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The Effect of the Concentrate of Polyunsaturated Fatty Acids on Indicators of Oxidative Stress in Experimental Dyslipidemy

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Abstract

This work was aimed at studying the effect of liposomal concentrate of polyunsaturated fatty acids (PUFA) on indicators of oxidative stress in experimental dyslipidemy. It was found that reproduction in rats in dyslipidemic state was accompanied by accumulation of end products of lipid peroxidation in blood serum and liver, and decreased activity of glutathione antioxidant protection in animals. The study showed that introduction of liposomal concentrate of PUFA into experimental animals against the background of dyslipidemy reduced parameters of oxidative stress in the organism.

Keywords

Oxidative stress; Dyslipidemy; Polyunsaturated fatty acids; Liposomes; Antioxidant system; Glutathione

Introduction

Alimentary dyslipidemy (DL) is recognized as one of modifiable risk factors of the ischemic heart disease. DL is characterized by a set of disorders that include increased level of lipids, intensification of their peroxidation, and changes in functioning of the immune and the antioxidant systems. These processes cause deviations in the entire organism, especially the immune system, mainly due to pathological changes in the composition of immune-competent cells membranes [1].

Changes in the structure and composition of cell membranes are associated with the processes of free-radical oxidation of lipids. In particular, oxidative modification of lipoproteins and very low density contribute to releasing cholesterol and embedding it into cell membranes. One of the reasons for the emergence of modified forms of low-density lipoproteins may be the formation of malondialdehyde (MDA), and the processes that are directly associated with interaction of active radicals. According to some researchers, a leading role in atherogenesis, along with other deviations, is played by the processes of lipids peroxidation (LPO) [2].

For preventive treatment and treatment of DL, biologically active additives (BAA) from lipids of marine hydrobionts can be used, which are rich in omega-3 polyunsaturated fatty acids (PUFA) with high physiological activity. In clinical and experimental studies, preparations based on lipids of marine aquatic organisms show anti-inflammatory, antidiabetic, hypolipidemic action and are recommended for comprehensive prevention and auxiliary treatment of a wide range of diseases [3]. As many studies show, omega-3 PUFA have a modulating effect on the structural and functional organization of cell membranes, activity of membrane-linking enzymes, and biosynthesis of icosanoids. According to several authors, PUFA feature high radical-intercepting activity and the ability to induce activity and expression of antioxidant enzymes genes [4].

The purpose of this study was to investigate the effect of liposomal concentrate of PUFA on some parameters of oxidative stress in case of experimental DL.

Materials and Methods

Liposomes derived from the phospholipids of Baikal seal's liver were used in the work [5]. The PUFA concentrate was obtained from

the Baikal seal's fat, using the method of complex formation with urea [6,7]. The fatty acid composition of the PUFA concentrate was determined by the method of gas-liquid chromatography using a chromatography-mass spectrometer 5973/6890 NMSD/DS produced by Agilent Technology (USA). Separation was performed with the use of capillary column HP-INNOWAX (30 m × 250 μm × 0.50 μm). The stationary phase is polyethylene glycol. Mobile phase: helium, gas flow rate: 1 ml/min. The temperature of the evaporator was 250°C, the temperature of ion source—230°C, and that of the line connecting the chromatograph and the mass spectrometer was 280°C. The scanning range was 41-450 atomic mass units. The volume of the injected sample was 1 μl, and flows were split 5:1. Chromatography was performed in isocratic mode at 200°C. Recording was performed with full ion current (SCAN mode).

The experiment was performed on sexually mature male rats of the Wistar breed with 130-150 g initial weight, obtained from the nursery of the Research Institute of Biophysics of the Angarsk State Technical Academy and kept in standard vivarium conditions in individual cages. Prior to the experiment, all used animals received the regular diet of the vivarium and water *ad libitum*. The study was performed in conformance with the requirements of legal regulations for the procedure of experimental work with the use of animals [8]. Experimental hyperlipidemy in rats was caused according to the methodical instructions (MG 2.3.2.721-98.2.3.2) developed by the Institute of Nutrition of the Russian Academy of Medical Sciences, using the model of atherogenic hyperlipidemy with introduction of the following into typical diet: 3-5% cholesterol (BioChemica, Applichem), 0.3% of 6-methylthiouracil, 1% of bile acid, and 5% of lard—for 21 days.

The experimental animals were divided into three groups (10 animals per group):

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Group I—intact (animals received standard fodder and water);
Group II—reference (animals received atherogenic diet for 21 days);
Group III—experimental (animals, after atherogenic diet, received oral liposomal suspension with the addition of PUFA concentrate for 14 days at the dosage of 20 mg per 1 kg of body weight).

In order to assess the hypolipidemic action of the concentrate of PUFA, the contents of total cholesterol (TC), triacylglyceride (TG), high density lipoprotein cholesterol (HDL cholesterol), low density lipoprotein cholesterol (LDL cholesterol), and very low density lipoprotein cholesterol (VLDL cholesterol) were determined in the blood serum of rats. Biochemical indicators were set with the use of standard reactants from “Abris+” and “Diaz” companies, using the automatic biochemical analyzer BS-400 (PRC). The atherogenic index (AI) was calculated for characterizing blood atherogenic properties.

The AI was calculated using the following formula:

$$AI = \frac{TC - HDLC}{HDLC}$$

The antioxidant capacity of the organism was assessed by the degree of inhibiting formation of lipid peroxidation products—MDA, the activity of glutathione reductase (GR), the activity of glutathione peroxidase (GSH-Px), the amount of reduced glutathione (GSH), and total content (TOC) of antioxidants in blood serum.

MDA concentration in the liver and blood serum was determined using the method based on MDA's ability to react with 2-thiobarbituric acid (TBA). Concentration of TBA-active products in the samples was measured at 532 nm wavelength by the degree of colored complex formation with TBA [9].

Determination of GR activity is based on changing NADPH oxidation rate, which is detected spectrophotometrically by decreasing optical density at 340 nm wavelength [10].

Defining activity of GSH-Px is based on GSH-Px's ability to catalyze GSH reaction with tert-butyl hydroperoxide (TBHP). Enzyme activity can be evaluated by measuring GSH content in the samples before and after incubation with the model substrate during color reaction with 5,5'-dithio-bis-2-nitrobenzoic acid (DTNBA) [11].

The amount of GSH was determined based on GSH interaction with DTNBA with formation of yellow-colored anion of 2-nitro-5-thiobenzoate. Increase in the concentration of yellow anion during this reaction was detected spectrophotometrically at 412 nm wavelength [12].

TOC was determined at the TsvetYauza-01-AA flow-injection system with a plunger pump. Samples were prepared according to the “Methods of measuring total content of fat-soluble antioxidants in food products using amperometric method.” Gallic acid was used as standard. TOC was determined with amperometric detector with the potential of the working electrode at 1.3 V and eluent feed rate of 1.2 cm³/min. Reproducibility indicator (relative mean square deviation of reproducibility) was 5%.

The results were statistically processed using the STATISTICA 6.0 software.

Results and Discussion

Concentrate of PUFA was obtained from Baikal seal's fat [13]. Examination of fatty acid composition in the obtained concentrate

showed that the total level of unsaturated fatty acids was 92.67%, including omega-3 PUFA at 12.20% and omega-6 PUFA at 15.91%, and the omega-6/omega-3 PUFA ratio was 1.3:1.

DL development was accompanied by changing composition of serum lipids. Figure 1 shows the levels of TC, HDL cholesterol, LDL cholesterol, VLDL cholesterol, triglycerides, and AI in the serum of animals.

As a result of 21 days of atherogenic diet for animals, the content of TC in serum increased by 62% as compared with the “intact” group. In order to lower cholesterol content, laboratory animals received liposomal suspension with PUFA concentrate orally for 14 days. TC in blood serum of animals decreased by 48%, as compared with the reference group, and by 15%, as compared with the respective indicators in the animals that didn't receive atherogenic diet.

Along with this, in the group of animals that received atherogenic diet, the decrease in the content of antiatherogenic HDL cholesterol fraction in blood serum by 45.7% was observed, as compared with the respective indicators of the intact group. After the introduction of liposomal suspensions with PUFA concentrate, the level of HDL-cholesterol increased by 42.7%, as compared with the reference group, and approached indicators of the intact group.

After introducing atherogenic diet, the indicators of LDL cholesterol and VLDL cholesterol in the reference group increased as compared with the same in the intact group by 43.2 and 54%, respectively. As compared with the reference group of animals, the indicators of LDL cholesterol and VLDL cholesterol in the experimental group decreased by 58.4 and 70.2%, respectively.

Triglycerides level in animals in the reference group increased by 40%, as compared with that in intact animals. In the experimental group, the level of triglycerides decreased by 35.9%, as compared with the respective indicators in the reference group.

AI in the reference group increased 7.3 times, as compared with the intact group of animals. In animals of the experimental group, AI value decreased 11.2 times, as compared with the “reference” group, and 1.53 times, as compared with the “intact” group.

Thus, the introduction of PUFA concentrate had a pronounced anti-atherogenic effect, which was manifested in restoring lipid profile of the blood serum in experimental animals. Hypolipidemic effect of the concentrate is, apparently, explained by restoring both structural and functional properties of membranes and antioxidant properties of PUFA.

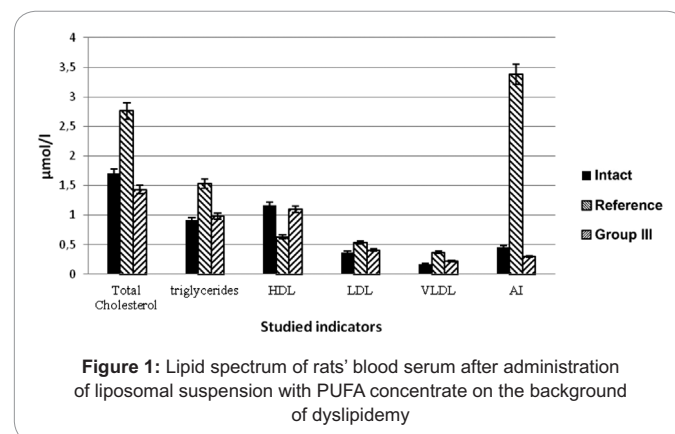
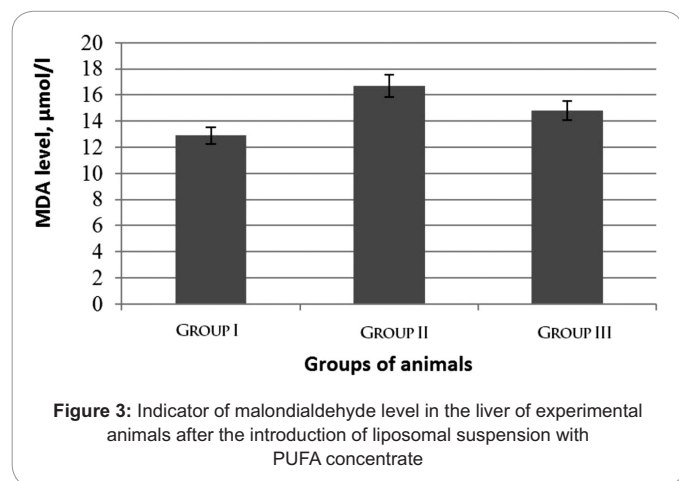
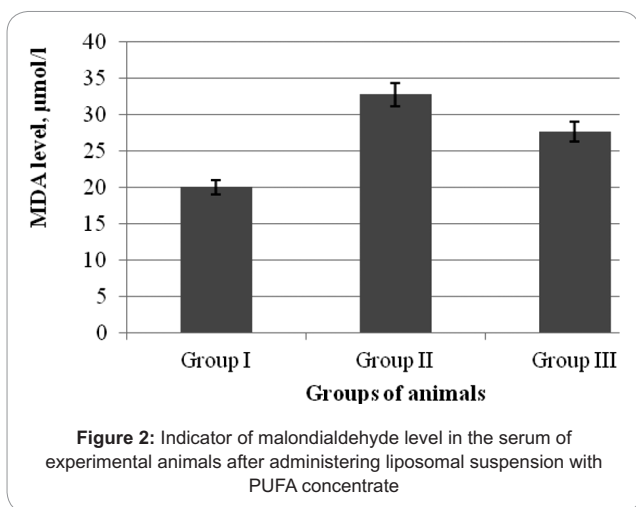


Figure 1: Lipid spectrum of rats' blood serum after administration of liposomal suspension with PUFA concentrate on the background of dyslipidemy



LPO is free-radical oxidation of PUFA in biological systems, and is one of the main processes in damaging biological membranes, which occurs in many pathological and pathobiochemical processes. MDA is one of the many LPO generated in relatively small quantities during peroxide oxidation of PUFA containing more than two double bonds.

As shown in Figure 2, the MDA content level in the serum of the animals that received atherogenic diet increased by 34.2%, as compared with the intact group. After feeding liposomal suspensions with PUFA concentrate, MDA content in the serum of the animals in group III decreased by 15.6%, as compared with the reference group.

Figure 3 shows the change of MDA level in the liver of experimental animals. In case of DL, MDA content in the liver of animals in the reference group significantly increased by 22.7%, as compared with that in the intact group of animals. After introducing liposomal suspension concentrate of PUFA, MDA level in the liver of animals decreased by 11.4%, as compared with the respective indicator in the reference group.

Based on the obtained results, indicators of MDA level in serum and liver, it was found that in case of DL development, animals were in the state of oxidative stress. The introduction of liposomal concentrate of PUFA into animals with DL had inhibitory effect on LPO, which was evidenced by decreasing MDA content. Decreasing MDA level in the tissues of animals after injecting PUFA is, apparently, caused by

activation of the antioxidant system in the organism. GSH-Px catalyzes this process by preventing transformation of lipid hydroperoxides into MDA (Table 1). Activity of protective mechanisms in oxidative stress is associated with both antioxidant enzymes and low-molecular components of cells.

As it follows from the data shown in Table 1, with DL development in animals in the reference group, activity of GSH-Px decreased by 48.9%, as compared with the intact group. After receiving liposomal form of PUFA, activity of GSH-Px in the experimental animals increased by 14%, as compared with the reference group.

In case of experimental DL in animals, activity of GR increased by 40.2%, as compared with the intact group. In the experimental group of animals, activity of GR decreased by 22.9%, as compared with the reference group.

As shown in Table 2, the content of reduced GSH in the erythrocytes of experimental animals in the reference group decreased by 46%, as compared with the intact group. In the experimental group, the content of the respective indicator decreased by 23.5%, as compared with the reference group.

Figure 4 shows data about the TOC of antioxidants in the serum of experimental animals. The obtained data indicate that in the reference group of animals, TOC decreased by 28.3%, as compared with the

Content of fatty acids	Seal fat	PUFA concentrate
Σ saturated fatty acids	19.16	7.33
Σ monounsaturated fatty acids	59.62	64.56
Σ polyunsaturated fatty acids	21.22	28.11
Σ omega-3 PUFA	10.33	12.20
Σ omega-6 PUFA	9.76	15.91
The omega-6/omega-3 ratio	0.94:1	1.3:1

Table 1: Total content of fatty acids in seal fat and in the PUFA concentrate obtained from seal fat

Groups of animals	Intact	Reference	Experimental
Indicator			
Activity of glutathione peroxidase (nmol/min g Hb)	14.6 ± 0.05	9.8 ± 0.08*	11.4 ± 0.06**
Activity of glutathione reductase (nmol/min g Hb)	5.8 ± 0.04	8.7 ± 0.07*	6.7 ± 0.02**

*Veracious value deviation compared with the intact ($p \leq 0.05$).

**Veracious deviation values compared with the reference ($p \leq 0.05$).

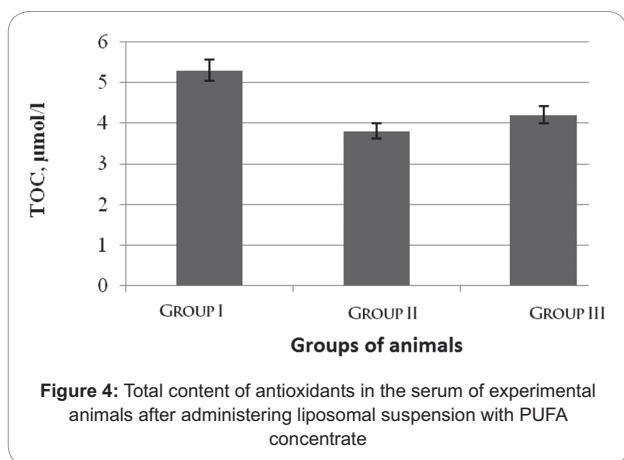
Table 2: Activity of enzymes in the erythrocytes of the rats treated with liposomal form of PUFA concentrate, on the background of experimental hyperlipidemy ($n = 10$)

Groups of animals	Intact	Reference	Experimental
Indicator			
Content of reduced glutathione (5-10 µmol/g Hb)	9.7 ± 0.04	5.2 ± 0.07*	6.8 ± 0.02**

*Veracious value deviation compared with the intact ($p \leq 0.05$).

**Veracious deviation values compared with the reference ($p \leq 0.05$).

Table 3: Content of reduced glutathione in the erythrocytes of the rats treated with liposomal form of PUFA concentrate, on the background of experimental hyperlipidemy ($n = 10$)



intact group. In the group of animals that had received liposomal form of PUFA concentrate, TOC increased by 9.5%, as compared with the reference group.

The activity of protective mechanisms in oxidative stress is evidently associated with the activity of antioxidant enzymes.

As mentioned above, decreasing parameters of oxidative stress after administering liposomal form of PUFA concentrate is, apparently, related to the radical-intercepting activity of omega-3 acids and induction of antioxidant enzymes activity, which result in increased content of reduced GSH as the main low-molecular component of the cells' antioxidant system.

Conclusion

The present experimental studies showed that the administration of liposomal forms of PUFA concentrate to animals against the background of experimental DL caused by the atherogenic diet resulted in the following phenomena: reducing TC, LDL cholesterol, VLDL cholesterol, triglycerides, and AI with simultaneous increase in HDL cholesterol.

A pronounced hypolipidemic effect was accompanied by decreasing content of peroxidation products, as indicated by the data about MDA content in the serum and the liver of experimental animals, as well as by increased activity of the enzymes in the GSH system, which increased content of reduced GSH.

The obtained results indicate viability of using PUFA concentrate in developing dietary supplements, pharmaceuticals, and functional nutrition products.

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