Progress in Nutrition 2017; Vol. 19, N. 2: 205-211 DOI: 10.23751/pn.v19i2.4976

Original article

Experimental correction of the oxidizing processes by lipids from freshwater hydrobiontes

Erzhena Vladimirovna Syngeeva[1], Sesegma Dashievna Zhamsaranova[2,3], Galina Petrovna Lamazhapova[2,3]

[1]Center of Biotechnology, East-Siberia State University of Technology and Management; [2]Department of Biotechnology, Institute of Food Engineering and Biotechnology, East-Siberia State University of Technology and Management; [3]Department of Pharmacology and Traditional Medicine, Medical Institute, Buryat State University - E-mail: lamazhap@mail.ru

Summary. *Background/Aim*: The influence of the liposomal form of seal fat and the concentrate of polyunsaturated fatty acids (PUFAs) on the indexes of pro-oxidant and antioxidant rat systems was studied with a representation of experimental dyslipidaemia. *Methods*: The concentrate of PUFAs was obtained by the complex formation method with urea. Liposomes were obtained by the extrusion method adding *alpha*-tocopherol. Hyperlipidaemia among the rats was induced by an atherogenic diet. *Results:* With the development of oxidative stress caused by the atherogenic diet, redox imbalance was observed within the animals, accompanied by a high intensity of lipid peroxidation processes and depletion of antioxidant protection. The level indicators of the oxidation products, the activity of the glutathione antioxidant system and the total content of antioxidants in the blood were restored to the level of intact rats by introducing liposomal suspension with the seal fat and concentrate of PUFAs to the experimental animals. *Conclusion*: Based on these data, the authors point to the prospects of the use of liposomal forms of essential polyunsaturated fatty acids in the development of dietary supplements and functional food.

Key words: liposomes, dyslipidaemia, oxidative stress, lipid peroxidation, antioxidant system, polyunsaturated fatty acids, Baikal seal (*Pusa sibirica*), food biotechnology

Introduction

Currently the populations of many countries, particularly in areas of environmental tension, don't receive proper nutrition. There is a shortage of essential nutrients – vitamins, minerals, components of a lipid nature – resulting in a reduction of the immune status of the organism and the development of many diseases. It is known that the main risk factor for various cardiovascular diseases is lipid metabolism disorder (atherogenic dyslipidaemia). In this connection, the search not only for medicines, but also biologically active food additives (BAA) with lipid corrective effects, is topical in order to prevent relapses of chronic diseases and their progression in the case of dyslipidaemia. In this respect, the food BAA obtained from fats of hydrobiontsare perspective. They contain a sufficient amount of *omega*-3 polyunsaturated fatty acids (PUFAs). The activity of PUFAs is expressed in the normalization of the lipid metabolism in living organisms (including reduction of the level of triglycerides and cholesterol in the blood), which determines the possibility of obtaining food BAA based on hydrobiont fats in order to prevent atherosclerosis, coronary heart disease and hypertension (1).

It is known that dyslipidaemia, regardless of its aetiopathogenesis, is accompanied by functional disorder of the key organ in the lipid homeostasis, the liver. The liver has the priority role because of the processes of regulation, resource mobilization, lipid biosynthesis and

inactivation of potentially toxic metabolites and compounds. In this case, the liver as the target organ of lipid metabolism disorders actively participates in the further progression of dyslipidaemia with the development of systemic pathological reactions of the whole organism.

The accumulation of lipid peroxidation products (LPO) in various tissues, including the liver, leads to the functional disorder of cell membranes, premature aging, reduced disease resistance, etc. Free-radical oxidation leads to the destruction of organic molecules, primarily lipids and, consequently, membrane cell structures, which often ends in their death. Therefore, the multicomponent system of inhibiting LPO effectively functions in the organism. The correction of LPO consequences is exposed by the influence on the cell membranes of various biologically active substances, including PUFAs, which are involved in the repair of membrane structures (2). Many data have been accumulated indicating the important role of these compounds in normal development and maintaining the balance between physiological and pathological processes in the organism.

One of the promising forms of delivery of biologically active substances is liposomes. From the point of view of liposomal biological compatibility, they are perfect as carriers – they are formed from natural lipids and therefore non-toxic, do not cause undesirable immune reactions and are biodegradable, i.e. they can be destroyed under the action of ordinary enzymes presented in the organism (3). The possibility of using raw fat materials of marine and freshwater hydrobionts for obtaining liposomes and other delivery systems has now been proved (4).

The aim of this study was to evaluate the effectiveness of liposomal forms of seal fat and concentrate of polyunsaturated fatty acids on lipid and antioxidant profiles of experimental animals with atherogenic dyslipidaemia.

Materials and Methods

PUFAs concentrate was obtained from the fat of the Baikal seal (Pusa sibirica) by the complex formation method with urea (5). Liposomes were obtained from phospholipids of the liver of the Baikal seal (6) by the extrusion method. A*lpha*-tocopherol was used as an inhibitor of fatty acid oxidation. The multilayer liposome was obtained by the method of hydration of the lipid film. Lipids (weighing 0.1 g of phospholipids obtained from the liver of the Baikal seal, and 0.05g of PUFAs concentrate obtained from the fat of the Baikal seal) in the ratio 2:1 and the antioxidant (*alpha*-tocopherol (BioChemica, Applichem)) at a rate of 5% of the total number of lipids were dissolved in chloroform and transferred into a round-bottom flask. The solution made from the components was evaporated on a rotary evaporator IKA RV 10 digital with a Diaphragm Vacuum Pump at a temperature of 35 °C in a water bath until the formation of a thin film of lipids on the flask walls. Then a buffer solution (pH 7.4) was added to the lipid film, and the mixture was shaken on a mechanical rocker for 30 minutes to obtain a homogeneous suspension. The obtained liposomal suspension was extruded through polycarbonate membranes with a pore diameter of 100 nm on a Liposomal Fast-Basic mini-extruder (AVESTIN, Canada).

The researches were carried out on 30 adult male Wistar rats weighing 130–150 g. They were obtained from the nursery of the Research Institute of Biophysics at the Angarsk State Technical Academy. Previously all animals used in the experiment had a standard vivarium diet and water *ad libitum*. Animals were kept in the vivarium in accordance with the "Sanitary rules for arrangement, equipment and maintenance of experimental biological clinics" of 06.04.1993. The experiments were carried out in accordance with the principles of the "European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes" (Strasbourg, 1986).

Experimental hyperlipidaemia within rats was induced according to the methodical guidelines (MUK 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 hyperlipidaemia and by introduction of 3–5 % cholesterol (BioChemica, Applichem), 0.3 % 6-methyl2-thiouracil (Alfa Aesar), 1% of cholic acid (Sigma) and 5% lard – within 21 days into the typical diet.

Experimental animals were divided into 4 experimental groups (10 animals per group): group I – intact (animals had standard feed and water); group II – con-

trol (the animals were on an atherogenic diet for 21 days); group III – experimental 1 (animals had oral liposomal suspension daily for 14 days with the addition of seal fat, at a dose of 20 mg/kg of the body weight of the rat after the atherogenic diet); group IV – experimental 2 (animals had the oral liposomal suspension daily for 14 days with the addition of the concentrate of polyunsaturated fatty acids obtained from the seal fat, at a dose of 20 mg/kg of the body weight of the rat after the atherogenic diet).

We defined the content of total cholesterol (TC), triglycerides (TG), cholesterol of high-density lipoproteins (CHDL), cholesterol of low-density lipoproteins (CLDL) and cholesterol of very low-density lipoproteins (CVLDL) in order to evaluate the hypolipidaemic action of liposomal agents on the fat and concentrate of PUFA in the rat blood serum. Also, the activity of the indicator enzymes of the cytolytic syndrome – alanine aminotransferase (ALT) and aspartate aminotransferase (AST) – was measured within animals with alimentary dyslipidaemia in the blood serum. Biochemical indices of the rat blood serum were determined by a BS-400 automatic biochemical analyser (PRC) using standard reagents of "Abris+" and "Dias" firms (Russia).

The atherogenic index (AI) was calculated to describe the atherogenic blood properties according to the following formula:

$$IA = \frac{TC - CHDL}{CHDL},$$

The concentrations of malondialdehyde (MDA) in the liver and in the serum were determined by the method based on the property of MDA to react with 2-thiobarbituric acid (TBA) (Sigma-Aldrich). The measurement of the concentration of TBA-active products in samples was carried out spectrophotometrically at a wavelength of 532 nm by analysing the degree of formation of coloured complex with TBA (7).

The determination of the glutathione reductase activity is based on the change in the oxidation rate of NADPH (MPBiomedicals, LLC), which is recorded spectrophotometrically by the decrease in optical density at a wavelength of 340 nm (8).

The determination of the restored glutathione (GSH) (Sigma-Aldrich) amount is based on the interaction of GSH and DTNBA (5,5'-dithio-bis-2-nitrobenzoic acid) by producing yellow anion of 2-nitro-5-thiobenzoate.The increase of the yellow anion concentration was recorded spectrophotometrically at a wavelength of 412 nm during the reaction (9). The determination of the total content of antioxidants (TCA) in the blood serum was carried out by the amperometric method using a "Tsvetyauza-01-AA" flow injection system with a plunger pump (Scientific Production Association "Khimavtomatika», Russia). Gallic acid (Closed Joint Stock Company "Vecton")was used as standard. The determination of the TCA was conducted by an amperometric detector "Tsvetyauza-01-AA" with a working electrode potential of 1.3 V and an eluent feed rate of 1.2 cm^3/min. The repeatability indicator (relative mean square deviation of repeatability) was 5%.

The statistical processing of the results was performed using the computer program STATISTICA 6.0.

Results and discussion

An experimental model of hyperlipidaemia was chosen for the assessment of the biological efficiency of liposomal agents on the basis of seal fat and PUFAs concentrate. This experimental model was developed by the Institute of Nutrition at the Russian Academy of Medical Sciences for food BAA recommended for the prevention, and as an aid in the treatment of, cardiovascular diseases.

The levels of TC, CHDL, CLDL, CVLDL, TG, ALT, AST and IA in the blood serum of experimental animals are presented in Table 1.

The content of total cholesterol in the blood serum was increased by 62% in comparison with the intact group as a result of using the atherogenic diet among experimental animals after 21 days. Along with this, a 45.7% decrease in the content of the anti-atherogenic fraction of CHDL in the blood serum was noticed in the group of animals treated with the atherogenic diet in comparison with the corresponding index of the intact group. At the same time the pro-atherogenic indices of CLDL and CVLDL of the control group rats increased in comparison with the intact group by 43.2% and 117.6%, respectively. The TG level within animals in the control group increased by 40% in com-

Table 1. The lipid profile and the activity of liver enzymes in the serum of different experimental groups of animals (n=10)

Biochemical indexes	intact	control	experimental 1	experimental 2
TC[mmol/L]	1.70±0.01	2.76±0.07[a]	1.84±0.13[b]	1.43±0.07[b,c]
CHDL[mmol/L]	1.16±0.05	0.63±0.0[a]	1.2±0.04[b]	1.1±0.05[b]
CLDL [mmol/L]	0.37±0.03	0.53±0.02[a]	0.37±0.03[b]	0.22±0.03[b,c]
CVLDL mmol/L]	0.17±0.02	0.37±0.01[a]	0.27±0.04[b]	0.11±0.02[b,c]
TG[mmol/L]	0.91±0.02	1.53±0.04[a]	1.2±0.11[b]	0.98±0.02[b,c]
IA[conv.units]	0.46±0.01	3.38±0.01[a]	0.53±0.03[b]	0.30±0.01[b,c]
ALT [U/L]	117.2±9.8	143±8.9[a]	97±10.6[b]	88±11.9[b]
AST [U/L]	221.8±20.2	278.8±25.6[a]	224.4±19.8[b]	214.5±18.6[b]

[a]– reliable value deviation in the control group in comparison with the intact value (p≤0.05); [b] – reliable value deviation in the experimental group 1 in comparison with the control (p≤0.05);[c] - reliable value deviation in the experimental group 2 in comparison with the value in the experimental group 1 (p≤0.05).

parison with the intact group animals. The IA in the control group increased 7.3 times in comparison with the intact group animals.

It is known that pro-oxidants (active oxygen forms and lipid peroxidation products) involved in the formation of oxidative stress induce damage of mitochondria and apoptosis or necrosis of hepatocytes. This ensures the cascade of reactions involving the disruption of stellate cells, matrix change and other cell tissue destruction, which, ultimately, leads to the development of steatohepatitis. One of the indicators of this liver disease is an increase in the intracellular enzymes AST and ALT in the blood, which get into the blood as a result of cytolysis. The ALT and AST indices increased significantly in the control group by 22% and 25.7% in comparison with the appropriate indices of the intact group.

Thus, all these data suggest that the consumption of large amounts of fat and cholesterol provoked the development of animals' dyslipidaemia state. The increase of the AST and ALT enzymes in the blood serum of the control animals may indicate the development of steatosis in liver cells.

The liposomal suspension with the seal fat and the concentrate of polyunsaturated fatty acids in liposomal form was administered orally for 14 days to reduce cholesterol within experimental animals.

The evaluation results of the hypolipidaemic action of the liposomal form of the Baikal seal fat and concentrate of PUFAs show significantly reduced proatherogenic fractions of cholesterol and an increase in the anti-atherogenic fraction of cholesterol in the blood serum of the experimental animals in comparison with the animals from the control group. So, the level of total cholesterol in the blood serum decreased by 33.3% among the animals of experimental group 1 which had the liposomal suspension with the seal fat in comparison with the control group. The introduction of the liposomal suspension with the concentrate of PUFAs decreased total cholesterol by 48% in the blood serum of the animals of experimental group 2 in comparison with the control group, and by 22.3% in comparison with experimental group 1.

The content of CHDL among rats from experimental groups 1 and 2 increased respectively by 90% and 74.6% in comparison with the control group and approached the indices of the intact group.

The level of CLDL within animals from experimental groups 1 and 2 decreased by 30.2% and 58.5% respectively in comparison with the indices of the control group. The index of CLDL of the experimental group 1 was equal to that of the intact group. In experimental group 2, the level of CLDL was significantly lower by 40.5% than that of experimental group 1. The levels of TG and CVLDL in the blood of animals decreased significantly in experimental group 1 by 21.6% and 27%, and in experimental group 2 by

35.9% and 70.3%, respectively, in comparison to the control group. The indices of TG and CVLDL in experimental group 2 were significantly lower than in experimental group 1.

Thus, the rats' IA, through the introduction of liposomal suspension with Baikal seal fat, declined 6.2 times in comparison with the control group and approached the indices of the intact group. Also, the IA decreased 11.2 times among animals that had the liposomal suspension with the concentrate of PUFAs, in comparison with the control group.

Alimentary dyslipidaemia within experimental animals was accompanied by increased activity of LPO processes in the blood serum and liver of rats, as evidenced by an increased content of lipid oxidation products.

As can be seen from the charts in Figure 1, the MDA level in the blood serum of the control group increased in comparison with animals that had the liposomal suspension with the seal fat orally for 14 days and reliably increased by 60% in comparison with the intact group, with an MDA level in the liver of 29.5%. A reliable decrease of the MDA level in the blood serum of experimental group 1 by 12.5% and experimental group 2 by 15.6% was observed in comparison with the control group when obtaining liposomes with the concentrate of PUFAs (introduction of additive through a probe). The MDA level in the liver of experimental group 1 decreased by 11.3% and that of experimental group 2 by 14.4% in comparison with the control group. Reliably significant differences were not found between the two experimental groups.

On the basis of the obtained results it follows that the oxidative processes developed during the realization of experimental dyslipidaemia among the rats. They were evident from the reliable increase of the MDA level in the blood serum and liver of the animals. The introduction of liposomal forms of the seal fat as well as the concentrate of PUFAs to animals against the background of dyslipidaemia exerted an inhibitory effect on the peroxidation of lipids, which is characterized by the decrease of the MDA content in the tissues of the body.

The activity of protective mechanisms during oxidative stress is connected with antioxidant enzymes as well as low-molecular components of cells, one of which is glutathione. Reduced glutathione is a coenzyme of several enzymes, whose activity is based on the change of the redox potential of glutathione, a direct function of which is the destruction of free radicals (10).

The activity indices of the glutathione system of experimental animals are presented in Table 2. As can be seen from the table, the activity of glutathione reductase decreased by 49% within the control group animals on the atherogenic diet in comparison with the intact group. Consequently, the content of the restored glutathione decreased by 46% among rats of the control group in comparison with the intact group.

The activity of glutathione reductase increased by 9.4% with the introduction of the liposomal suspension with the Baikal seal fat (experimental group 1). As

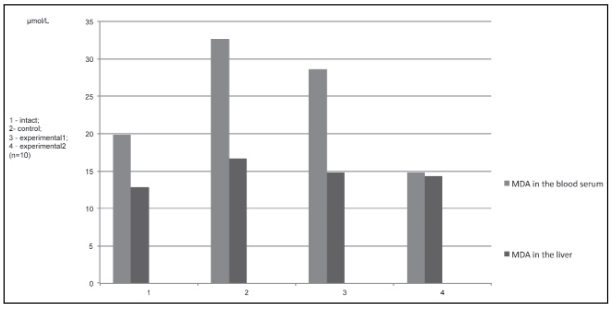

Figure 1. The level index of MDA in the blood serum and in the liver of the rats after the introduction of the liposomal suspension with the fat of the Baikal seal and liposomal suspension with the PUFAs concentrate obtained from the seal fat (n=10).

Table 2. The activity of glutathione reductase, the content of reduced glutathione in the rat blood serum after administration of the liposomal suspension with the fat of the Baikal seal and liposomal suspension with the PUFAs concentrate obtained from the seal fat (n=10)

	Index	Intact	Control	Experimental 1	Experimental 2
1	Glutathione reductase nmol/min/g Hb	5.7±0.24	2.9±0.11[a]	3.2±0.17[b]	3.8±0.19[b]
2	Reduced glutathione nmol/g Hb	9.7±0.56	5.2±0.37[a]	6.8±0.49[b]	7.6±0.17[b]

[a] – reliable value deviation in comparison with the intact group (p≤0.05); [b] – reliable value deviation in comparison with the control group (p≤0.05).

for experimental group 2 (liposomes with the PUFAs concentrate), activity increased by 23.6% in comparison with the control group. The content of reduced glutathione in experimental group 1 increased by 23.5% and in experimental group 2 by 31.5% in comparison with the control group.

As can be seen from the diagram in Figure 2, TCA decreased by 39.6% in the control group in comparison with the intact group. TCA increased by 13.6% with the introduction of the liposomal suspension in experimental group 1. TCA increased by 26.9% with the introduction of the liposomal suspension with the PUFAs concentrate in experimental group 2 in comparison with the control group.

A redox imbalance was observed in the animals during the development of oxidative stress induced by the atherogenic diet. It was accompanied by a high intensity of LPO and exhaustion of antioxidant protection (AOP). The liposome antioxidant effect with the concentrate was higher than with the Baikal seal fat. The liposomal suspension with the PUFAs concentrate can be recommended as an agent of natural origin in choosing means for correcting disturbances in the antioxidant defence system within rats under conditions of oxidative stress. Also, the obtained results show that liposomal forms of drugs can be effective in the complex treatment of diseases with free radical pathologies. The results indicate that the liposomal forms of biologically active substances can be effective in the treatment and prevention of diseases associated with free radical processes.

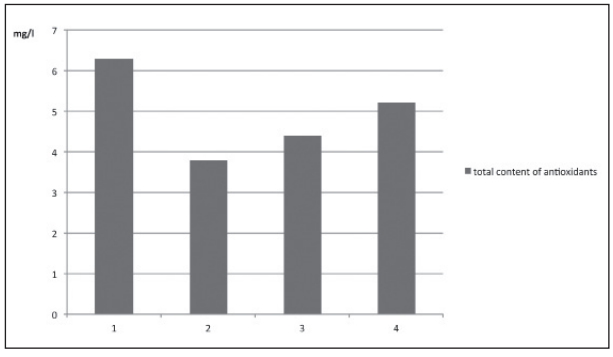

Figure 2. The total content of antioxidants in the blood serum after administration of the liposomal suspension with the Baikal seal fat and liposomal suspension with the PUFAs concentrate obtained from the seal fat(n=10).

Conclusion

The use of biologically active substances of freshwater hydrobionts helped the correction of oxidative stress in experimental dyslipidaemia. The antioxidant activity was expressed by a decrease of LPO products, indicated by MDA data in the blood serum and liver of the experimental animals, and an increase in the content and activity of antioxidant system components. The decrease of the oxidative stress parameters during the introduction of the PUFAs liposomal form is apparently connected with the radical-interceptive activity of *omega*-3 fatty acids and increased activity of antioxidant enzymes, which leads to reduced glutathione increase in the content, as a major low molecular component of the antioxidant cell system. These results are consistent with the statement of Vasilyev AV et al. (11) that the formation of nutrilipidomic pools is determined by alimentary impact on the cascading metabolic changes that affect lipoprotein exchange, formation of numerous derivatives of PUFAs, and the dynamics of free radical oxidation.

Analysis of the obtained results indicates the perspective of the PUFAs concentrate used in the development of BAA, drugs and functional food products.

Acknowledgements

This work was funded by a grant for "Young Scientists of the East Siberian State University of Technology and Management, 2015".

References

1. Kravchenko LV, Aksenov IV, Avrenyeva LI,Beketova NA, Trusov NV, Guseva GV. Effects of omega-3 polyunsaturated fatty acids on antioxidant capacity in rats. Vopr Pitan 2013; 2: 4–9.
2. Riediger ND, Othman RA, Suh M, Moghadasian MH. A systemic review of the roles of n-3 fatty acids in health and disease. J Acad Nutr Diet 2009; 109(4): 668–679. DOI 10.1016/j.jada.2008.12.022.
3. Barsukov LI. Liposoms. Soros Educ J 1998; 10: 2–9.
4. Averina ES, Kutyrev IA. Perspectives on the use of marine and fresh-water hydrobiont oils for development of drug delivery systems. Biotech Adv 2011; 29: 548–557. DOI: 10.1016/j.biotechadv.2011.01.009.
5. Zhamsaranova SD, Lamazhapova GP, Syngeeva EV. Develop-

ment of a method to produce a concentrate of polyunsaturated fatty acids. Biosci, Biotechnol Res Asia 2014; 11: 59–64. DOI 10.13005/bbra/1440.
6. East Siberia State University of Technology and Management (Russia). The way of liposom obtaining, possessing immunocorrecting and hepatoprotective action. Inventors: Lamazhapova GP, Zhamsaranova SD, Tsyrenzhapov AV, Nikolaev SM. IPC A61K9/127 RU Patent Application RU2308940. 16.05. 2006.
7. Uchiyama M,Mihara M. Determination of malonaldehyde precursor in tissues by thiobarbituric acid test. Anal Biochem 1978; 86: 271–278.
8. Comhair Suzy AA, Erzurum Serpil C. The regulation and role of extracellular glutathione peroxidase. Antioxid Redox Signal 2005; 7(1–2): 72–79.
9. Glatzle D, Vuilleumier JP, Weber F, Decker K. Glutathione reductase test with whole blood, a convenient procedure for the assessment of riboflavin status in humans. Experientia 1974; 30: 665–667.
10. Kulinskiy VI, Kolesnichenko LS. Glutation system I. Synthesis, transport, glutation, glutation peroxidase. Biomed Chem 2009; 55 (3): 255–277.
11. Vasilyev AV, Sharanova NE, Kulakova SN. Nutrimetabolomics - the new stage of biochemistry of nutrition. The role of nutrilipidomic analysis. Vopr Pitan 2014; 1: 4-11.

Correspondence:
Galina Petrovna Lamazhapova
Klyuchevskaya str., 42B, 670013 Ulan-Ude
Republic of Buryatia, Russian Federation
Tel.: 0073012 431415
E-mail: lamazhap@mail.ru