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Effect of 1-O-Alcylglycerols from sea hydrobionts on the metabolic status of rats with alimentary dyslipidemia

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ABSTRACT

Objective: Sea hydrobionts are a rich source of biologically active lipid compounds. Search for new biologically active substances to determine their pharmacological effectiveness is of current interest.

Background: In recent interest held pharmaceuticals from marine hydrobionts containing 1-*O*-alkyl-diacylglycerol (ADG). Significant amounts of ADG found in the tissues of some marine organisms of Pacific ocean - squid *Berryteuthis magister* (up to 50% in the lipids of the liver), crab *Paralithodes camtschatica* (10% lipids of the hepatopancreas). This makes it possible to use these aquatic animals as new sources of dietary supplements. In rats with alimentary dyslipidemia (DLP) examined the effect of nature 1-*O*-alkyl-glycerol (AG) on the metabolism of lipids, the state of the hepatobiliary, antioxidant systems and hematological parameters of blood.

Method: Alimentary model DLP caused high-fat diet of beef fat and cholesterol. Were injected AG in rats with DLP a dose of the 0.4 g/kg for 30 days. 1-*O*-alkyl-glycerol were obtained by hydrolysis of the lipids of the liver ADG squid *Berryteuthis magister*. Biochemical parameters of lipid and carbohydrate metabolism, and liver enzymes measured in blood serum. Investigated the total antioxidant activity (TAA) of blood plasma, the activity of catalase in erythrocytes, glutathione reductase (GR) and glutathione peroxidase (GP) activity, glutathione (GSH) lever. The content of initial and final products of lipid peroxidation – hydroperoxides of lipids (HPL), malondialdehydes (MDA) in the blood were

investigated. Determination of hematological parameters is carried out on «Abacus» (USA). Statistical significance of differences was calculated by Student's t-test.

Results: Introduction AG resulted in a reduction in triglycerides in the blood serum of rats by 24.2% compared with rats with DLP ($p < 0.05$), increase in HDL-C by 63% ($p < 0.001$). There was an increase in blood glucose concentration by 21.3% ($p < 0.001$), and lactate dehydrogenase (LDG) activity by 30% ($p < 0.05$), ALT – 24% ($p < 0.001$) compared with rats with DLP. After use AG in rats showed an increase in the activity of catalase, reduction of lipid hydroperoxides in plasma. Showed normalization of the TAA and the trend to reduce the concentration of MDA. In the glutathione-redox system under the influence of AG increased activity GR, GP, GSH levels. After use AG an increase in the total number of red blood cells in the blood by 40% ($p < 0,001$), total hemoglobin by 38% ($p < 0.001$), platelet count by 30% ($p < 0.001$), lymphocytes - 43% ($p < 0.001$), blood clotting time increased by 57%.

Conclusion: The study showed that the use of AG causes increased protective functions - hematopoietic, immune-stimulating and antioxidant. These data suggest the widespread use of AG from lipid liver squid *Berryteuthis magister* in rehabilitation practice of various pathologies.

Keywords: natural 1-O-alkyl-lglycerols, dislipidemia, metabolic status

BACKGROUND:

Marine hydrobionts are important sources of n-3 polyunsaturated fatty acids (n-3 PUFA). n-3 PUFAs have hypolipidemic, anti-atherogenic, anti-hypertensive, anti-arrhythmic and thrombolytic effects [1]. Therefore, justified the use of fat containing n-3 PUFAs in the prevention and treatment of coronary heart disease, hypertension, dyslipidemia, arthritis and other inflammatory and autoimmune disorders [1, 2]. However, the presence of complex lipid mixtures from marine hydrobionts other active ingredients cause more detailed study of the biological properties of these drugs. These lipids are of interest to medicine are 1-*O*-alkyl-diacylglycerol (ADG) - compounds formed by fatty acids and 1-*O*-alkyl-glycerol (AG) (Fig. 1-2). Biological properties of ADG less studied than the n-3 PUFA [3, 4].

In the world medical practice for over 40 years is used shark liver oil, which contains more than 20% of the alkyl-diacylglycerols [5, 6]. The most well studied the effect of shark oil in cancer. This is due to antiproliferative, hematopoietic, radioprotective properties of lipids with alkyl bond. Analysis of the literature showed that ADG have immunostimulating properties [6-12]. ADG are precursors in the biosynthesis of platelet activating factor (PAF) (Fig. 3) [13, 14], which indirectly affect the functional activity of cells in the blood, cardiovascular, immune, and reproductive systems. In a few publications found information about the antioxidant properties of the drug which rich ADG [14]. Suggest that an important role in the manifestation of the antioxidant properties of these pharmacological agents is assigned to hypertension. Significant amounts of ADG found in the tissues of some marine organisms of Pacific ocean - squid *Berryteuthis magister* (up to 50% in the lipids of the liver), crab *Paralithodes camtschatica* (10% lipid hepatopancreas) [4, 15, 16]. This makes it possible to use these aquatic animals as new sources of dietary supplements.

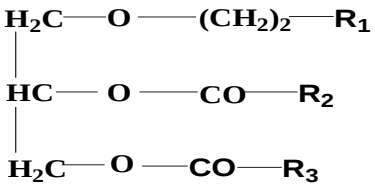

Fig. 1. 1-*O*-alkyl-diacylglycerols (ADG)

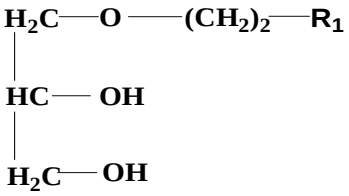

Fig. 2. 1-*O*-alkyl-glycerols (AG)

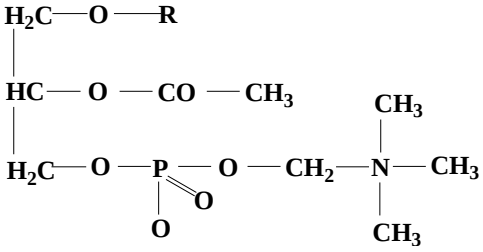

Fig. 3. Platelet activating factor (PAF) (1-alkyl-2-acyl-sn-glycero-3-phosphocholine).

In our earlier studies on the biological effects of lipids from squid *Berryteuthis magister* liver in experimental cardiomyopathy in rats identified some negative effects [17, 18, 19]. Was found hypertryglitserinemiya, hyperlipoperoxidemiya, fatty liver, and red blood lymphocytosis, decreased blood clotting time. These effects are not specific for the known properties of n-3 PUFAs. We assumed that the biologically active principles responsible for the specific actions of lipids squid this is 1-O-alkyl-glycerol (AG), which are formed from ADG (Fig. 2). As a precursor in the biosynthesis of platelet activating factor, AG can provoke cardiorespiratory disorders, increase platelet degranulation. A detailed study of AG, isolated from the total lipid fraction of marine aquatic organisms, will reveal the characteristics of the biological activity of lipid drugs and dietary supplements based on them.

The aim: to study the effect of 1-*O*-alkyl-glycerols obtained from squid *Berryteuthis magister* liver on lipid metabolism, the state of the hepatobiliary, antioxidant systems and hematological parameters in the blood of rats under experimental alimentary dyslipidemia.

METHODS:

1-*O*-alkyl-glycerols were obtained by hydrolysis of the lipids of the liver ADG squid *Berryteuthis magister* [15]. Gas-liquid chromatography was performed on AG ether gas chromatograph GC-14B ("Shimadzu", Japan) equipped with a capillary column (30 m × 0.35 mm, 0.25 mm) with a stationary phase Supelcowax 10M ("Supelco", USA) and column (30 m × 0.35 mm, 0.25 mm) with a stationary phase SPB-5 ("Supelco", USA) and a flame ionization detector in the mode: the column temperature, the injector and detector - 190^{o}C, 220^{o}C and 220^{o}C respectively. Chromatogram processing carried out on the basis of calculation of data Chromatopac CR4-A ("Shimadzu", Japan). Identification silyl ethers AG are on the standard mixture of 1-O-alkyl-glycerol. In Table 1 shows the composition of AG, isolated from liver lipid squid.

From the literature it is known that the most biologically active AG are C16:0, C18:0, C18:1 [8]. In our study content of AG follows: C16:0 - 55.2%, C18:0 - 6.9%, C18:1 - 17.3%.

Table 1. AG content in lipids of *Berryteuthis magister* liver

Alkyl substituents in AG	AG content in lipids of *Berryteuthis magister* liver (in % in the total AG)
14:0	1,1
15:0	0,4
16:0	55,2
17:0	2,2
18:0	6,9
19:0	1,5
20:0	0,5
14:1	0,1
16:1	1,7
18:1	17,3
20:1	9,6
Saturated	67,8
Monoenoic	28,7
Unidentified	3,5

The study was conducted on 30 adult white male Wistar rats with an initial mass of 280 ± 20. It was generated 3 groups of animals with 10 rats in each: control group - intact rats which were on a standard (normal) diet, the experimental group 1 - rats with alimentary dyslipidemia, experimental group 2 - rats with alimentary DLP, treated AG at a dose of 0.4 g/kg body weight of rats. DLP model called unbalanced nutritional composition fat diet with the inclusion of high-calorie foods and cholesterol (Table 2). AG is administered to the animals of the experimental group 2 for 30 days. Euthanasia of animals was carried out by decapitation under ether anesthesia in accordance with the requirements of the European Convention for the protection of experimental animals 86/609 EEC.

Table 2. Composition of high-fat diet (g/kg of animal weight)

Ingredients	High-fat diet	Normal diet
Tallow	**42.5**	5
Cholesterol	**4.3**	-
Sunflower oil	5	5
Grain mixture	50	50
Bread	20	20
Grits	13	13
Beef	20	20
Skim cheese	8	8
Carrot	33	33
Greens	33	33

Lipid profile and level of glucose of the serum was studied on the biochemical analyzer FP-901 firms «Labsistems» (Finland). Total cholesterol (TC), triglyceride (TG), high-density lipoprotein cholesterol (HDL-C) was measured. Studied the activity of enzymes - alanine aminotransferase (ALT), aspartate aminotransferase (AST) and lactate dehydrogenase (LDH).

The total plasma antioxidant activity was assessed by the accumulation in a model system of yolk lipoprotein peroxidation end products. As the initiator of the reaction using iron sulfate [20-22]. The activity of glutathione redox-system in erythrocytes were analyzed by the lever GSH, glutathione reductase activity, glutathione peroxidase activity. GSH was measured according to the Ellman method [23]. Glutathione reductase activity was measured according to the method described [24], glutathione peroxidase activity [25]. The intensity of lipid peroxidation in blood investigated by spectrophotometric method for the content of the initial and final products of lipid peroxidation – hydroperoxides of lipids (HPL), malondialdehyde (MDA) [20].

Determination of hematological parameters is carried out on Abacus (USA). All data were analyzed by ANOVA using computer program Statistika 6.1 (series 1203C for Windows). Data present as means ± SEM. (M). Differences between means were assessed by Student's significance test.

RESULTS AND DISCUSSION:

The results of the study are presented in Table 3. Simulation DLP on rats led to increased levels of cholesterol, triglycerides, glucose on the blood. There was an increase of the enzymatic activity of the liver, the accumulation of lipid peroxidation products, reduced the total antioxidant activity of blood. Noted a drop of activity of catalase and glutathione enzymes level. Development of a model DLP accompanied by changes in hematological parameters of peripheral blood to decrease red blood cells, blood clotting time, decrease the number of immune cells.

Introduction AG resulted in a reduction in triglycerides in the blood serum of rats by 24.2% relative to the experimental group 1 ($p < 0.05$), increase in HDL-C by 63% ($p < 0.001$). Total cholesterol (TC) levels under the influence of nature AG did not change. Glucose concentration increased by 21.3% ($p < 0.001$). In the experimental group 2 there was an increase of LDH activity by 30% ($p < 0.05$), ALT - 24% ($p < 0.001$) compared with rats of the experimental group 1.

Antioxidant system after applying AG characterized by high catalase activity. However, the total antioxidant activity remained at the level of the experimental group 1. HPL level in the blood decreased by 3 times ($p < 0.001$). There was a weak tendency to decrease in the concentration of MDA in erythrocytes. In the glutathione-redox system under the influence of AG increased the activity of GR, GP, GSH level.

Study of hematological parameters of peripheral blood revealed a growth in the number of red blood cells in the blood by 40% ($p < 0,001$) after using AG. Total hemoglobin level increased by 38% ($p < 0.001$), platelet count increased by 30% ($p < 0.001$), lymphocytes - 43% ($p < 0,001$), blood clotting time increased by 57%. The number of white blood cells under the influence of nutritional AG did not change.

Results of the study revealed hypotriglyceridemic effect of AG. The level of total cholesterol in the blood was high, due to the ability of lipids to an alkyl group inhibit etherification of cholesterol-lecithin cholesterol acyltransferase [14].

Table 3. Biochemical and hematological parameters of blood of rats before and after administration of AG (M ± m)

Measure	**Control, n=10**	**DLP**	
		experimental group 1 (model DLP)	**experimental group 2 (model DLP+AG)**
Glucose, mmol/L	5,47±0,11	***8,78±0,29	***10,65±0,29***
TC, mmol/L	1,57±0,04	***3,34±0,04	***2,93±0,2
TG, mmol/L	1,12±0,04	***1,95±0,06	*1,48±0,14*
HDL-C, mmol/L	0,67±0,04	***0,26±0,02	0,7±0,025***
ALT, mmol	52,21±3,50	***108,9±3,57	***144,5±12,22**
AST, mmol	118,61±6,10	***243,9±9,10	***237,7±16,55
LDG, mmol	936,61±133,08	**1799±176,86	***2599±249,75*
TAA, %	23,91±0,19	***41,71±3,09	***37,4±2,20
MDA, nmol /gHb	5,12±0,22	***9,01±0,20	***8,41±0,26
HPL, s.u.	0,79±0,03	***3,493±0,11	***1,206±0,04***
Catalase, %	85,35±0,58	***73,21±1,01	84,2±1,29***
GSH, umol/gHb	5,4±0,4	**3,4±0,1	4,9±0,2**
GP, nmolGL/min*mg proteins	44,43±1,20	***32,51±1,31	46,50±1,10***
GR, nmolNADPH/min*mg proteins	75,15±2,14	**68,04±1,28	74,31±1,23**
Erythrocytes,*10^{12}/l	22,95±0,91	*20,24±0,65	**28,86±0,52***
Hemoglobin, g/l	110±2,74	*93,9±4,9	***150,7±2,46***
Platelets, 10^9/л	315±9,68	*349±10,27	**455±19,22***
Clotting time, second	22,8±0,71	***10,6±0,65	**24,5±0,58***
Leukocytes, G/l	7,16±0,14	***8,48±0,12	*8,13±0,50
Lymphocytes, %	22,2±0,92	**18±0,34	***31,4±1,64***

Comparison between groups was made using Student test. (*) left – statistic significance of differences in to control group; right – experimental group 1; * $p < 0.05$; ** – $p < 0.01$; *** – $p < 0.001$.

Detected after the application of AG increase in catalase activity and reduced lipoperoxides shows antioxidant properties of AG. This is also evidenced by the few published data, which shows the ability of shark oil show antioxidant effects[6]. Use 1-O-alkyl-glycerol resulted in increased red blood cells, immune system cells, hemoglobin synthesis. Possible mechanism for increasing the number of blood cells due to the influence of AG hematopoietic by activating the synthesis of PAF. This is the trigger for the formation of secondary mediators that provide the proliferation of hematopoietic cells [13, 14].

Previous studies from our institute [17, 19] have been found mixed effects of lipid mixtures of AG and n3 PUFA. It was shown that the greatest positive effect was achieved at necessary participation n3 PUFA, which offset some of the negative effects of AG.

Conclusion: This study demonstrated that the use of AG to cause an adequate response of the metabolic system - hematopoietic, immune and antioxidant. Obviously, these factors must be considered as one of the most important in assessing the therapeutic effect of drugs intended

for the regulation of metabolic disorders. Identified hyperglycemia, high cholesterol and other effects are not critical and can be reduced by co-application with n3 fatty acids, which originally present in the natural lipid complexes.

It was identified important metabolic properties of AG from liver lipid squid in DLP. These data suggest the widespread use of AG in rehabilitation practice of various pathologies.

Abbreviations: ADG, 1-*O*-alkyl-diacylglycerols; AG, 1-*O*-alkyl-lglycerols; ALT, alanine aminotransferase; AST, aspartate aminotransferase; DLP, dislipidemia; GP, glutation peroxidase; HDL-C, high-density lipoprotein cholesterol; HPL, hydroperoxide of lipids; GR, glutation reductase; GSH, glutation; LDH, lactate dehydrogenase; MDA, malondialdehyde; PAF, platelet activating factor; n-3 PUFA, n-3 polyunsaturated fatty acids; TAA, total antioxidant activity; TC, total cholesterol; TG, triglyceride;

Author's contribution: All authors have been contributed

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