUFA was fish oil in the form of a commercial drug Vitrum Cardio (manufactured by Unipharm, Inc., USA), which contained 32% eicosapentaenoic acid (EPA) and 24% docosahexaenoic acid (DHA) (Table).

Fatty acids in fish oil were identified by gas chromatography on a HRGC 5300 chromatograph (Italy). For the analysis of individual fatty acids used standard drugs from Sigma. ω-3 PUFA was administered per os at a daily dose of 120 mg/kg body weight of animals, because at this dose exhibit antioxidant and membrane-protective effects [13].

Euthanasia of the animal was performed under light ether anesthesia on the 7th and 14th day of laser diode irradiation.

The mitochondrial fraction of rat liver was obtained by differential centrifugation [14]. The level of oxidative modification of proteins and enzymatic activity of superoxide dismutase (SOD, 1.15.1.1) and catalase (CAT, 1.11.16) was determined in the mitochondrial fraction. Oxidative modification of proteins was determined by the content of carbonyl derivatives (method based on the reaction of the interaction of carbonyl derivatives of proteins with 2,4-dinitrophenylhydrazine with the formation of 2,4-dinitrophenylhydrazones) [15] and the level of sulfhydryl groups (SH-groups) (the method is based on the interaction of Elman reagent with protein SH-groups) [16]. The formation of a superoxide radical ($O_2^{\bullet-}$) was recorded in a test with Nitro blue tetrazolium (NBT) [17]. The principle of the method is that NBT in the presence of a superoxide radical is reduced to hydrazinetetrazolium, with a maximum depth at 540 nm. The rate of $O_2^{\bullet-}$ formation was ex-

Table. The content of polyunsaturated fatty acids of the family ω-3 and ω-6 in the ω-3 PUFA

The content of ω-6 and ω-3 PUFA					The degree of unsaturation
ω-6, %	ω-3, %			ω-6/ω-3, %	
LA	α-LNA	EPA	DHA		
1.63	0.67	32.57	24.13	0.03	191.70

pressed in nmol of hydrazinetetrazolium formed per minute per mg of protein (nmol/min per mg of protein), given that the extinction of 0.325 corresponds to 325 nmol of super oxide radical [17].

Determination of superoxide dismutase activity in the mitochondrial fraction of the liver is carried out by the method [18], which is based on the ability of SOD to inhibit the oxidation of adrenaline. The enzymatic activity of SOD was expressed in (c.u.)/ mg protein. Determination of catalase activity was performed by the method [19], which is based on the ability of hydrogen peroxide to form a stable colored complex with ammonium molybdate with an absorption maximum at $\lambda = 410$ nm. Catalase activity was expressed in mmol/min per mg of protein.

Statistical processing of the results was performed using one-way analysis of variance (ANOVA) followed by Tukey's HSD post hoc test. The differences were considered significant if $P \leq 0.05$.

Results and Discussion

Oxidative stress plays a key role in the pathogenesis of liver disease. Dysfunction of this organ potentates inflammatory-degenerative processes of tissues. During normal liver function, a dynamic balance is maintained between the prooxidant and antioxidant systems [20].

Due to the action of various factors on the body, in particular laser irradiation, the balance between free radical processes and the antioxidant system is disturbed [21], which will enhance the processes of oxidative modification of proteins. These changes may be one of the main reasons for the inactivation of enzymes and changes in the structural organization of proteins.

One of the main markers of oxidative modification of proteins is carbonyl derivatives, which are formed by oxidation of amino acid residues of tryptophan, phenylalanine, histidine, proline, arginine, lysine and threonine. Accumulation of carbonyl groups of proteins can be an early indicator of tissue damage [22].

As a result of the conducted researches it is established that under the action of laser irradiation in the area of the abdominal cavity that anatomically corresponds to the localization of the liver, there is an increase in the level of carbonyl derivatives in the mitochondrial fraction of the liver, which is amplified as the laser irradiation period increases as compared with non-irradiated animals ($P \leq 0.05$) (Fig. 1). Obviously, under the action of a laser diode on each absorbed photon in the photochemical reaction, an activated particle (free radical, atom, molecule) is formed [23]. After activation, a cellular reaction (primary) occurs, which is reflected in the increase of protein carbonyl derivatives.

The increase in the level of carbonyl groups of proteins may be the result of a decrease in the activity of cellular protease systems. Because it is known that the decrease in proteosome function may be accompanied by the accumulation of damaged proteins.

Along with the oxidation of protein amino groups, oxidation of sulfhydryl groups is observed in the mitochondrial fraction of the liver. After seven days of irradiation with a laser diode, the level of SH-groups of proteins decreases 2.7 times, and after fourteen days – 8 times as compared with non-irradiated animals ($P \leq 0.05$) (Fig. 2).

Enhancement of oxidative modification of proteins probably occurs due to the intensive generation of superoxide anion radical components of the electron transport chain of mitochondria, which is confirmed by our results. With increasing duration of irradiation in the mitochondrial fraction of the liver of rats increases the rate of formation of $O_2^{\bullet-}$ with the maximum values of the 14th day of irradiation (Fig. 3), which probably triggers the free radical destruction of proteins under the action of laser irradiation.

Thus, the daily four-minute directional action of the laser diode in the abdominal cavity leads to the initiation of free radical processes in the mitochondrial fraction of the liver, enhancing the generation of $O_2^{\bullet-}$ and oxidative modification of proteins. Such a primary cellular reaction can turn into a generalized (systemic, secondary) reaction with prolonged exposure to low-intensity laser radiation on the body [23]. To reduce the prooxidant effect of a laser diode, the effect of which is determined by the physical properties of radiation, its features and the properties of the biological object of influence, we used ω-3 PUFA, which have antioxidant properties [8].

The use of different schemes of administration of ω-3 PUFA has shown that the highest antioxidant effect of ω-3 PUFAs have their seven-day preintroduction into the body before the start of laser irradiation. In this group of animals, the levels of carbonyl derivatives (Fig. 1) and SH-groups of proteins (Fig. 2) do not differ from the control indicators on the 7th day of irradiation, and on the 14th day of irradiation the content of carbonyl derivatives in-

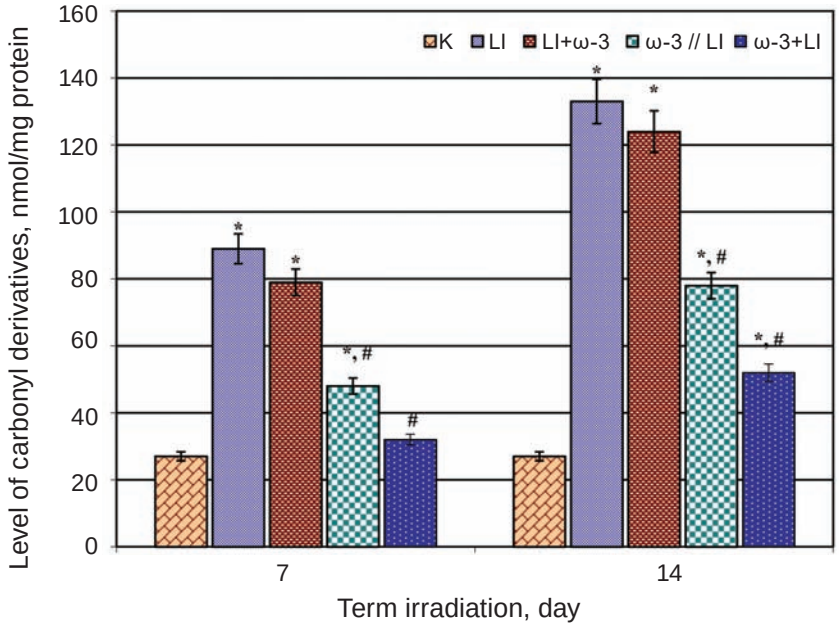

*Fig. 1. The level of carbonyl derivatives in the mitochondrial fraction of the rat liver under the action of laser irradiation and the introduction of ω-3 polyunsaturated fatty acids. K – intact animals (control); LI – rats exposed to a laser diode; LI + ω-3 – rats, which were injected with ω-3 PUFA after laser irradiation; ω-3 // LI – rats, which ω-3 PUFA was injected daily two hours before laser irradiation; ω-3 + LI – rats, which ω-3 PUFA was injected 7 days before the application of laser irradiation; *statistically significant difference compared to intact animals ($P \leq 0.05$); #statistically significant difference compared to irradiated rats ($P \leq 0.05$)*

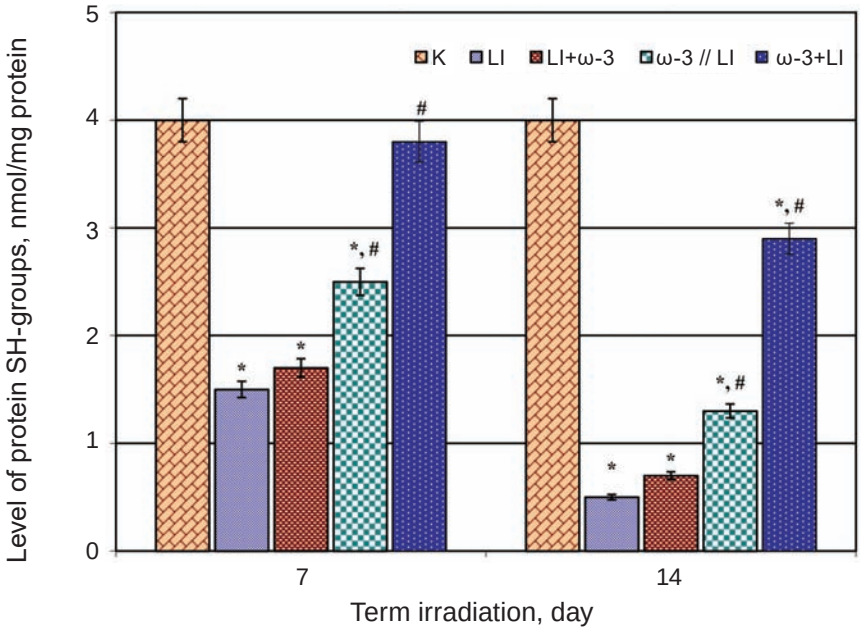

*Fig. 2. The level of protein SH-groups in the mitochondrial fraction of rat liver under the action of laser irradiation and the introduction of ω-3 polyunsaturated fatty acids. K – intact animals (control); LI – rats exposed to a laser diode; LI + ω-3 – rats, which were injected with ω-3 PUFA after laser irradiation; ω-3 // LI – rats, which ω-3 PUFA was injected daily two hours before laser irradiation; ω-3 + LI – rats, which ω-3 PUFA was injected 7 days before the application of laser irradiation; *statistically significant difference compared to intact animals ($P \leq 0.05$); #statistically significant difference compared to irradiated rats ($P \leq 0.05$)*

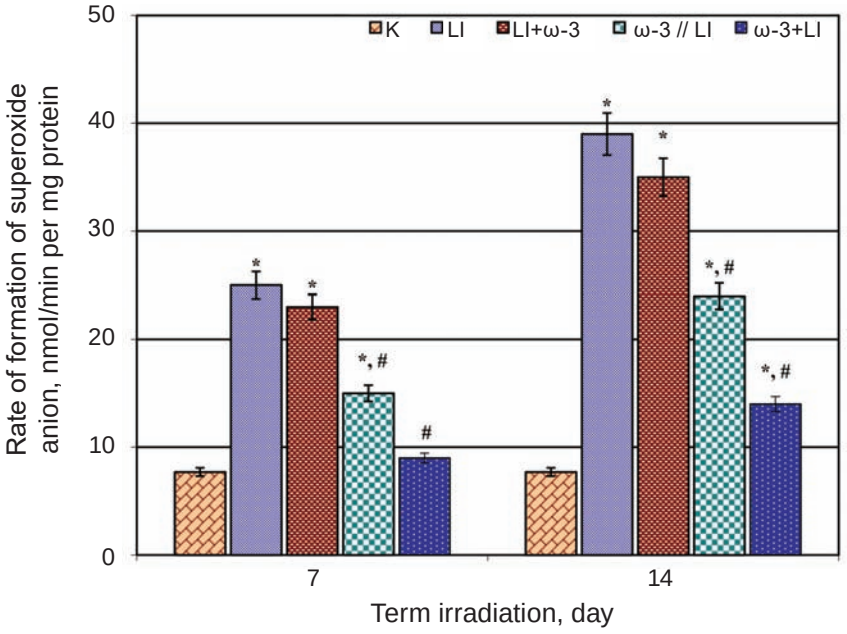

*Fig. 3. The rate of formation of superoxide anion radical in the mitochondrial fraction of rat liver under the action of laser irradiation and the introduction of ω-3 polyunsaturated fatty acids. K – intact animals (control); LI – rats exposed to a laser diode; LI + ω-3 – rats, which were injected with ω-3 PUFA after laser irradiation; ω-3 // LI – rats, which ω-3 PUFA was injected daily two hours before laser irradiation; ω-3 + LI – rats, which ω-3 PUFA was injected 7 days before the application of laser irradiation; *statistically significant difference compared to intact animals ($P \leq 0.05$); #statistically significant difference compared to irradiated rats ($P \leq 0.05$)*

creases 1.9 times ($P \leq 0.05$) (Fig. 1), and SH-groups decreases 1.4 times ($P \leq 0.05$) (Fig. 2) compared with the control. At the same time, the rate of $O_2^{\bullet-}$ generation remains low (Fig. 3), as this indicator does not differ statistically significantly from the control indicator on the 7th day of irradiation and increases 1.8 times - on the 14th day of irradiation compared to the control ($P \leq 0.05$) (Fig. 3). Obviously, the previous introduction of ω-3 PUFA promotes their incorporation into the membrane structure of cells, which makes them resistant to free radical destruction of macromolecules [13]. On the other hand, the bioregulatory role of ω-3 PUFAs on mitochondrial function may be manifested through eicosanoids, which are synthesized from ω-3 PUFAs.

During the use of ω-3 PUFA after irradiation, there is an increase in the generation of $O_2^{\bullet-}$ (Fig. 3) and oxidative modification of proteins, which is expressed by an increase in the level of carbonyl derivatives (Fig. 1) and a decrease in SH-groups of proteins (Fig. 2). However, these results do not differ from those of the group of irradiated animals that were not injected with ω-3 PUFA. The result of oxidative modification of proteins may be a decrease in their structural and functional organization and disruption of the respiratory chain. As a result, there is a generation of superoxide anion radical in the respiratory chain of mitochondria, which indicates the emergence of prooxidant status in these groups of animals [24].

The introduction of ω-3 PUFA two hours before the action of laser irradiation leads to an increase in the level of protein carbonyl groups ($P \leq 0.05$) (Fig. 1) and a decrease in SH-groups of proteins ($P \leq 0.05$) (Fig. 2) in the mitochondrial fraction of rat liver compared to intact animals. However, such changes in markers of oxidative modification of proteins do not reach the level of the group of irradiated animals that were not injected with ω-3 PUFA. Obviously, in the first stages of irradiation, pre-administration of ω-3 PUFA has an antioxidant effect, and as the duration of irradiation with ω-3 PUFA become substrates of reactive oxygen species (ROS). An excessive ROS generation may also be associated with low activity of the enzymatic and non-enzymatic components of antioxidant protection.

The main enzymes of the antioxidant system are SOD and CAT. SOD is the first link in antioxi-

dant protection against ROS, which catalyzes the dismutation of the superoxide radical. Two SOD isoforms were detected in the cell. The Mn-containing isoform is localized in mitochondria, the function of which is to inactivate $O_2^{\bullet-}$, formed as a result of the functioning of the mitochondrial electron transport chain [25].

As a result of research, an increase in superoxide dismutase activity was found in the group of experimental animals exposed to a laser diode and in those treated with ω-3 PUFA after the laser irradiation as compared with non-irradiated animals ($P \leq 0.05$) (Fig. 4). The increase in the enzymatic activity of SOD can occur in response to the increased formation of $O_2^{\bullet-}$ (Fig. 3).

However, on the 14th day of irradiation there is a sharp decrease in superoxide dismutase activity (Fig. 4), which may occur with the accumulation of the reaction product - hydrogen peroxide and/or with oxidative inactivation of the enzyme [25].

The increase and subsequent decrease in the enzymatic activity of SOD may be a consequence of the development of pathological processes in the body. As a result of the superoxide dismutase reaction, H_2O_2 is formed, which is further metabolized to O_2 and H_2O by the action of the heme-containing enzyme catalase [26]. The decrease in the content of this enzyme in the cells leads to an increase in the cytotoxic effect of H_2O_2, which is formed as a result of dismutation of the superoxide radical [25].

Analysis of the results showed that in the initial stages of laser irradiation (7 days) there is an increase in the enzymatic activity of catalase in the mitochondrial fraction of rat liver ($P \leq 0.05$) (Fig. 5).

Because CAT is a heme-containing protein, it can be directly activated by the photons of a laser diode. On the other hand, the increase in catalase activity may occur in response to the formation of H_2O_2, which is a substrate for catalase. On the 14th day, there is a decrease in catalase activity by 2.2 times compared with the control ($P \leq 0.05$) (Fig. 5). Decreased enzyme activity of catalase in the mitochondrial fraction of rat liver may be due to reduced synthesis and increased degradation of its molecules as a result of oxidative inactivation of the enzyme by ROS – $O_2^{\bullet-}$, ·OH and H_2O_2, the number of which increases under oxidative stress [24] .

The highest antioxidant effect of ω-3 PUFA have under the conditions of their previous seven-day administration before irradiation. Thus, on the

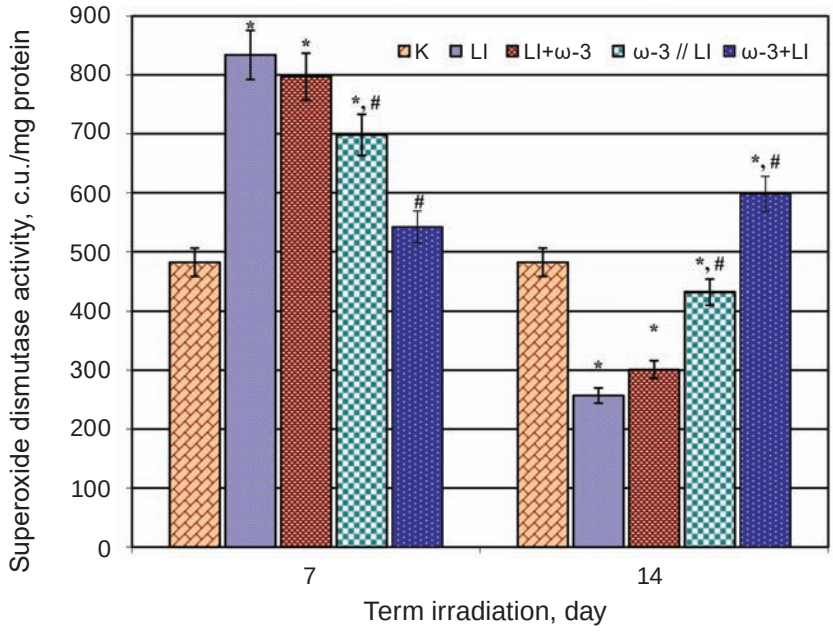

*Fig. 4. Superoxide dismutase activity in the mitochondrial fraction of rats under the action of laser irradiation and the introduction of ω-3 polyunsaturated fatty acids. K – intact animals (control); LI – rats exposed to a laser diode; LI + ω-3 – rats, which were injected with ω-3 PUFA after laser irradiation; ω-3 // LI – rats, which ω-3 PUFA was injected daily two hours before laser irradiation; ω-3 + LI – rats, which ω-3 PUFA was injected 7 days before the application of laser irradiation; *statistically significant difference compared to intact animals ($P \leq 0.05$); #statistically significant difference compared to irradiated rats ($P \leq 0.05$)*

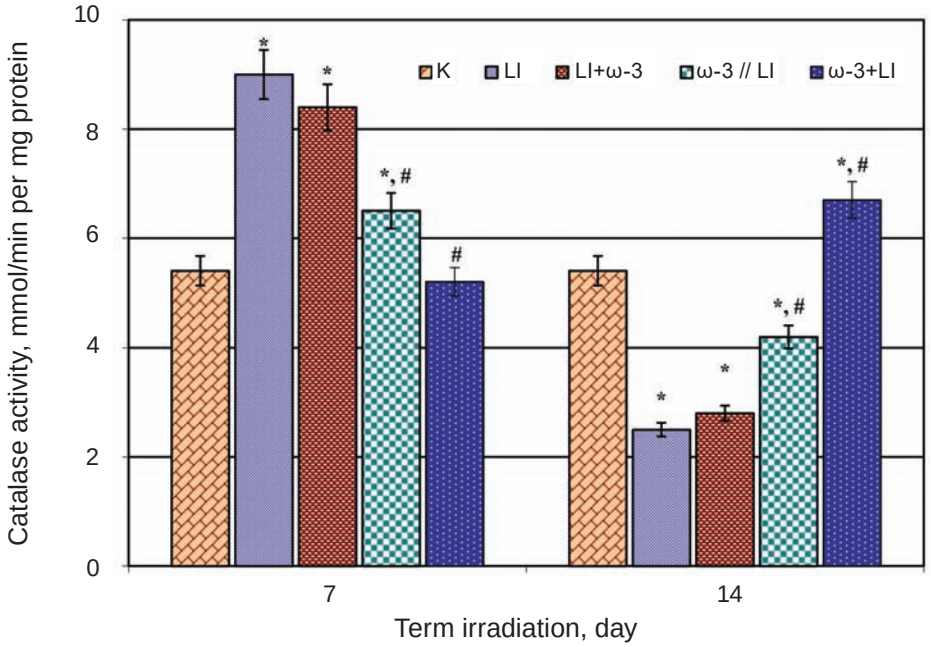

*Fig. 5. Catalase activity in the mitochondrial fractions of the liver of rats under the action of laser irradiation and the introduction of ω-3 polyunsaturated fatty acids. K – intact animals (control); LI – rats exposed to a laser diode; LI + ω-3 – rats, which were injected with ω-3 PUFA after laser irradiation; ω-3 // LI – rats, which ω-3 PUFA was injected daily two hours before laser irradiation; ω-3 + LI – rats, which ω-3 PUFA was injected 7 days before the application of laser irradiation; *statistically significant difference compared to intact animals (P ≤ 0.05); #statistically significant difference compared to irradiated rats (P ≤ 0.05)*

7th day of irradiation the level of catalase activity does not change compared to the control, and on the 14th day there is a slight increase (1.2 times), apparently in response to the generation of ROS (Fig. 5). The introduction of ω-3 PUFA two hours before irradiation and after irradiation first increases the catalase activity, and then reduces it on the 14th day of irradiation. These changes are more pronounced in animals to which ω-3 PUFA was administered after irradiation, because the indicators of this group of animals do not differ from irradiated animals that were not injected with ω-3 PUFA (Fig. 5). The established changes will lead to an increase in the concentration of H_2O_2 in liver cells, which will trigger a cascade of reactions of free radical oxidation of lipids, thereby damaging the membranes of mitochondria [24].

Thus, the daily four-minute action of the laser diode leads to the initiation of oxidative modification of proteins with simultaneous depletion of antioxidant reserves in the mitochondrial fraction, as evidenced by a decrease in the enzymatic activities of SOD and CAT. An increase in the rate of $O_2^{\bullet-}$ formation may indicate a violation of the mitochondria in the electron transport chain and the leakage of electrons from it [24]. $O_2^{\bullet-}$ will initiate free radical oxidation of proteins.

The introduction of ω-3 PUFA reduces the prooxidant-antioxidant imbalance in the mitochondrial fraction of the liver, but this effect largely depends on the scheme of their introduction and irradiation. ω-3 PUFA does not show antioxidant effect under the conditions of their introduction after irradiation. Daily administration of ω-3 PUFA two hours before irradiation shows a slight antioxidant effect in the initial stages of irradiation, as evidenced by indicators of protein modification and generation of $O_2^{\bullet-}$. Preliminary seven-day administration of ω-3 PUFA before laser irradiation helps to reduce the level of carbonyl groups of proteins, $O_2^{\bullet-}$ generation and increase the content of SH-groups, while stimulating the enzymatic link of antioxidant protection. The protective effect of the previous administration of omega-3 PUFAs is obviously related to their incorporation into the mitochondrial membranes, which stabilizes the latter and makes them resistant to radiation.

Conflict of interest. Authors have completed the Unified Conflicts of Interest form at http://ukr-biochemjournal.org/wp-content/uploads/2018/12/coi_disclosure.pdf and declare no conflict of interest.

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ОКИСНА МОДИФІКАЦІЯ ПРОТЕЇНІВ ТА АКТИВНІСТЬ АНТИОКСИДАНТНИХ ЕНЗИМІВ У МІТОХОНДРІЯХ ПЕЧІНКИ ЩУРІВ ЗА ЛАЗЕРНОГО ОПРОМІНЕННЯ ТА ВВЕДЕННЯ ω-3 ПОЛІНЕНАСИЧЕНИХ ЖИРНИХ КИСЛОТ

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Досліджено вплив лазерного опромінення щурів у поєднанні з введенням омега-3 поліненасичених жирних кислот (ω-3 ПНЖК) на окисну модифікацію протеїнів і на активність супероксиддисмутази та каталази у мітохондріальній фракції печінки. Тварин опромінювали 650 нм лазерним діодом щодня у ділянці черевної порожнини протягом 4 хв на відстані 10 см від поверхні шкіри. ω-3 ПНЖК вводили per os у добовій дозі 120 мг/кг маси тіла. Жирні кислоти у риб'ячому жирі ідентифікували методом газової хроматографії. Тварин було поділено на п'ять груп (по 12 у кожній): I – інтактні щури (контроль); II – щури, яких опромінювали щодня лазером протягом 7 або 14 днів; III – щури, які отримували ω-3 ПНЖК через дві години після опромінення; IV – щури, які отримували ω-3 ПНЖК за дві години до опромінення; V – щури, які отримували ω-3 ПНЖК упродовж 7 днів до опромінення. Мітохондріальну фракцію печінки щурів отримували диференціальним центрифугуванням. Після семиденного лазерного опромінення у щурів виявлено збільшення вмісту карбонільних та зменшення вмісту тіолових груп протеїнів у мітохондріях печінки. Зі збільшенням тривалості опромінення активність супероксиддисмутази та каталази зменшувалася, що свідчило про виснаження мітохондріальних антиоксидантних резервів. За введення ω-3 ПНЖК через дві години після лазерного опромінення антиоксидантного ефекту не спостерігали, а за введенні ω-3 ПНЖК за 2 години до опромінення було виявлено незначний антиоксидантний ефект. Введення ω-3 ПНЖК (протягом 7 днів) перед лазерним опроміненням було найефективнішим, оскільки супроводжувалось зниженням вмісту карбонільних похідних та пригніченням утворення $O_2^{\bullet-}$, підвищенням вмісту SH-груп протеїнів та активності антиоксидантних ензимів.

Ключові слова: SH-групи, карбонільні похідні, супероксидний радикал, лазерне опромінення, ω-3 поліненасичені жирні кислоти, мітохондрії печінки, супероксиддисмутазна та каталазна активність.

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