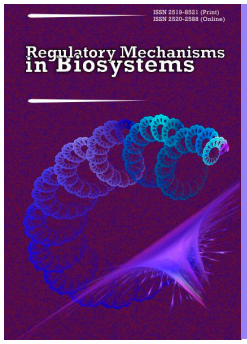

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Changes in the spectrum of proteins and phospholipids in tissues of rats exposed to thiosulfonates

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Esters of thiosulfoacids demonstrate a wide range of biological activity. One of their effects is the influence on the metabolism of proteins and lipids in the body. Therefore, the purpose of our experiment was to study the impact of synthesized thiosulfonates on the total content of proteins and phospholipids, as well as their spectrum in the blood, liver, and kidney of rats. For the experiment, allyl, ethyl, and methyl esters of thiosulfoacid were used. The protein profile of rat tissues was investigated by electrophoresis, and the ratio of different fractions of phospholipids – by thin-layer chromatography. Our results have shown that short-term administration of thiosulfonates in a dose of 300 mg/kg of body weight did not cause significant changes in the content of total protein and its fractions in liver tissue, whereas the effect of allyl and ethyl esters of thiosulfoacid was accompanied by an increase in the total protein and albumin in the blood plasma. The decrease in total protein was found in the kidney tissue of rats injected with allyl- and methyl thiosulfonates. The newly synthesized compounds did not lead to significant changes in the total content of phospholipids in blood plasma and tissues of rats, except for methyl thiosulfonate, the effect of which was accompanied by an increase in the total phospholipids in the liver of rats. These data may indicate an adaptive reaction of the rat's organism. Tissue-specific features of the phospholipid spectrum were detected in rats after short-term exposure to thiosulfonates. The most significant effect on the phospholipid profile in the blood was shown for allyl- and ethyl esters of thiosulfoacid. Their action was accompanied by a decrease in the phosphatidylserine and phosphatidylinositol fractions, while phosphatidylethanolamine and phosphatidylcholine increased, respectively. Esters of thiosulfoacid significantly influenced the ratio of different fractions of phospholipids in the liver and kidney tissues. The phospholipid composition of the liver was more influenced by the allyl and methyl esters of thiosulfoacid, whereas for the kidney tissue a greater effect was observed for ethyl and methyl esters. Thus, the action of allyl ester of thiosulfoacid caused a decrease in the asymmetry coefficient of hepatocyte membranes, indicating an elevation of the lipid bilayer saturation and the increase of membrane microviscosity. Similar changes were found in the kidney of rats treated with allyl- and ethyl thiosulfonates.

Keywords: S-esters of thiosulfoacids; protein profile; phospholipids ratio; blood; liver; kidney

Introduction

Esters of thiosulfoacids are structural analogues of natural phytoncides of garlic, onion etc. These synthesized compounds demonstrate a wide range of biological activity. They are effective against various microorganisms, in particular bacteria and fungi (Lubenets et al., 2017; Oriabinska et al., 2017; Reiter et al., 2017; Leontiev et al., 2018). These substances are characterized by anti-inflammatory (Zenkov et al., 2007; Arreola et al., 2015), antioxidant (Nepravishta et al., 2012; Chan et al., 2013; Bhuiyan et al., 2015), and cytotoxic (Smith et al., 2016; Saini et al., 2017; Siyo et al., 2017) activities. Due to these properties, synthesized esters of thiosulfoacid may be considered as promising substances for the development of effective therapeutic agents.

Literature data of recent years have shown that organic sulfur-containing compounds, isolated from garlic manifest anti-carcinogenic effect (Zou et al., 2016; Petrovic et al., 2018). Existing data indicate that oil-soluble compounds, such as diallyl sulfide, diallyl disulfide, diallyl trisulfide, and ajoene more effectively inhibit the proliferation of different types of cancer cells than their water-soluble compounds (Shintyapina et al., 2017). The molecular mechanisms of their effects are associated with the activation of enzymes detoxifying carcinogens, inhibition of the formation of DNA adducts, antioxidant effects, cell cycle regulation, induction of apoptosis, histone modification, and inhibition of angiogenesis (Yi & Su, 2013; Li et al., 2017; Puccinelli et al., 2017). On the other hand, water-soluble salts of thiosulfoacids are the most effective antioxidants and anti-inflammatory agents among a series of alkyl thiosulfonate compounds. They not only inhibit peroxidation processes in cells but also induce expression of genes that encode antioxidant enzymes. Thus, 3-(3'-tert-butyl-4'-hydroxyphenyl)propyl thiosulfonate sodium-induced three times greater expression of the GSTP 1 gene in human hepatocytes HepG2 than the synthetic antioxidant such as tert-butyl hydroquinone (Zenkov et al., 2007; Shintyapina et al., 2014). Experiments *in vivo* have shown that, in response to the action of new compounds of the thiosulfanilate structure, in the liver of mice, an expression not only of the genes encoding glutathione-S-transferase increased, but also of NADPH: quinone oxidoreductase through the activation of the Nrf2 pathway (Vavilin et al., 2014).

The important feature of the compounds of the thiosulfonate structure is their ability to lower lipid levels in the blood (Kumari & Augusti, 2007; Ried et al., 2013). Thus, in contrast to classical statins that inhibit the activity of 3-hydroxy-3-methylglutaryl-coenzyme-A-reductase, allicin and its derivatives can depress cholesterol biosynthesis by inhibiting such enzymes as squalene monooxygenase (Gupta et al., 2001) and acetyl CoA synthetase (Focke et al., 1990). Taking into account the fact that coenzyme A contains a thiol group, it can be assumed that allicin will interact directly with coenzyme A, making it unavailable for biosynthetic processes, including sterols (Borlinghaus et al., 2014).

We have shown previously that the administration of S-alkyl esters of thiosulfoacid did not significantly affect the total lipid content in their blood, liver and kidney tissues of rats, but led to the redistribution of their classes (Pylypets et al., 2017).

Literary data reported that allicin and its derivatives freely penetrate through cell membranes and easily interact with SH- and NH_2-groups of protein, realizing their biological effects. However, there is no information about the influence of these compounds on the protein content and phospholipid spectrum in various tissues of the body. Therefore, the purpose of our work was to study the effect of short-term exposure of S-esters of thiosulfoacids to the protein and phospholipid profile of blood, liver, and kidney of rats.

Materials and methods

Synthesis of thiosulfanilates. Methyl-, ethyl- and allyl thiosulfonates were used in the experiment. The chemical formulae of the synthesized bioactive substances are shown in Figures 1–3. Synthesis, physical and chemical properties of ethyl thiosulfonate are described in detail in the article (Lubenets, 2013).

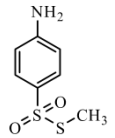

Fig. 1. Chemical structure of methyl-4-aminobenzenethiosulfonate (M), molecular weight 203.27

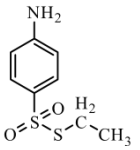

Fig. 2. Chemical structure of ethyl-4-aminobenzenethiosulfonate (E), molecular weight 217.30

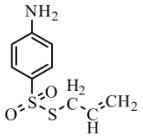

Fig. 3. Chemical structure of allyl-4-aminobenzenethiosulfonate (A), molecular weight 229.31

Methyl- and allyl thiosulfonates are synthesized by analogous methods. The scheme of these reactions is given below (Fig. 4).

Animals. Wistar male rats with body weight of 190–210 g were used in this study and each group included five animals. The rats were housed under standard laboratory conditions of the animal house (Institute of Animal Biology of the National Academy of Agrarian Sciences of Ukraine) with free access to food (normal pellet diet) and water.

The animals were treated in accordance with the requirements of European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (Strasbourg, 1986) and the national General Ethical Principles of Experiments on Animals (Ukraine, 2001).

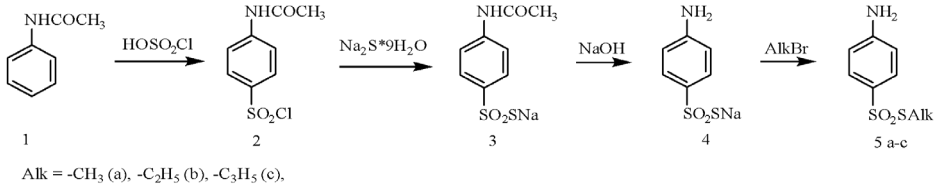

Fig. 4. Scheme of obtaining methyl- and allyl thiosulfonates

Experimental design. The animals were randomly divided into four groups, each consisting of five animals: control K and three experimental – A, E, M. For the administration to animals, thiosulfonates were dissolved in sterile oil (trademark "Oleyna", DSTU ISO 14024, Ukraine). The solution was freshly prepared and administered intraperitoneally at a dose 300 mg/kg of body weight once a day: group A – allyl thiosulfonate, group E – ethyl thiosulfonate, group M – methyl thiosulfonate. Animals of the control group were injected an appropriate dose of sterile oil. After three days of the experiment, the rats were decapitated under ether anesthesia. Samples of blood, liver, and kidney were collected. All procedures on tissues were carried out at 4 °C.

Protein study. The total protein content in the blood and tissue homogenates was determined by the Lowry method (Lowry et al., 1951). The fractional composition of blood plasma proteins and soluble proteins of liver and kidney tissues was investigated by the electrophoresis method in 7.5% polyacrylamide gel (Laemmli, 1970). After electrophoresis, electropherograms were fixed in 10% trichloroacetic acid solution, washed with distilled water and stained with Coomassie G-250 solution (Serva, Sweden). After staining, the electropherograms were washed with 6% acetic acid solution and scanned on a scanner HP Scanjet G2710 (China). Fractions of soluble protein were identified compared with the mobility of blood plasma proteins (Vlizlo et al., 2012). The protein content was determined using TotalLab TL120 (Nonlinear Dynamics Limited, UK) and expressed as a percentage of the total pool.

Lipids study. Blood plasma and homogenates of tissues were extracted with chloroform-methanol mixture (2 : 1, V/V) by the Folch method (Folch et al., 1957). A solution of KCl (0.74 M) was added to each sample of the lipid extract. After 24 hours, upper phase containing hydrophobic peptides was removed by a water pump and lower phase, which contained the lipids, was filtered. The lipid extracts were evaporated to dryness and then weighed on an analytical balance.

The phospholipid content was determined by the amount of inorganic phosphorus in the lipid extract, as described by (Vaskovsky et al., 1975). The separation of phospholipids into subclasses was performed using thin-layer chromatography on silica gel plates (silica gel L 5/40μ, LSL 5/40μ, Chemapol, Czech Republic). A mixture of chloroform-methanol-water (65 : 25 : 4, V/V/V) was used as an eluent system (Kates, 1986). Classes of phospholipids were revealed in vapours of crystalline iodine. Identification of individual phospholipids was carried out with Rf-values. Quantitative analysis of the phospholipid subclasses was performed using the TotalLab TL120 software (Nonlinear Dynamics Limited, UK) and expressed as a percentage of the total pool.

Statistical analysis. Statistical evaluation of the results was conducted using the arithmetic mean ± standard error (x ± SE). The results on the figures are expressed as x ± SD. The data were processed statistically by ANOVA. Bonferroni pairwise test was carried out after ANOVA for the determination of significant differences ($P < 0.05$) between the means of the values obtained for each groups.

Results

Existing data indicate that the protein content in the blood and its fractional composition may change in response to the action of various factors. Our results showed that intraperitoneal administration of esters of thiosulfoacid had no effect on the total protein content in the blood plasma of rats except for animals administered by allyl thiosulfonate (Table 1). We also found the tendency to increase its level in animals of experimental groups treated by ethyl- and methyl thiosulfonates. It is important that this indicator in animals of both the experimental and control groups was within the physiological range. Electrophoretic analysis of blood plasma proteins showed some changes in the protein spectrum of blood plasma. Thus, the increase in albumin was observed in the

groups of animals administered by allyl- and ethyl thiosulfonates, at the same time, β-globulins decreased compared to the control group in the animals treated with ethyl- and methyl thiosulfonates. As can be seen from the results of Table 2, all esters of thiosulfoacid did not significantly affect the protein synthesis in the liver, but there was a tendency to increase in the total protein content in animals injected by ethyl- and methyl thiosulfonates. A fraction of pre-albumin was found in the soluble protein spectrum of the liver, but its content did not exceed 2 %.

Table 1
Protein spectrum in blood plasma of rats,
treated with esters of thiosulfoacid (x ± SE, n = 5)

Indicators	Groups			
	control	allyl thiosulfonate	ethyl thiosulfonate	methyl thiosulfonate
Total protein, g/l	63.70 ± 6.96	79.87 ± 10.24*	70.00 ± 8.65	69.30 ± 4.37
Albumin, %	36.90 ± 6.67	43.55 ± 2.43*	49.31 ± 2.43#	37.28 ± 5.39
α-globulin, %	20.97 ± 2.41	18.93 ± 4.88	18.72 ± 1.60	23.67 ± 1.83
β-globulin, %	30.67 ± 5.10	25.71 ± 1.54	23.32 ± 1.89#	25.00 ± 0.92*#
γ-globulin, %	11.46 ± 4.13	11.81 ± 1.30	8.65 ± 0.97	14.07 ± 1.45

Note: * – the differences are statistically significant between control animals and animals treated with allyl thiosulfonate (P < 0.05); # – the differences are statistically significant between control animals and animals treated with ethyl thiosulfonate (P < 0.05); *# – the differences are statistically significant between control animals and animals treated with methyl thiosulfonate (P < 0.05).

Table 2
Soluble protein spectrum in the liver of rats,
treated with esters of thiosulfoacid (x ± SE, n = 5)

Indicators	Groups			
	control	allyl thiosulfonate	ethyl thiosulfonate	methyl thiosulfonate
Total protein, g/kg	305.4 ± 12.0	303.5 ± 11.7	318.9 ± 9.9	321.6 ± 14.9
Pre-albumin, %	1.36 ± 0.76	1.83 ± 0.18	1.49 ± 0.16	1.16 ± 0.49
Albumin, %	18.73 ± 1.69	17.71 ± 1.23	18.51 ± 2.64	17.47 ± 1.07
α-globulin, %	15.96 ± 1.90	16.91 ± 1.27	17.60 ± 1.86	17.19 ± 3.04
β-globulin, %	32.18 ± 3.38	30.10 ± 2.36	32.63 ± 1.34	31.80 ± 3.00
γ-globulin, %	31.78 ± 2.75	33.45 ± 4.06	29.76 ± 3.64	32.38 ± 4.04

Note: statistically significant differences between all experimental groups and control group by ANOVA test were not detected.

Table 3 shows that the action of thiosulfonates is accompanied by the decrease in the protein content in the kidney tissue in animals administered by allyl- and methyl thiosulfonates. Thus, the total protein content was lower by more than 6% (P < 0.05) in the kidney of rats administered allyl- and methyl thiosulfonate. At the same time, only the dynamics to increase in the total protein content in the kidney was observed in the rats treated with ethyl thiosulfonate. It should be noted no significant changes in the soluble protein composition of the kidney, except for the decrease by 28%, 22%, and 20% (P < 0.05) in protein fraction whose electrophoretic characteristics correspond to α-globulins of blood plasma, in rats of all experimental groups. As can be seen from the results of the Table 3, the content of the pre-albumin fraction in the kidney tissue varies within 6–9% and is significantly higher compared to the pre-albumin fraction in protein spectrum of liver tissue.

The results of our studies showed that intraperitoneal administration of the synthesized thiosulfonates did not lead to significant changes in the phospholipids content in the blood plasma and tissues of rats (Fig. 5–7). It is worth noting only the increase in the total phospholipids of liver tissue in rats injected with methyl thiosulfonate. These data may indicate an adaptive reaction of the rat's organism under the action of this substance.

As can be seen from the results presented in Table 4, the main fractions of phospholipids in the blood are phosphatidylcholine and phosphatidylethanolamine, the content of which accounts for more than 50% of their total content. The main impact on the phospholipid spectrum of blood is found for allyl and ethyl esters of thiosulfoacid. Thus, the content of phosphatidylserine and phosphatidylinositol decreased by 47% and 23% respectively, while the amount of phosphatidylethanolamine increased by 14% (P < 0.05), lysophosphatidylcholine – by 41% (P < 0.05) and phosphatidic acid – by 37% (P < 0.05) in the blood plasma of rat administered allyl thiosulfonate.

Table 3
Soluble protein spectrum in the kidney of rats,
treated with esters of thiosulfoacid (x ± SE, n = 5)

Indicators	Groups			
	control	allyl thiosulfonate	ethyl thiosulfonate	methyl thiosulfonate
Total protein, g/kg	305.80 ± 2.47	287.07 ± 5.38*	315.04 ± 9.00	285.61 ± 5.55*#
Pre-albumin, %	5.78 ± 1.62	7.83 ± 1.29	8.10 ± 1.57	9.19 ± 3.81
Albumin, %	7.97 ± 1.66	11.52 ± 2.31	9.80 ± 2.06	11.44 ± 5.12
α-globulin, %	24.62 ± 1.40	17.72 ± 1.58*	19.19 ± 1.70#	19.65 ± 3.81*#
β-globulin, %	27.31 ± 1.10	26.11 ± 2.47	26.46 ± 2.85	26.45 ± 4.15
γ-globulin, %	34.42 ± 2.40	36.82 ± 2.53	36.45 ± 1.81	33.27 ± 3.27

Note: see Table 1.

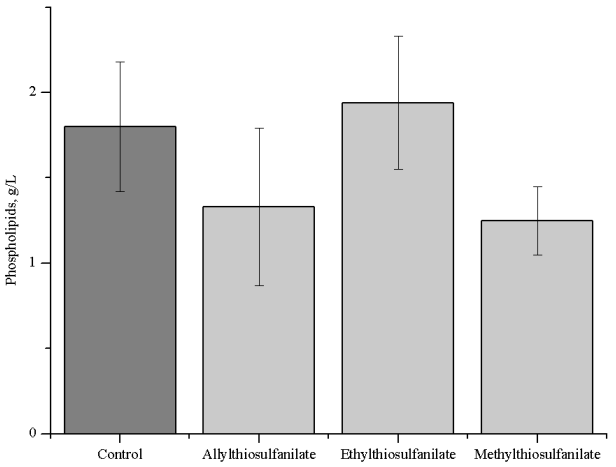

Fig. 5. Effect of thiosulfonates on the total phospholipids in the blood plasma of rats: statistically significant differences between all experimental groups and control group by ANOVA test were not detected; x ± SD, n = 5

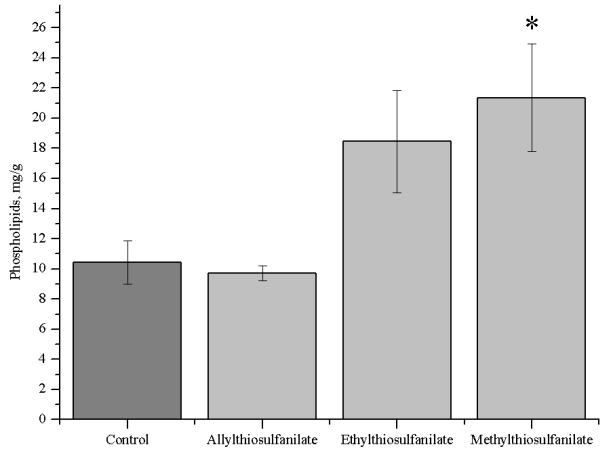

Fig. 6. Effect of thiosulfonates on the total phospholipids in the liver of rats: * – the difference is statistically significant between control animals and animals treated with methyl thiosulfonate (P < 0.05); x ± SD, n = 5

The administration of ethyl thiosulfonate to animals was accompanied by a decrease in the content of phosphatidic acid by 29% (P < 0.05), phosphatidylinositol by 21% (P < 0.05) and phosphatidylserine by 51% (P < 0.05) in comparison with the control group, and phosphatidylethanolamine and phosphatidylcholine, on the contrary, increased significantly. A decrease in the content of phosphatidylserine by 46% (P < 0.05) was observed in the blood plasma of rats treated with methyl thiosulfonate too.

It is important that the administration of all esters of thiosulfoacid led to the increase in the lysophosphatidylcholine fraction.

Our results presented in Table 5 showed that the dominant fraction of phospholipids in the liver is phosphatidylcholine, whose content varied within 42–45%. As can be seen from the data there was a decrease in

the fractions of phosphatidylethanolamine by 11% ($P < 0.05$) in the liver of animals injected with allyl thiosulfonate, compared to the control. At the same time, an increase in phosphatidylinositol (47%, $P < 0.05$) and sphingomyelin (46%, $P < 0.05$) was observed in these animals.

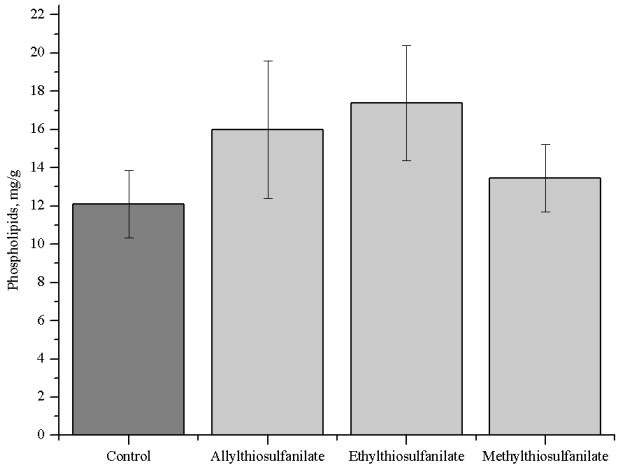

Fig. 7. Effect of thiosulfonates on the total phospholipids in the kidney of rats: statistically significant differences between all experimental groups and control group by ANOVA test were not detected; x ± SD, n = 5

Table 4
Fractional composition of phospholipids in blood plasma (%, x ± SE, n = 5)

Class of lipids	Groups			
	control	allyl thiosulfonate	ethyl thiosulfonate	methyl thiosulfonate
Phosphatidic acid	12.77 ± 0.77	17.52 ± 0.12*	9.10 ± 0.40#	12.07 ± 0.13
Phosphatidylethanolamine	21.16 ± 0.86	24.12 ± 0.71*	23.16 ± 0.81#	21.57 ± 0.55
Phosphatidylinositol	10.44 ± 0.54	7.99 ± 0.57*	8.30 ± 0.71#	10.68 ± 0.55
Phosphatidylcholine	29.08 ± 0.63	28.67 ± 0.81	34.86 ± 0.63#	28.13 ± 0.77
Phosphatidylserine	13.56 ± 0.51	7.21 ± 0.21*	6.61 ± 0.37#	7.26 ± 0.04*#
Sphingomyelin	6.96 ± 0.36	5.92 ± 0.37*	6.86 ± 0.78	7.42 ± 0.43
Lysophosphatidylcholine	6.04 ± 0.26	8.58 ± 0.04*	11.10 ± 0.64#	12.89 ± 0.45*#

Note: see Table 1.

A significant increase in phosphatidylethanolamine and phosphatedylserine fractions was recorded in methyl thiosulfonate-treated rats whereas the amount of lysophosphatidylcholine and phosphatidic acid was significantly reduced. Similar changes of lysophosphatidylcholine and phosphatidylserine were also found in ethyl thiosulfonate- treated rats. Therefore, this redistribution of phospholipid fractions in the liver may be apparently associated with changes in the activity of the concerned enzymes.

Table 5
Fractional composition of phospholipids in the liver (%,x ± SE, n = 5)

Class of lipids	Groups			
	control	allyl thiosulfonate	ethyl thiosulfonate	methyl thiosulfonate
Phosphatidic acid	7.91 ± 0.27	7.32 ± 0.17	7.07 ± 0.31	4.24 ± 0.90*#
Phosphatidylethanolamine	22.95 ± 0.57	20.43 ± 0.41*	22.77 ± 1.42	25.82 ± 0.70*#
Phosphatidylinositol	5.72 ± 0.29	8.40 ± 0.28*	5.07 ± 0.40	5.78 ± 0.18
Phosphatidylcholine	42.50 ± 0.49	42.33 ± 3.99	44.35 ± 4.60	44.56 ± 3.93
Phosphatidylserine	7.07 ± 0.20	6.56 ± 0.13	7.88 ± 0.36#	8.12 ± 0.64*#
Sphingomyelin	5.36 ± 0.44	7.82 ± 0.40*	6.77 ± 0.97#	5.61 ± 0.21
Lysophosphatidylcholine	8.49 ± 0.08	7.14 ± 0.69*	6.09 ± 0.70#	5.87 ± 0.90*#

Note: see Table 1.

In the present study, the administration of all synthesized thiosulfonates had a significant effect on the phospholipid spectrum in the kidney (Table 6). Thus, a significant reduction in the content of one of the most important phospholipids of cell membranes – phosphatidylcholine was observed in animals of all experimental groups.

It is obvious that such changes could occur due to an increase in the fraction of sphingomyelin in these animals. In addition, an increase in phosphatidylethanolamine content by 15% ($P < 0.05$) and 25% ($P < 0.05$), respectively, was found in rats injected with ethyl- and methyl thiosulfonates. At the same time, the level of phosphatidylinositol was signifycantly higher in animals treated with allyl- and ethylthiosulfonates.

Table 6
Fractional composition of phospholipids in the kidney (%, x ± SE, n = 5)

Class of lipids	Groups			
	control	allyl thiosulfonate	ethyl thiosulfonate	methyl thiosulfonate
Phosphatidic acid	9.57 ± 0.59	10.10 ± 0.66	9.29 ± 0.36	10.57 ± 0.25*#
Phosphatidylethanolamine	16.87 ± 0.66	16.98 ± 0.74	19.41 ± 0.79#	21.10 ± 1.69*#
Phosphatidylinositol	9.17 ± 0.26	12.51 ± 0.63*	7.78 ± 0.24#	12.58 ± 0.32*#
Phosphatidylcholine	39.65 ± 2.30	32.67 ± 1.65*	30.59 ± 1.46#	35.97 ± 0.42*#
Phosphatidylserine	9.38 ± 0.59	9.22 ± 0.79	12.51 ± 1.18#	7.27 ± 0.25*#
Sphingomyelin	6.46 ± 0.42	9.52 ± 0.23*	9.83 ± 0.44#	6.61 ± 0.72
Lysophosphatidylcholine	8.90 ± 0.79	9.00 ± 0.59	10.59 ± 0.90#	5.90 ± 0.20*#

Note: see Table 1.

Discussion

Computerized predictive analysis of the toxicity of synthesized esters of thiosulfoacid using the GUSAR program has shown that all of these compounds belong to Class IV of toxicity (Lagunin et al., 2011). Synthetic thiosulfonates are also more stable than natural organic sulfur compounds of garlic, onions etc. Literature data report the different biological effect of these substances due to their participation in various biochemical processes (Iciek, 2009; Kaschula et al., 2016).

The genotoxicity of propyl thiosulfinate oxide in rats after oral administration in doses 5.5, 17.4, and 55.0 mg/kg was assessed by authors (Mellado-García et al., 2016). This compound exhibited no *in vivo* genotoxicity, and the histopathological analysis revealed only slight modifications, such as increased glycogen storage in the liver and a degenerative process in the stomach, with vacuolization of cell membranes, only at the highest dose.

In the present study, the effect of synthesized methyl-, ethyl- and allyl esters of thiosulfoacid on the protein and phospholipid profile in blood plasma, liver, and kidney tissues of rats was determined.

It is well known that albumin and globulins are functional proteins in blood plasma. Albumin is a homogeneous fraction, the main function of which is to maintain oncotic blood pressure, as well as the transport of various substances in tissues. Instead, the globulins are a group of proteins with different level of dispersity and molecular weight. Many studies have suggested that protein fractions with different electrophoretic mobility may be markers of various compensatory and adaptive reactions, and pathological processes in the organism (Tkachenko et al., 2014; Koropec'ka et al., 2015).

Our data have shown that the protein spectrum of the liver did not change under the short exposure to S-alkyl esters of thiosulfoacid, indicating no influences of these biologically active compounds on protein synthesis in the liver. It is worth noting that in the tissues of the liver and kidney, on contrast to the blood plasm, most soluble proteins were located in the zone of blood plasma β- and γ-globulins mobility, and the smaller part – in the area of albumin and pre-albumin mobility. In our experiment, the total protein content decreased in the tissues of kidney of groups of animals administered intraperitoneally with methyl and allyl thiosulfonates. The obtained results indicate that a high dose of these esters of thiosulfoacid may have nephrotoxic effects, even after the short-term exposure.

Taking into account the lipophilic nature of the synthesized thiosulfonates, it is obvious that these compounds can quickly penetrate through the plasma membranes by passive diffusion (Miron et al., 2000). It is well-known that phospholipids form stable double-layer membrane structures that regulate the flow of ions and substances inside the cell (Marquardt et al., 2015). Thus, phospholipid composition of biomembranes is an important structural and functional characteristic in all cells of the body. The ratio of individual subclasses of phospholipids, the degree of their saturation with fatty acids determine the viscosity of membrane lipid bilayer, affects the ordering of lipid molecules, as well as the character of lipid-lipid and protein-lipid interactions. These factors significantly

influence the physicochemical properties, permeability, and lability of biomembranes (Gula & Margitych, 2009). Therefore, the special attention was paid to the study of phospholipid composition in the blood, liver, and kidney in response to the action of these biologically active compounds.

In the present study, we found that the administration of alkyl thiosulfonates causes certain changes in the ratio of phospholipids in blood plasma. Phosphatidylethanolamine and phosphatidylserine characterize the internal membrane monolayer (Yamaji-Hasegawa & Tsujimoto, 2006). Under the influence of allyl thiosulfonate, the amount of phosphatidylethanolamine in animals significantly increased, at the same time phosphatidylserine decreased in the blood. Our results are consistent with the literature data; since it is known that about 80% phosphatidylethanolamine is formed by decarboxylation of phosphatidylserine (Semenova, 2006). The detected increase in phosphatidic acid in these animals may be, on the one hand, due to the degradation of phospholipids, and on the other hand, a disturbance of synthetic processes. It is important that the administration of all esters of thiosulfoacid led to the increase in the lysophosphatidylcholine fraction, which may indicate an increase in phospholipase activity (Baba et al, 2014). On the other hand, the formation of large amounts of lysophosphatidylcholine, which is characterized by a detergent function, reduces the viscosity and increases the fluidity of the membrane. Consequently, such changes in the phospholipid blood spectrum may be an adaptive-compensatory response to the short-term effect of synthesized thiosulfonates.

Analysis of the phospholipid spectrum in the liver cells has shown that the greatest effect on the ratio of different fractions of phospholipids was detected for the allyl ester of thiosulfoacid. Thus, a decrease in phosphatidylethanolamine and phosphatidylserine may indicate that these fractions are rapidly oxidized with the enhancement of lipid peroxidation processes. Increasing phosphatidylinositol under these conditions indicates its adaptive synthesis since this phospholipid is involved in the effects of many hormones (Manna & Jain, 2015).

An important indicator that characterizes the lability of the lipid bilayer is the coefficient of membrane asymmetry, which is defined as (phosphatidylethanolamine + phosphatidylserine) / phosphatidylcholine + sphingomyelin. The ratio of the number of phospholipids with less saturated fatty acids, which are mainly found in the inner monolayer of the lipid membrane, to saturated phospholipids that are located in the outer monolayer, gives an understanding of the properties of the cell membrane (Srubilin et al., 2016). Thus, the coefficient of asymmetry in hepatocyte membranes after administration of allyl thiosulfonate is lower than in the control by 14%. These changes lead to increasing of saturation of lipid bilayer and its microviscosity. In contrast to allyl thiosulfonate, methyl ester of thiosulfoacid has a positive effect on the structural and functional characteristics of cell membranes, increasing their fluidity (coefficients of asymmetry, respectively, 0.63 and 0.76 for control group and methyl thiosulfonate-treated rats).

Thus, the observed redistribution of different fractions of phospholipids in blood plasma and liver in response to the administration of all thiosulfonates in rats can be closely related, first of all, to their involvement in the energy metabolism, the regulation of the activity of various transmembrane proteins, especially the activity of cytochrome P_{450} by modulating its structure during detoxification (Ghosh & Ray, 2014).

It is well-known that there is a close relationship between the lipid metabolism in the liver and kidneys; therefore, the adaptive changes in the phospholipid spectra of kidney cell in response to the influence of the thiosulfonates largely depends on the characteristics of the lipid metabolism in the liver. Thus, in the present study, the ratio of phospholipid fractions in the kidneys changed due to the decrease in phosphatidylcholine of the outer membrane layer. At the same time, the decrease in phosphatidylcholine compensated by increasing the content of sphingomyelin. Taking into account the high saturation of sphingomyelin, large amounts of cholesterol can embed into clusters that form this phospholipid in the membranes. As a result, the decrease in permeability of the cell membrane, changes in the processes of active transport and transport of substances were observed (Quinn, 2014). Literature data indicate that the ratio of phosphatidylcholine to sphingomyelin may reflect changes in the structure of the membrane bilayer. Under the administration of thiosulfonates, this ratio was 3.43, 3.11, 5.44 respectively for groups of animals treated with allyl-, ethyl-, and methyl thiosulfonates compared to 6.14 in the control. The reduction of this coefficient by 44% and 49% in animals, injected by allyl- and ethyl thiosulfonates may indicate a decrease in fluid properties and an increase in the microviscosity of the lipid phase of the nephrocyte membranes. However, the increase in phosphatidylinositol in animals treated with allyl- and methyl thiosulfonates indicates their involvement in signal transduction, since it is well-known the role of this phospholipid in control of membrane-cytosolic processes, regulation of cellular permeability and the provision of intranuclear processes (Di Paolo & De Camilli, 2006).

Summing up our experimental results, we can assume that the divergence of changes in the content of individual phospholipids explains minor changes in their total amount under experimental conditions. These data may indicate a different sensitivity of the metabolic pathways responsible for the synthesis and degradation of individual phospholipids.

Conclusion

Intraperitoneal administration of methyl, ethyl and allyl esters of thiosulfoacid to rats did not significantly affect the total protein and their spectrum in the liver tissue but led to an increase in the total protein and albumin in the blood of rats injected with allyl- and ethyl thiosulfonates. The detected reduction in total protein in the kidney tissue of animals after short-term exposures of methyl and allyl thiosulfonates may indicate their potentially nephrotoxic effect.

The administration of synthesized thiosulfonates at a dose of 300 mg/kg of body weight did not cause significant changes in the total phospholipids of blood plasma, liver and kidneys, but led to tissue-specific changes in the ratio of their fractions. The most significant changes in the phospholipid spectrum of rats tissues, which accompanied by changes in the structural and functional characteristics of cell membranes, were observed for the allyl ester of thiosulfoacid, and to a lesser extent – for methyl- and ethylthiosulfonates. Increase in the saturation of lipid bilayer and the microviscosity of the liver and kidney cell membranes in animals administered by allyl ester of thiosulfoacid may cause alteration of membrane transport systems and the activity of membrane-bound enzymes.

Redistribution of different fractions of phospholipids in blood plasma, liver and kidney tissue in response to the administration of synthesized biologically active compounds is the result of adaptive-compensatory reactions of the body and associated with their participation in various physiological processes.

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