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STUDY OF THE PLEITROPIC EFFECTS OF SODIUM 2-((4-PHENYL-5-THIOPHEN-3-YLMETHYL)-1,2,4-TRIAZOL-3-YL)THIO)ETHANOATE IN CONDITIONS OF EXPERIMENTAL HYPERLIPIDEMIA

Actuality. *Atherosclerosis remains one of the leading global medical problems underlying most cardiovascular diseases, in particular coronary heart disease, hypertension, stroke, obliterating atherosclerosis of the lower extremities According to the World Health Organization, cardiovascular diseases are the leading cause of death in the world: they claim the lives of approximately 17.9 million people each year, accounting for about 32% of all deaths. Of these, more than 85% are caused by myocardial infarctions and strokes, most of which develop against the background of atherosclerotic vascular changes. In developed countries, atherosclerosis occurs in more than 50% of men and about 40% of women over the age of 50, and this trend continues despite advances in prevention and treatment. In Ukraine, according to official data of the Ministry of health, cardiovascular diseases account for more than 65% of the mortality structure, and the number of complications caused by atherosclerosis is growing every year.*

In this regard, an in-depth study of the mechanisms of atherosclerosis development and the identification of sensitive biomarkers that allow not only early detection of pathological changes, but also accurate prediction of the risk of complications is extremely important. Of particular value are so – called pleiotropic biomarkers-molecules that simultaneously reflect several key pathophysiological processes, such as inflammation (CRP), activation of the hemostatic system (D-dimer), ischemic myocardial damage (MV-CPK) and the functional state of the endothelium (eNOS).

In this context, given the significant hypocholesterolemic and hypotriglyceridemic effects found in the ''leader compound'', as well as the ability to increase the level of high-density lipoprotein cholesterol (HDL cholesterol), it is of obvious scientific interest to study the potential pleiotropic effects of sodium 2-((4-phenyl-5-(thiophene-3-ylmethyl)-1,2,4-triazole-3-yl)thio)ethanoate under experimental hyperlipidemia. In particular, the study of its effect on key biomarkers of inflammation, coagulation state, ischemic myocardial injury and endothelial function can reveal new mechanisms of action of the compound and the potential for its use as a drug for the treatment of atherosclerosis.

The aim *of the study is to study the effect of sodium 2-((4-phenyl-5-(thiophene-3-ylmethyl)-1,2,4-triazole-3-yl)thio)ethanoate compound on pleiotropic biomarkers under experimental hyperlipidemia.*

Materials and methods. *The study of the biological activity of sodium salt 2-((4-phenyl-5-(thiophene-3-ylmethyl)-1,2,4-triazole-3-yl)thio) ethanoic acid was conducted on 20 white nonlinear rats weighing 160–310 g, obtained from the nursery of the Institute of Pharmacology and toxicology of the National Academy of Medical Sciences of Ukraine. Experiments were performed on the basis of a certified vivarium and the Department of Clinical Pharmacy, Pharmacotherapy, Pharmacognosy and Pharmaceutical chemistry of Zaporizhia State Medical and Pharmaceutical University. The animals were kept under standard conditions in accordance with the requirements of EU directive 2010/63/EC and order of the Ministry of Education and Science No. 249 of 01.03.2012. Experimental hyperlipidemia was modeled by administration of cholesterol and ergocalciferol for 5 days. Serum levels of eNOS (enzyme immunoassay), CRP, MV-CPK, and D-dimer (ACCENT-200 biochemical analyzer, corresponding reagents produced by Cloud-Clone, Cormay, and Vector-best) were determined.*

Results. *In the experimental hyperlipidemia model, a decrease in eNOS levels and an increase in CRP, D-dimer, and MV-CPK in rat blood serum were observed, which indicated endothelial dysfunction, inflammation, activation of the hemostatic system, reduction of ischemic myocardial damage, and thrombotic processes. Administration of sodium 2-((4-phenyl-5-(thiophene-3-ylmethyl)-1,2,4-triazole-3-yl)thio) ethanoate significantly increased eNOS activity (by 236.46%), exceeding the effect of atorvastatin and improving the functional state of the endothelium. The level of D-dimer when using the test compound decreased by 34.39%, which was more effective compared to atorvastatin and indicated a decrease in the activation of the hemostatic system. A significant decrease in MV-CPK activity and CRP levels was also recorded, which confirms reduction in ischemic myocardial damage and anti-inflammatory properties of the compound. Thus, the studied compound shows pronounced pleiotropic effects, in particular, endothelioprotective, antithrombotic, metabolitotropic and anti-inflammatory.*

Conclusions. *The sodium compound 2-((4-phenyl-5-(thiophene-3-ylmethyl)-1,2,4-triazole-3-yl)thio)ethanoate exhibits pronounced endothelioprotective and antithrombotic activity, which was confirmed by an increase in eNOS levels and a decrease in the D-dimer. Its anti-inflammatory and reduces ischemic myocardial damage have been established. The studied compound was not inferior in strength of pharmacodynamic effects to the reference drug atorvastatin.*

Key words: *1,2,4-triazoles, hyperlipidemia, pleiotropic effects.*

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ДОСЛІДЖЕННЯ ПЛЕЙОТРОПНИХ ЕФЕКТІВ НАТРІЮ 2-((4-ФЕНІЛ-5-ТІОФЕН-3-ІЛМЕТИЛ)-1,2,4-ТРИАЗОЛ-3-ІЛ)ТІО) ЕТАНОАТУ В УМОВАХ ЕКСПЕРИМЕНТАЛЬНОЇ ГІПЕРЛІПІДЕМІЇ

Вступ. *Атеросклероз залишається однією з провідних глобальних медичних проблем, яка лежить в основі більшості серцево-судинних захворювань, зокрема ішемічної хвороби серця, інсульту та периферичної артеріальної хвороби. За даними*

Всесвітньої організації охорони здоров'я, серцево-судинні захворювання є основною причиною смертності у світі: щороку вони забирають життя приблизно 17,9 мільйона людей, що становить близько 32 % усіх випадків смерті. З них понад 85 % спричинені саме інфарктами міокарда й інсультами, більшість з яких розвиваються на тлі атеросклеротичних змін судин. У розвинених країнах атеросклероз виявляється у понад 50 % чоловіків і близько 40 % жінок віком понад 50 років, і ця тенденція зберігається, попри успіхи в профілактиці та лікуванні. В Україні, за офіційними даними МОЗ, серцево-судинні захворювання становлять понад 65 % у структурі смертності, а кількість ускладнень, зумовлених атеросклерозом, щороку зростає.

У зв'язку із цим надзвичайно важливим є поглиблене вивчення механізмів розвитку атеросклерозу та виявлення чутливих біомаркерів, які дають можливість не лише раннього виявлення патологічних змін, а й точного прогнозування ризику ускладнень. Особливу цінність мають так звані плейотропні біомаркери – молекули, які одночасно відображають кілька ключових патофізіологічних процесів, як-от запалення (СРБ), активація системи гемостазу (Д-дімер), ішемічного пошкодження міокарду; (МВ-КФК) та функціональний стан ендотелію (eNOS).

У цьому контексті, з огляду на виявлену у «сполуки лідера» істотну гіпохолестеринемічну та гіпотригліцеридемічну дію, а також здатність підвищувати рівень холестерину ліпопротеїнів високої щільності (ХС ЛПВЩ), цілком очевидний науковий інтерес становить вивчення потенційних плейотропних ефектів натрію 2-((4-феніл-5-(тіофен-3-ілметил)-1,2,4-триазол-3-іл)тіо) етаноату в умовах експериментальної гіперліпідемії. Зокрема, дослідження його впливу на ключові біомаркери запалення, коагуляційного стану, ішемічного пошкодження міокарду та ендотеліальної функції може розкрити нові механізми дії сполуки та потенціал її використання як багатофакторного засобу профілактики або терапії атеросклерозу.

***Метою** дослідження є: вивчення дії сполуки натрій 2-((4-феніл-5-(тіофен-3-ілметил)-1,2,4-триазол-3-іл)тіо) етаноату на окремі плейотропні біомаркери, в умовах експериментальної гіперліпідемії.*

***Матеріали та методи.** Дослідження біологічної активності натрієвої солі 2-((4-феніл-5-(тіофен-3-ілметил)-1,2,4-триазол-3-іл)тіо)етанової кислоти проведено на 20 білих нелінійних щурах масою 160–310 г, отриманих з розпліднника Інституту фармакології та токсикології АМН України. Експерименти виконували на базі атестованого віварію та кафедри клінічної фармації, фармакотерапії, фармакогнозії та фармацевтичної хімії; Запорізького державного медико-фармацевтичного університету. Тварини утримувалися в стандартних умовах, згідно з вимогами Директиви ЄС 2010/63/ЄС та наказу МОН № 249 від 01.03.2012. У сироватці крові визначали рівні eNOS (імуноферментний метод), СРБ, МВ-КФК і Д-дімеру (біохімічний аналізатор ACCENT-200, відповідні реагенти виробництва Cloud-Clone, Cormay, Вектор-Бест).*

***Результати дослідження.** У моделі експериментальної гіперліпідемії спостерігалося зниження рівня eNOS та підвищення показників СРБ, Д-дімеру й МВ-КФК у сироватці крові щурів, що свідчить про ендотеліальну дисфункцію, запалення, ішемічне пошкодження міокарду та тромботичні процеси;введення натрію 2-((4-феніл-5-(тіофен-3-ілметил)-1,2,4-триазол-3-іл)тіо) етаноату суттєво підвищувало активність eNOS (на 236,46 %), перевищуючи ефект аторвастатину. Рівень Д-дімеру в разі застосування досліджуваної сполуки знижувався на 34,39 %, що було ефективніше порівняно з аторвастатином. Також зафіксовано істотне зниження активності МВ-КФК і рівня СРБ, що підтверджує зменшення ішемічного пошкодження міокарду та протизапальні властивості сполуки. Таким чином, досліджувана сполука демонструє виражені плейотропні ефекти, зокрема ендотеліопротекторну, антитромботичну, протизапальну дію та зменшує ішемічне пошкодження міокарду.*

***Висновки.** Сполука натрію 2-((4-феніл-5-(тіофен-3-ілметил)-1,2,4-триазол-3-іл)тіо) етаноату демонструє виражену ендотеліопротекторну та антитромботичну активність, що підтверджується підвищенням рівня eNOS та зниженням Д-дімеру. Встановлено її протизапальні властивості, а також здатність зменшувати ішемічне пошкодження міокарду. Загалом сполука проявляє багатовекторну плейотропну дію, не поступаючись ефективністю аторвастатину.*

***Ключові слова:** 1,2,4-тріазоли, гіперліпідемія, плейотропні ефекти.*

Actuality. Atherosclerosis remains one of the leading global medical problems underlying most cardiovascular diseases, in particular coronary heart disease, hypertension, stroke, obliterating atherosclerosis of the lower extremities (Віничук та ін., 2015; Mishra et al., 2025). According to the World Health Organization, cardiovascular diseases are the leading cause of death in the world: they claim the lives of approximately 17.9 million people each year, accounting for about 32% of all deaths. Of these, more than 85% are caused by myocardial infarctions and strokes, most of which develop against the background of atherosclerotic vascular changes (Kawai et al., 2024; Kazymyrko et al., 2022). In developed countries, atherosclerosis occurs in more than 50% of men and about 40% of women over the age of 50, and this trend continues despite advances in prevention and treatment. In Ukraine, according to official data of the Ministry of Health, cardiovascular diseases account for more than 65% of the mortality structure, and the number of complications caused by atherosclerosis is growing every year.

In this regard, an in-depth study of the mechanisms of atherosclerosis development and the identification of sensitive biomarkers that allow not only early detection of pathological changes, but also accurate prediction of the risk of complications is extremely important (Murphy et al., 2020). Of particular value are so – called pleiotropic biomarkers-molecules that simultaneously reflect several key pathophysiological processes, such as inflammation, thrombosis, endothelial dysfunction, and energy imbalance (Okyay, 2021).

D-dimer as a product of fibrin degradation is an indicator of activation of Hemostasis and fibrinolysis, and its increase may indicate a hypercoagulation state that contributes to thrombosis against the background of atherosclerotic changes (Carolyne Elbaz, 2024). CRP, known as a sensitive marker of systemic inflammation, plays an important role in the formation of atherosclerotic plaques and their instability (Kaptoge et al, 2010; Belenichev et al, 2022). The indicator of energy production – MV-CPK – is traditionally considered in the context of

myocardial damage, but its changes may reflect deeper disorders of cellular metabolism that accompany the progression of atherosclerosis (Farid Ghorbaninezhad et al., 2022). Special attention should be paid to eNOS, a key enzyme in the synthesis of nitrogen oxide (NO), which regulates vascular tone, inhibits platelet aggregation and counteracts inflammation. Decreased Enos activity or expression is considered one of the earliest markers of endothelial dysfunction, which is a critical stage in the development of atherosclerotic lesions (Widmer et al., 2014; Silva et al., 2006; Бондар та ін., 2019). The study of the relationships between these pleiotropic indicators – D-dimer, CRP, MV-CPK, and eNOS – allows us to go deeper.

In previous work, we identified a "leader compound" among newly synthesized thiophene derivatives of 1,2,4-triazoles, namely Sodium 2-((4-phenyl-5-(thiophene-3-ylmethyl)-1,2,4-triazole-3-yl)thio)ethanoate. This compound demonstrated significant hypocholesterolemic and hypotriglyceridemic effects, as well as the ability to increase the level of high-density lipoprotein cholesterol in experimental hyperlipidemia, as well as very low toxicity (Хільковець, 2024; Khilkovets, Bilai, 2023). That is why the study of potential pleiotropic effects is of obvious scientific interest, in particular, the study of its effect on key biomarkers of inflammation, coagulation state, ischemic myocardial damage and endothelial function can reveal new mechanisms of action of the compound and the potential for its use as a drug for the treatment of atherosclerosis.

The aim of this study is to study the effect of sodium compound 2-((4-phenyl-5-(thiophene-3-ylmethyl)-1,2,4-triazole-3-yl)thio)ethanoate in experimental hyperlipidemia on pleiotropic effects .

Materials and methods of research. Studies of the biological activity of the sodium salt of 2-((4-phenyl-5-(thiophen-3-ylmethyl)-1,2,4-triazol-3-yl)thio)ethanoic acid were conducted on the basis of the Educational and Scientific Medical Laboratory Center with a Vivarium of the Zaporizhia State Medical and Pharmaceutical University. The center has certification from the State Expert Center of the Ministry of Health of Ukraine (certificate No. 181/23), which confirms compliance with the assessment requirements, and also certifies its technical competence and ability to perform measurements in the field of legally regulated metrology. Additionally, studies were performed at the Department of Clinical Pharmacy, Pharmacotherapy, Pharmacognosy and Pharmaceutical Chemistry under the supervision of Doctor of Medical Sciences, Professor I.M. Bilay and Doctor of Biological Sciences, Professor I.F. Belenichev, Head of the Department of Pharmacology and Medical Formulation with a course in Normal Physiology. Studies of specific pharmacological activity were conducted on 20 white nonlinear rats weighing 160–310 g in accordance with bioethical standards (protocol of the Bioethics Commission No. 11 dated November 26, 2020). The animals were obtained from the nursery of the State Institution "Institute of Pharmacology and Toxicology of the National Academy of Medical Sciences of Ukraine" and were kept in standard conditions with free access to water, balanced diet and natural light.

All procedures were carried out in accordance with the "Regulations on the Use of Animals in Biomedical Research", "Rules for Preclinical Safety Assessment of Medicinal Products (GLP)", methodological recommendations of the State Center of the Ministry of Health of Ukraine and taking into account the "General Ethical Principles of Animal Experiments", consistent with the provisions of the "European Convention for the Protection of Vertebrate Animals", the resolution of the First National Congress on Bioethics and the requirements of the Bioethics Commission of the ZDMFU. Euthanasia was carried out in accordance with the methodological recommendations for the withdrawal of animals from the experiment.

The test compound was administered intragastrically as an aqueous suspension (0.3–0.7 ml of distilled water with Tween-80) at a dose of 1/10 of the LD_{50} determined in the acute toxicity study (Bilai, 2023). On the sixth day after anesthesia with ethyl ether, blood was collected from the aortic bifurcation and the heart was isolated.

Experimental hyperlipidemia ("vitamin" model) was reproduced according to the method of Yousufzai S.Y.K., Siddiqi M. by five-fold intraperitoneal administration of a mixture of cholesterol with 0.125% oil solution of ergocalciferol at a dose of 350,000 U/kg (0.8 ml/kg). Animals were divided into four groups: intact, pathology control (cholesterol + vitamin D_2), control with a reference drug (atorvastatin 10 mg/kg) and a group with the test compound. Blood was centrifuged for 20 minutes at 1500 rpm in an Eppendorfcentrifuge 5810 R centrifuge (Germany). The resulting blood serum was poured into 0.5 ml Eppendorf tubes and stored in a freezer NZ – 280/75 A at –40˚C. Subsequently, the samples were thawed and used for biochemical studies – 0.2 ml, for enzyme-linked immunosorbent assays – 0.1 ml.

In the blood serum of rats, the levels of endothelial NO synthase (eNOS) were determined by the enzyme-linked immunosorbent assay using Cloud-Clone Corporation reagents on the Immunochem-2200 analyzer (USA). The level of C-reactive protein (CRP) was determined by the immunoturbidimetric method using a set of reagents manufactured by Cormay on the

ACCENT-200 biochemical analyzer (Poland). Using the same device and Cormay kits, the content of malondialdehyde (MV-CPK) was investigated, and the level of D-dimer was determined using reagents from the Vector-Best company on the same biochemical analyzer. The results obtained were processed by the statistical method STATISTICA®forWindows 13.00 (StatsoftInc., USA, license NjPZ8041382130ARCN10 – J).

Results. As part of the study, the pleiotropic properties of the compound sodium 2-((4-phenyl-5-(thiophene-3-ylmethyl)-1,2,4-triazole-3-yl)thio)ethanoate were analyzed with special attention to its effect on the main pathophysiological mechanisms involved in the formation of atherosclerosis.

In particular, to assess the hemostatic activity of the compound, the concentration of D-dimer in the blood serum was determined, which acts as an important indicator of the formation of a fibrin clot and the subsequent fibrinolysis process. An increase in the level of D-dimer indicated activation of the blood coagulation system and the presence of thrombotic changes characteristic of atherosclerotic vascular damage. The state of vascular endothelium, as a critical factor in maintaining vascular homeostasis, was assessed by the level of eNOS activity in the blood serum. A decrease in the activity of this enzyme indicates a violation of the synthesis of nitrogen oxide, the main vasodilator with anti – inflammatory and antithrombotic properties.

Chronic vascular inflammation, as one of the key links in atherogenesis, was determined by the content of CRP, a generally recognized sensitive biomarker of the systemic inflammatory process associated with the risk of cardiovascular events.

Assessment of the energy supply of myocardial contractile function was performed by measuring the activity of the cardiac isoform MV-CPK in blood serum. This enzyme catalyzes the reactions of ATP resynthesis in conditions of high energy demand of the heart muscle, and its changes may indicate a violation of cellular metabolism in the myocardium.

As a result of the study, it was revealed (table) that in experimental hyperlipidemia, eNOS activity decreased in rat blood serum (by 52.89%), which indicated inhibition of no synthesis and protective properties of the endothelium, which plays an important role in maintaining vascular hemostasis. At the same time, MV-CPK activity increased in rat blood serum (by 146.88%), which indicated a decrease in hyperproduction of heart muscle contractions. Under this condition, the level of D-dimer, a marker of thrombosis, also increased in the blood serum (by 50.65%). At the same time, an increase in CRP levels (by 183.76%) in the control group with hyperlipidemia indicated activation of a chronic inflammatory process in the vascular wall.

Thus, simulated experimental hyperlipidemia was accompanied by a decrease in serum eNOS levels. At the same time, an increase in serum D-dimer, CRP, and MV-CPK activity was observed.

As a result of the study, it was revealed that in experimental hyperlipidemia, the activity of eNOS in the blood serum was most significantly increased when sodium 2-((4-phenyl-5-(thiophene-3-ylmethyl)-1,2,4-triazole-3-yl)thio)ethanoate was administered to rats (by 236.46%), which significantly exceeded the strength of this indicator atorvastatin (by 144.18%) and the Intact Group. This fact indicates the importance of maintaining vascular homeostasis in rats when the leader compound under study is administered.

The antithrombotic activity of sodium 2-((4-phenyl-5-(thiophene-3-ylmethyl)-1,2,4-triazole-3-yl)thio) ethanoate was indicated by a significant decrease in the level of D-dimer in the blood serum by 34.39%, which significantly exceeded the strength of this effect of the reference drug (by 22.3%) and reached the normal level (124.42 ± 2.64 ng/ml and 125.88 ± 4.99 ng/ml).

Table

Effect of sodium 2-((4-phenyl-5-(thiophene-3-ylmethyl)-1,2,4-triazole-3-yl)thio)ethanoate on pleiotropic effects in rat serum in experimental hyperlipidemia

Group	eNOS (ng/ml)	D-dimer (ng/ml)	MB-CPK (U/L)	CRP (mg/L)
Intact group, M ± m	33,54 ± 1,47	125,88 ± 4,99	19,20 ± 0,92	6,28 ± 0,25
Control group, M ± m, Δ % P	15,80 ± 0,75 –52,89% p ≤ 0,05	189,64 ± 1,46 +50,65% p ≤ 0,05	47,40 ± 0,80 +146,88% p ≤ 0,05	17,82 ± 0,25 +183,76% p ≤ 0,05
Atorvastatin, M ± m, Δ %	38,58 ± 3,43* +144,18	147,34 ± 5,94* –22,31	11,80 ± 0,77* –75,11	12,06 ± 0,79* –32,32
Compound, M ± m, Δ %	53,16 ± 3,54*/** +236,46	124,42 ± 2,64*/** –34,39	13,40 ± 0,64*/** –71,73	8,04 ± 0,37*/** –54,88

Note: p < 0,05-reliability in relation to the Intact Group; * – reliability in relation to the control group; ** – reliability in relation to atorvastatin.

At the same time, a significant probable decrease in MV-CPK levels was observed in rat blood serum when sodium 2-((4-phenyl-5-(thiophene-3-ylmethyl)-1,2,4-triazole-3-yl)thio)ethanoate (by 71.73%) and atorvastatin (by 75.11%) was administered, which was lower than normal (19.20 ± 0.92 IU/L).

The introduction of the test compound most significantly reduced the CRP content (by 54.88%), indicating the presence of an anti-inflammatory effect in the substance. Atorvastatin moderately reduced the level of this molecular marker of inflammation by 32.32% and was significantly inferior in strength to sodium 2-((4-phenyl-5-(thiophene-3-ylmethyl)-1,2,4-triazole-3-yl)thio) ethanoate.

Discussion. The results of the study indicated the multifaceted pharmacological potential of the sodium 2-((4-phenyl-5-(thiophene-3-ylmethyl)-1,2,4-triazole-3-yl)thio)ethanoate compound under experimental hyperlipidemia. It affected the key pathogenetic links of atherosclerosis, in particular, it contributed to the normalization of vascular homeostasis, improved the functional state of the endothelium and demonstrated angioprotective properties.

Due to a decrease in the activity of markers of inflammation and thrombosis, the studied compound showed a pronounced anti-inflammatory and antithrombotic effect. There was also a stabilization of energy metabolism in the myocardium, which indicated a positive effect on the functional reserve of the heart muscle.

The combination of identified effects-antithrombotic, endotheliotropic, anti – inflammatory and reduces myocardial damage us to consider the compound as a promising agent with pleiotropic action for complex correction of disorders characteristic of the atherosclerotic process. In most indicators, it was not inferior, and sometimes even exceeded the effectiveness of the reference drug.

Conclusions

1. It was found that the sodium compound2-((4-phenyl-5-(thiophene-3-ylmethyl)-1,2,4-triazole-3-yl) thio)ethanoate showed pronounced endotheliotropic and antithrombotic activity, which was manifested in a significant increase in eNOS activity and a decrease in the level of D-dimer in the blood serum of rats with experimental hyperlipidemia.

2. Test substance has an anti-inflammatory and reduces ischemic myocardial damage, which was indicated by a decrease in the level of C-reactive protein, as well as a decrease in the activity of MV-CPK.

3. The sodium compound 2-((4-phenyl-5-(thiophene-3-ylmethyl)-1,2,4-triazole-3-yl)thio)ethanoate showed pleiotropic activity not inferior in effectiveness to the reference drug atorvastatin.

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