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Biochemical Characteristics of the Effect of Tiosulfonic Acid Esters on Lipid Content in Rat Blood Plasma

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Abstract: This study investigated the biochemical effects of synthetic thiosulfonate derivatives — S-ethyl-4-aminobenzenethiosulfonate (ETS), S-allyl-4-aminobenzenethiosulfonate (ATS), and S-allyl-4-acetylaminobenzenethiosulfonate (AATS) — on lipid metabolism in rat blood plasma. Over a 21-day period, animals in the experimental groups received oil-based solutions of the compounds (500 µL daily) at a dose of 100 mg/kg body weight. A reduction in total plasma lipid content was observed, primarily due to decreased levels of triacylglycerols and esterified cholesterol. At the same time, increases in di- and monoacylglycerols, unesterified cholesterol, free fatty acids, and phospholipids were recorded. The findings indicate that these compounds can modulate specific pathways of lipid metabolism and may be useful in correcting metabolic disorders and preventing complications associated with lipid imbalance.

Keywords: s-ethyl-4-aminobenzenethiosulfonate; s-allyl-4- aminobenzenethiosulfonate; s-allyl-4-acetylaminobenzenethiosulfonate; lipids.

1. Introduction

S-esters of thiosulfonic acids ($RSO_2SR`$) are effective sulfonating and sulfonylating reagents [1, 2]; they are compounds with high biological activity [3, 4]. The high reactivity of S-esters of thiosulfonic acids is a result of the structure of the thiosulfonic group, due to the high polarity of the -S-S- bond, which depends on the nature of the acid and thiol components, which in turn affects their reactivity [5, 6] and determines the scope of their possible applications. These compounds can be used as effective biologically active substances, namely as insecticides, plant protection products, and growth regulators [7], as well as a basis for pharmaceuticals [8–10].

Natural sulfur-containing compounds found in garlic and onion extracts, such as allicin, alliin, and others, exhibit antioxidant activity to neutralize free radicals in vitro [6, 11, 12].

Literature data suggest that garlic extract enhances the anticancer activity of dexamethasone in rats with multiple myeloma [13–15]. Some authors have shown that anti-inflammatory effects in mice may be associated with garlic and onion extracts [16–18].

Additionally, the use of garlic and onion supplements has improved the condition of people with oxidative stress-related diseases, such as rheumatoid arthritis or obesity [19, 20].

Previously, we have shown that under the influence of synthesized S-alkyl esters of thiosulfonic acids at doses of 50 and 100 mg/kg administered to rats for 21 days, multidirectional changes in lipid peroxidation and the antioxidant system in tissues were detected [21–23]. This suggests a varying degree of inhibition or activation of lipid peroxidation processes, depending on the type of tissue and the structure of the thiosulfonic acid ester [24, 25].

The results of other authors showed that short-term administration of thiosulfonic acid methyl ester at a dose of 300 mg/kg body weight did not cause significant changes in the content of total protein and its fractions in liver tissue, while the effect of allyl and ethyl esters of thiosulfonic acid was accompanied by an increase in total protein and albumin in blood plasma. The action of these compounds did not cause significant changes in the total phospholipid content in the blood plasma and tissues of rats, except for methyl thiosulfonate, the effect of which was accompanied by an increase in the total phospholipid content in the liver of rats [26].

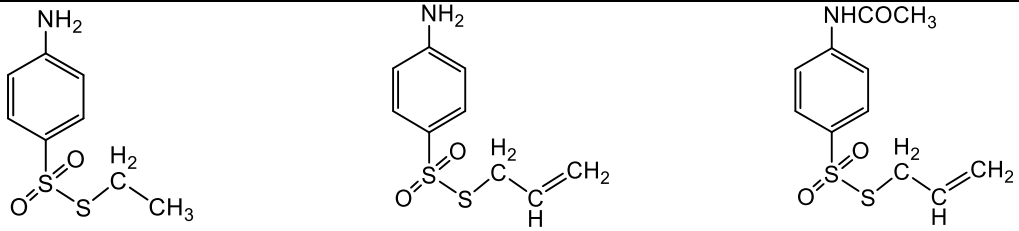
Among modern lipid-lowering agents, statins remain the gold standard due to their effective inhibition of HMG-CoA reductase, resulting in significant reductions in LDL cholesterol levels [27]. Fibrates, which activate PPAR-α receptors, primarily reduce triglycerides and raise HDL cholesterol [28]. More recently, PCSK9 inhibitors, such as alirocumab and evolocumab, have shown remarkable efficacy in lowering LDL cholesterol, especially in patients with familial hypercholesterolemia or statin intolerance [29]. Ezetimibe, an inhibitor of intestinal cholesterol absorption, is often combined with statins to enhance lipid reduction [30]. Omega-3 polyunsaturated fatty acids effectively lower triglycerides and have anti-inflammatory effects [31]. Novel agents like bempedoic acid and inclisiran provide alternative or adjunctive therapy options, particularly for patients with resistant dyslipidemia [32, 33]. Additionally, ANGPTL3 inhibitors, such as evinacumab, target both LDL cholesterol and triglycerides, representing an innovative approach in lipid management [34]. The literature contains studies of adverse reactions of various medications that affect lipid metabolism, including cholesteryl ester transfer protein inhibitors, ezetimibe, fenofibrate, HMG-CoA reductase inhibitors (statins), and nicotinic acid derivatives. Moreover, the main focus is now on research involving the study of various types of lipid-modifying drugs that have minimal side effects [35].

The multifaceted effect of synthetic sulfonic acid esters sparked our interest in determining their influence on the content of individual lipids in rat blood plasma.

2. Materials and Methods

2.1. Thiosulfonates.

Our task was to study the biochemical features of the effect of S-ethyl-4-aminobenzenethiosulfonate (ETS), S-allyl-4-aminobenzenethiosulfonate (ATS), and S-allyl-4-acetylaminobenzenethiosulfonate (AATS) on lipid content in blood plasma. These compounds were synthesized at the Department of Technology of biologically active compounds, pharmacy and biotechnology of National University “Lviv Polytechnic” according to the protocol described previously [5]. The structural formulas of thiosulfate esters are shown in Figure 1.



(a) **(b)** **(c)**

Figure 1. Structural formulas of thiosulfonate esters: **(a)** – S-ethyl-4-aminobenzenethiosulfonate (ETS); **(b)** – S-allyl-4-aminobenzenethiosulfonate (ATS); **(c)** – S-allyl-4-acetylaminobenzenethiosulfonate (AATS).

2.2. Experimental animals.

The research was conducted on white male Wistar rats (190–210 g). The study was carried out in compliance with the general ethical principles of animal experiments adopted by the First National Congress on Bioethics (Kyiv, Ukraine, 2001) and in accordance with the provisions of the "European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes" (Strasbourg, France, 1986). A permission to conduct the study was obtained from the Bioethics Committee of the Institute of Animal Biology NAAS (№ 128/ 27.02.2023). The animals were kept in a vivarium under appropriate lighting and temperature conditions.

2.3. Design of experiments.

Comparison of animal groups. The animals were divided into four groups: group I - control, groups II, III, and IV - experimental (7 rats in each). Group I was a control in relation to experimental groups II, III, and IV, which received an oil solution of thiosulfonates. The control and experimental groups received standard pelleted food for laboratory rats. All animals in the experimental groups were administered 500 µL of an oil solution of thiosulfonates at a rate of 100 mg/kg body weight (20.0 mg/day for a rat weighing 200 g). Rats in group II received ETS with food, group III received ATS, and group IV received AATS. Animals in the control group were similarly given 500 µL of oil once a day with their diet. For preparing the oil solutions of synthesized compounds, "Oleina" oil (traditional: refined, deodorized, frozen; Producer of PJSC with II "DOEP"; certified according to State Standard of Ukraine 4492: 2017, complies with ISO 14024) was used. The experiment lasted 21 days. Rats from all groups were decapitated under thiopental anesthesia on the 22nd day of the experiment. All procedures were performed at a temperature of +4°C. Blood plasma of rats was obtained by centrifugation of heparinized blood at 300 rpm for 10 min.

2.4. Biochemical analysis.

Obtaining common lipids. Blood plasma (1 cm^3) was extracted with a chloroform-methanol mixture in the ratio 2:1 (v/v) according to the Folch method [36]. To clean the lipid extract, a 0.74 M KCl solution was added. The total amount of lipids was determined by weighing dry using a residue gravimetric method [37]. Separation of lipids into classes. The separation of lipids into classes was carried out using thin-layer chromatography (TLC) on silica gel (silica gel L 5/40 µ, LSL 5/40 µ, Chemapol, Slovakia) as the mobile phase. A hexane-diethyl ether–acetic acid mixture in a ratio of 70:30:1 (v/v/v) was used [37]. Plates were obtained using the vapors of crystalline iodine. Identification of individual lipids was carried out by Rf values [37]. The developed plates were scanned (HP Scanjet G2710, China).

Quantitative analysis and counting of the contents of the lipid classes were performed by computer processing of foregrams using the TotalLab TL120 software (Nonlinear Dynamics Limited, UK) and expressed as a percentage of the total pool.

The content of non-esterified cholesterol (NEC) and esterified cholesterol (EC), phospholipids (PL), monoglycerols and diacylglycerols (MDAG), triacylglycerols (TAG), and non-esterified fatty acids (NEFA) was identified in rats' blood plasma. For separation of the phospholipids by the TLC method on a silica gel, a solvent system of chloroform-methanol-water in a ratio 65:25:4 (v/v/v) was used [38]. Crystalline iodine vapors were used as a developer. The identification of individual phospholipids was carried out by Rf values [39]. The obtained plates were scanned. Quantitative analysis and counting of the individual lipid content were performed by computer processing of the foregrams using the TotalLab TL120 software (Nonlinear Dynamics Limited, UK) and expressed as a percentage of the total pool. Blood plasma of rats was examined to identify the content of the phosphatidic acid (PA), cardiolipin (CL), phosphatidylethanolamine (PE), phosphatidylinositol (PI), phosphatidylcholine (PC), phosphatidylserine (PS), sphingomyelin (SM), and lysophosphatidylcholine (LPC) in rats' blood plasma.

2.5. Statistical analysis.

Statistical analysis. The obtained digital data were processed statistically using the Microsoft Excel 2016 package. We calculated the mean and standard error of the mean (M±m). To determine probable differences between the statistical groups, the Student's criterion is used.

3. Results and Discussion

In experimental studies, under the influence of AATS at a dose of 100 mg/kg body weight, a significant decrease in the total lipid content in rat blood plasma was observed by 6.76% (Table 1).

However, under the influence of ETS and ATS, there was only a tendency to reduce the content of total lipids in blood plasma by 5.40% and 6.56%, respectively. The results obtained may indicate an increase in the activity of catabolic processes and the mobilization of lipids as a source of energy due to the activation of the enzyme lipoprotein lipase, which degrades blood lipids, or their use in adaptive changes in the lipid layer of cell membranes. Other authors have found a decrease in the total lipid content of rat liver under the influence of synthetic thiosulfonates [39].

The relative content of the following lipid classes in rat blood plasma was studied: unesterified and esterified cholesterol, mono- and diacylglycerols, triacylglycerols, non-esterified fatty acids, and phospholipids (Table 1).

Table 1. The content of total lipids and the fractional composition of total lipids in blood plasma.

Indicators	Control group	Experimental group		
		D1 (ETS)	D2 (ATS)	D3 (AATS)
Total lipids (g/L)	5.18±0.34	4.90 ± 0.23	4.84±0.54	4.83±0.18*
Class of lipids, %				
Triacylglycerols	7.48±0.43	6.91±0.57*	4.34±0.67**	5.99±0.44***
Monoacylglycerols and diacylglycerols	16.89±1.20	20.05±2.04*	16.96±1.18	19.85±1.99**
Esterified cholesterol	18.03±2.18	10.02±1.46***	4.67±0.81***	3.92±0.97***
Nonesterified cholesterol	6.85±1.26	7.18 ±1.38	13.18±1.17***	7.63±1.42

Indicators	Control group	Experimental group		
		D1 (ETS)	D2 (ATS)	D3 (AATS)
Non-esterified fatty acids	4.21 ±0.72	6.48±0.24***	4.94±0.67	8.94±0.96***
Phospholipids	46.55±2.24	49.36±2.66	55.92±3.97**	53.69±4.63**

*, **, *** the differences are statistically significant compared to the control group (P < 0.05-0.001)

The decrease in total lipid content in the blood plasma of animals under the influence of sulfonic acid esters can be attributed to a reduction in triacylglycerol levels. This suggests that the compounds studied accelerate the breakdown of storage lipids, specifically cholesterol esters. This conclusion is supported by the results of the study, which showed a significant reduction in the relative content of triacylglycerols in the blood plasma of animals exposed to ETS, ATS, and AATS at a dose of 100 mg/kg by 7.62%, 41.98%, and 19.92%, respectively, compared with the control group (Table 1).

At the same time, the relative content of monoacylglycerols and diacylglycerols increased significantly under the influence of ETS (by 18.71%) and AATS (by 17.52%). A decrease in the level of triacylglycerols in the blood may indicate their breakdown into diacylglycerols and monoacylglycerols, a process confirmed by the results obtained.

The study demonstrated a substantial decrease in the relative content of esterified cholesterol in the presence of ETS, ATS, and AATS at a dose of 100 mg/kg, with reductions of 44.42%, 74.10%, and 78.25%, respectively (Table 1). This observation suggests the possibility of an increase in the hydrolysis of cholesterol esters, which function as storage and transport forms of cholesterol, facilitated by cholesterol esterase. Consequently, the esterified cholesterol is hydrolyzed to unesterified cholesterol and fatty acids.

The studies revealed a significant increase in the content of unesterified cholesterol in animals under the influence of ATS by 92.41%, as well as a slight increase under the influence of ETS and AATS by 4.82% and 11.39%, respectively, compared to the control group. It is also noteworthy that the content of unesterified cholesterol was higher in group III animals receiving ATS than in groups II and IV receiving ETS and AATS, respectively.

The results obtained indicate that the activity of the enzyme responsible for cholesterol esterification, lecithin (phosphatidylcholine)-cholesterol acyltransferase, is low in the blood of animals in the experimental groups. A decrease in the level of esterified cholesterol in the blood reduces the risk of cholesterol plaque formation in the blood vessels and the development of cardiovascular disease. The body regulates the level of esterified cholesterol through enzymes responsible for cholesterol esterification and hydrolysis, as well as receptors that control its metabolism in cells and tissues, thereby ensuring the normal function of these processes.

As a result of the hydrolysis of esterified cholesterol, non-esterified fatty acids (NEFA) are formed, and an increase in their content was observed in the blood of animals from the experimental groups. Specifically, under the influence of ETS and AATS at a dose of 100 mg/kg, a significant increase in NEFA was observed in the blood of rats by 53.92% and 112.35%, respectively (Table 1). This can be attributed not only to hydrolysis of esterified cholesterol but also to increased lipolysis and degradation of triacylglycerols. Typically, NEFA can then be transported to the liver, where they undergo β-oxidation and partially provide energy to cells, or they can be esterified and stored as triacylglycerols.

Phospholipids are the main lipid components of biological membranes [40]. The content of phospholipids in the membrane of a mature red blood cell is continuously renewed during the circulation of blood. Erythrocytes are incapable of de novo biosynthesis of phospholipids, but regulate their composition by passive exchange of intact phospholipids with plasma lipoproteins [41]. An alternative pathway for phospholipid replenishment involves the

acylation of fatty acids, either endogenous or exogenous lysophospholipids, followed by their incorporation into the erythrocyte membrane.

The studies showed that the content of phospholipids in the blood plasma of rats was significantly increased by 20.13% and 8.77%, respectively, under the influence of ATS and AATS at a dose of 100 mg/kg, and slightly increased by 6.04% under the influence of ETS (Table 1). Since phospholipids in blood plasma have a transport function, their increase plays a crucial role in transporting cholesterol, fatty acids, and newly synthesized sulfonic acid esters into the organism.

The level of phospholipids in the blood plasma increases. This is due to an increase in the levels of phosphatidylethanolamine and phosphatidylcholine (D1 and D2), phosphatidylinositol and phingomyelin (D3) (Table 2).

Similar results were obtained by other researchers, who found that allyl- and ethylthiosulfonate esters caused an increase in the levels of phosphatidylethanolamine and phosphatidylcholine fractions but a decrease in phosphatidylinositol in the tissues of rats [26].

Table 2. Fractional composition of phospholipids in blood plasma.

Class of lipids, %	Control group	Experimental group		
		D1 (ETS)	D2 (ATS)	D3 (AATS)
Phosphatidic acid	38.84±2.04	7.86±1.10***	2.90±0.27***	1.23±0.39***
Cardiolipin	5.44±0.48	3.14±0.41***	1.86±0.30***	4.47±0.68**
Phosphatidylethanolamine	13.90±1.11	40.82±2.78***	63.21±1.10***	8.27±1.02***
Phosphatidylinositol	19.16±2.17	6.52 ±0.42***	10.87±1.48***	65.12±2.13***
Phosphatidylcholine	4.35 ±0.43	22.56±2.26***	8.96±0.54***	2.97±0.88**
Phosphatidylserine	6.67±0.49	7.86±1.22	3.10±0.78***	6.11±1.00
Sphingomyelin	3.75±0.72	6.13±1.03**	3.50±0.83	7.89±1.66***
Lysophosphatidylcholine	7.52±0.83	5.11±0.77**	5.60±0.63**	3.96±0.28***

*, **, *** the differences are statistically significant compared to the control group (P < 0.05-0.001)

In our studies, there was an increase in phosphatidylethanolamine levels in groups D1 and D2, but a decrease in group D3. This phospholipid is involved in the synthesis of phosphatidylcholine. Therefore, it is obvious that the phosphatidylcholine content also increased in groups D1 and D2, but decreased in group D3. Phosphatidylcholine, one of the most important phospholipids, has lipotropic and regulatory effects and serves as a structural component of essential biological elements (cell membranes, myelin sheaths). It can transport excess cholesterol from tissues and blood to the liver, facilitating its elimination from the body and accelerating redox processes.

The level of phosphatidylinositol decreased in the blood of rats in groups D1 and D2, but increased in D3. This phospholipid and its derivatives represent only about 10-20% of all phospholipids. Despite their low abundance, they play a crucial role as important intracellular secondary messengers, being involved in universal signaling systems within cells, including lipid distribution and metabolic processes [42].

Sphingolipids are potential biomarkers of metabolic disorders in blood plasma [43]. Sphingomyelins play important structural and functional roles in many signaling pathways. The increase in sphingomyelin content under the influence of ETS and AATS may result from reduced ceramide formation, which inhibits the activation of the Akt signaling pathway and consequently promotes the restoration of receptor signaling.

Phosphatidylserine is a phospholipid that is highly concentrated in metabolically active organs, including the brain, lungs, heart, liver, and skeletal muscle. It is primarily found in the inner layer of the cell membrane and has unique regulatory and structural functions. Primarily,

phosphatidylserine modulates the activity of receptors, ion channels, enzymes, and signaling molecules, and regulates membrane fluidity [44, 45]. In the blood plasma of rats exposed to ATS, the levels of phosphatidylserine and phosphatidylinositol decreased. Since these phospholipids are directly involved in the activation of atypical forms of protein kinase C and PI3K, this may contribute to the restoration of membrane receptor sensitivity.

Cardiolipin is the major polyglycerophospholipid in mammalian tissues, constituting approximately 7-15% of the total phospholipid content of mitochondria. Cardiolipin is present in blood plasma as a component of lipoproteins. Changes in cardiolipin levels may be associated with various pathophysiological conditions, including diabetes, obesity, heart failure, hyperthyroidism, and aging [46]. The content of cardiolipin significantly decreased due to the influence of the studied compounds in the blood plasma of rats, which may indicate a probable increase in cardiolipin in mitochondrial membranes. Since cardiolipin modulates the activity of many mitochondrial enzymes involved in ATP production and regulates the mitochondrial electron transport chain, this is significant for oxidation processes in the body [47]. However, cardiolipin has also been implicated in the regulation of numerous other cellular processes, including apoptosis, autophagy, mitophagy, and glucose transport across the membrane [48].

ETS, ATS, and AATS induced changes in the lipid profile of blood plasma. This may be due to the ability of the tested compounds to modulate several key regulators of lipid metabolism by increasing the expression of peroxisome proliferator-activated receptor (PPARγ) and activating the activated protein kinase AMPK. The PI3K/AKT/mTOR signaling pathway is involved in the biosynthesis of macromolecules and regulates lipogenesis, as well as the expression of lipogenic genes. AMPK is an energy sensor that regulates metabolism and is activated when stored ATP is depleted, and is responsible for the suppression of several key lipogenic factors in the liver related to cholesterol and fatty acid synthesis. SREBPs control lipogenic gene expression and cholesterol metabolism and are involved in dietary regulation of fatty acids and triglycerides.

However, we have not found direct evidence of the action of S-esters of thiosulfonic acids on the expression of PPARγ or the activation of AMPK. Therefore, the study of the effect of S-esters of thiosulfuric acids on individual indicators of lipid metabolism, their reactivity in biochemical reactions, which is based on the specific effects of the sulfonyl and thiol components of the studied compounds, may be of interest in further studies of the mechanisms of their action on cellular signaling pathways associated with lipid metabolism. Thus, these studies may help develop strategies for treating and regulating metabolic disorders of lipid metabolism and their associated complications in the future.

4. Conclusions

Experimental studies have shown that S-ethyl-4-aminobenzenethiosulfonate, S-allyl-4-aminobenzenethiosulfonate, and S-allyl-4-acetylaminobenzenethiosulfonate at a dose of 100 mg/kg body weight decreased the total lipid content in the blood of experimental animals. This was due to a decrease in the content of triacylglycerols and esterified cholesterol, with an increase in di- and monoacylglycerols, unesterified cholesterol, and fatty acids. The results obtained indicate an increase in lipolysis and hydrolysis of esterified cholesterol under the influence of the sulfonic acid esters studied. The increase in the level of total phospholipids in blood plasma may be due to an increase in the content of phosphatidylethanolamine and phosphatidylcholine (ETS and ATS influence), as well as phosphatidylinositol and

sphingomyelin (AATS influence). Therefore, understanding the effects of synthetic sulfonic acid esters on individual components of lipid metabolism may help regulate metabolic disorders and complications that occur in the body.

In the future, we plan to conduct studies that will reveal and confirm our hypotheses that the studied S-esters of thiosulfuric acids exert a lipid-regulating effect by improving the expression of the peroxisome proliferator-activated receptor (PPARγ) and activating the AMPK.

Author Contributions

Conceptualization, R.I. and N.L.; methodology, R.I. and N.L.; validation, N.L., data curation, R.I.; formal analysis, R.I.; investigation, N.L.; writing—original draft preparation, R.I.; writing—review and editing, N.L. All authors have read and agreed to the published version of the manuscript.

Institutional Review Board Statement

The animal study protocol was approved by the Institutional Review Board of the Institute of Animal Biology of NAAS (protocol code 128 and date of approval 27.02.2023).

Informed Consent Statement

Not applicable.

Data Availability Statement

Data supporting the findings of this study are available upon reasonable request from the corresponding author.

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Conflicts of Interest

The authors declare no conflict of interest.

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