Study on the influence of 7-β-hydroxy-γ-aryloxypropylxanthinyl-8-thioalkanic acid derivatives on the lipid metabolism in experiment

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A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation; D – writing the article; E – critical revision of the article; F – final approval of the article

Key words: xanthines, hyperlipidemia, lipoproteins.

Statin side effects are not a rare occurrence, in particular dyspeptic disorders, insomnia, headache, skin erythema, rash are often noted. All of this determines scientists to find new effective and low-toxic hypolipidemic agents. Various natural and synthetic xanthine derivatives have been recognized as therapeutically potential compounds and reported to control various diseases. Therefore, the study of new xanthine derivatives and their hypolipidemic effects, which would have a significant therapeutic effect with minimal side effects, is relevant.

The aim of the study was to examine the effect of 7-β-hydroxy-γ-aryloxypropylxanthinyl-8-thioalkanic acid derivatives on lipido-gram parameters in experimental laboratory rats.

Materials and methods. The objects of the study were 7-β-hydroxy-γ-aryloxypropylxanthinyl-8-thioalkanic acid derivatives. The experiments were performed in white laboratory Wistar rats weighing 180–220 g. Experimental modeling of hyperlipidemia – tween model: intraperitoneal administration of tween-80 at a dose of 200 mg/100 g body weight. The test compounds were administered orally, simultaneously with tween, at a dose of 1/10 of LD₅₀ (previously calculated by Prozorovsky express method) for 6 days. The following indicators of lipidogram were determined: total cholesterol (TC), high-density lipoprotein cholesterol (HDL cholesterol), low-density lipoprotein cholesterol (LDL cholesterol), triglycerides (TG) and atherogenic index of plasma: TC/HDLC = HDL cholesterol / LDL cholesterol. The experiments were carried out with respect to Bioethical rules and norms.

Results. The studies have shown data on the hypolipidemic activity of 7-β-hydroxy-γ-aryloxypropylxanthinyl-8-thioalkanic acid derivatives. According to the conditional efficiency index Σ, which included the overall percentage of the following indicators – total cholesterol, low-density lipoprotein cholesterol and triglycerides, the leading compounds were 2439 (87.47%), 6047 (82.30%). The reference drug atorvastatin had a value of 82.98%.

Conclusions. The major compound was 2439 identified among all compared to the control group. The prospect of further research is a more detailed study on the ability of xanthine derivatives to exhibit hypolipidemic effects and to influence oxidative stress in various hyperlipidemic models.
Исследование влияния производных 7-β-гидрокси-γ-арилоксипропилксантинил-8-тиоалкановых кислот на показатели липидограммы в эксперименте

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Побочные эффекты статинов — нередкое явление, часто отмечают диспепсические расстройства, бессонницу, головную боль, зрителную дрожь, сыпь. Это мотивирует ученых к поиску новых эффективных и малоотоксических гиполипидемических средств. Различные природные и синтетические производные ксантина признаны терапевтически сильнодействующими соединениями, описаны как средства для борьбы с различными заболеваниями. Поэтому изучение новых производных ксантина и их гиполипидемических эффектов, которые имели бы значительный терапевтический эффект при минимальном побочном действии, является актуальным.

Цель работы — изучение влияния производных 7-β-гидрокси-γ-арилоксипропилксантинил-8-тиоалкановых кислот на показатели липидограммы в эксперименте на лабораторных крысах.

Материалы и методы. Объекты исследования — производные 7-β-гидрокси-γ-арилоксипропилксантинил-8-тиоалкановых кислот. Эксперименты проведены на белых лабораторных крысах линии Вистар массой 180–220 г. Экспериментальное моделирование гиперлипидемии — твиновая модель: внутрибрюшинное введение твин-80 в дозе 200 мг/100 г веса.

Исследуемые вещества вводили внутрь одновременно с твином в течение 6 дней в дозе 1/10 от LD₅₀ моделирование гиперлипидемии — твиновая модель: внутрибрюшинное введение твин-80 в дозе 200 мг/100 г веса.

Результаты. В результате исследований получены данные о гиполипидемической активности производных 7-β-гидрокси-γ-арилоксипропилксантинил-8-тиоалкановых кислот. По данным эксперимента, по показателю условного индекса эффективности Σ, который включал сумму процентов таких показателей, как общий холестерин, холестерин липопротеидов низкой плотности (ХС ЛПНП), холестерин липопротеидов низкой плотности (ХС ЛПНП), трiglyceridy (ТГ) и холестериновый индекс атерогенности (ХИА) по формуле: ХИА = ОХС − ХС ЛПВП / ХС ЛПВП. В ходе эксперимента придерживались биохимических правил и норм.

Выводы. Преобладающее по сравнению с контрольной группой соединение — вещество 2439. Перспективы дальнейших исследований предусматривают более глубокое изучение производных ксантина относительно способности проявлять гиполипидемическое действие и влиять на показатели оксидативного стресса на разных моделях гиперлипидемии.
Table 1. Structural formula of 7-β-hydroxy-γ-aryloxypropylxanthinyl-8-thioalkane acid derivatives

| №  | Compound code | R   | R’  | n   | R2  |
|----|---------------|-----|-----|-----|-----|
| 1  | 2439          | H   | CH3-o| 1   | NH2 |
| 2  | 2486          | H   | CH3-o| 1   | OH  |
| 3  | 2487          | H   | CH3-o| 2   | OH  |
| 4  | 5705          | CH3 | OCH3-η| 1   | OH  |
| 5  | 6042          | H   | CH3-η| 1   | OH  |
| 6  | 6043          | H   | CH3-η| 1   | OH  |
| 7  | 6047          | CH3 | CH3-η| 1   | OH  |
| 8  | 6049          | CH3 | CH3-η| 1   | OH  |
| 9  | 6286          | CH3 | CH3-η| 1   | OH  |
| 10 | 8402          | H   | C(CH3)3-η| 1 | OH  |
| 11 | 8403          | CH3 | C(CH3)3-η| 1 | OH  |

professionals and consumers. Therefore, the study on new xanthine derivatives and their hypolipidemic effects, which would have a significant therapeutic effect with minimal side effects, is relevant [9].

**Aim**

The aim of the study was to investigate the effects of 7-β-hydroxy-γ-aryloxypropylxanthinyl-8-thioalkane acid derivatives on the lipid profile in laboratory rats experimentally.

**Materials and methods**

The objects of the study were 11 derivatives of 7-β-hydroxy-γ-aryloxypropylxanthinyl-8-thioalkane acids (Table 1), synthesized at the Department of Biological Chemistry of Zaporizhzhia State Medical University headed by Professor M. I. Romanenko, which are promising in search for hypolipidemic medicines. A number of modern physicochemical methods of analysis confirmed the structure of the compounds: IR-, PMR-spectroscopy, mass-spectrometry. The experiments were performed in 112 white laboratory Wistar rats aged between 6-8 months, weighing 180–220 g. The rats were divided into groups: group 1 (normal) – intact animals; group 2 (control) – animals with experimental hyperlipidemia (without treatment); groups 3–13 – animals with hyperlipidemia and tested compounds; group 14 – comparison drug – atorvastatin. Each group consisted of 8 animals. The rats were obtained from the animal farm of the State Institution “Institute of Pharmacology and Toxicology of the Academy of Medical Sciences of Ukraine”. The animals were kept on the standard diet in natural light regime “day and night”.

The tween model as an experimental hyperlipidemia was used: intraperitoneal administration of the tween-80 in a dose of 200 mg/100 g body weight. Tested compounds were administered orally, simultaneously with tween, for 6 days. 12 hours after the last administration of tween-80 or tested compounds, the animals underwent laparotomy under ether narcosis, blood was taken from glomerular artery, centrifuged to obtain a serum. This model is advantageous since leads to a rapid (8–10 hours) increase in blood lipids (especially triglycerides) and a decrease in HDL cholesterol. The tested compounds were administered at the dose of 1/10 of the LD₅₀ (previously calculated by the express method of Prozorovsky) [10]. The comparison option was the medicine from the group of statins – atorvastatin (“Atorvastatin” (Ananta Medicare (India)) at the dose of 20 mg/kg body weight.

The level of total cholesterol (TC) was determined by colorimetric, enzymatic methods, diagnostic kits Cormay (Poland) (mmol/l), HDL cholesterol (α-cholesterol) – by colorimetric and precipitating methods, HDL cholesterol – precipitating reagent 1 × 50 ml (Cormay, Poland) (mmol/l), LDL cholesterol – colorimetric and precipitating methods, LDL cholesterol – precipitating reagent 1 × 20 ml (Cormay, Poland) (mmol/l), TG – colorimetric, enzymatic methods, diagnostic kits 150 ml (Cormay, Poland) (mmol/l) and cholesterol atherogenic index (CAI) (conventional units) × according to the formula:

\[
\text{CAI} = \frac{\text{TC} - \text{HDL cholesterol}}{\text{HDL cholesterol}}
\]

Laboratory tests were carried out on the semi-automatic biochemical analyzer “Screen point” (Italy).

For the integral assessment of the derivative hypolipidemic action, the conditional efficacy index Σ (EI) was used representing the generalized value of lipid metabolism (sum of reductions in percentages of TC, LDL cholesterol, TG: EI = TC (%) + LDL cholesterol (%) + TG (%)[11].

During the experiment, the rules and regulations were followed according to the Protocol no. 5 (April 17, 2019) of the Commission on Bioethics Session of ZSMU.

The results of the study were processed on a personal computer using the statistical package of the licensed program Statistica, version 13 (Copyright 1984-2018 TIBCO Software Inc. All rights reserved. License No. JP28041382130ARCN10-J). The normality of the quantitative variables distribution was analyzed using the Shapiro–Wilk test. Descriptive statistics was provided in the form of a median with interquartile range – Me [Q₇₅-Q₇₅], because the parameters had the distribution different from the normal. The Mann–Whitney test was used to find differences between the groups. A level of P < 0.05 was considered statistically significant.

**Results**

The studies have demonstrated the data on the hypolipidemic activity of 7-β-hydroxy-γ-aryloxypropylxanthinyl-8-thioalkane acid derivatives (Tables 2, 3).

The highest hypolipidemic activity (TC) relative to the control group was shown by compounds 6043 (27.5 %), 6047 (27.1 %) and 2439 (25.3 %). The value of comparison drug atorvastatin, relative to the control group, was 23.6 %. The most effective derivatives to increase the HDL-cholesterol levels were: 8402 (67.1 %), 6286 (54.4 %), 6042 (50.6 %), compounds with moderate
activity – 2486 (33.0 %) and 2439 (30.4 %), relative to the control group.

The best results in the lowering of LDL-cholesterol relative to the control group were shown by derivatives: 8402 (45.0 %), 6043 (36.3 %) and 2439 (34.3 %). Compounds 6047 (30.2 %) and 2486 (28.1 %) exhibited moderate activity in respect of this value.

The most effective hypotriglyceridemic activity was shown by compounds 2439 (27.9 %), 2486 (26.9 %), 6047 (25.0 %). Derivative 6286 was characterized by moderate activity (19.2 %) relative to the control group.

When assessing the CAI, the most active compounds, compared to the control group, were 8402 (82.1 %), 6049 (69.3 %), 6043 (64.4 %), 2439 (63.2 %), 2486 (60.4 %), 2487 (53.4 %). Compounds 6047 (56.7 %) and 6286 (45.0 %) showed moderate activity, compound 6042 (33.4 %) had a slight effect.

By the generalized value of lipid metabolism indicator – EI Σ, the best hypolipidemic effect, relative to the control group, had compounds 2439 (87.5 %), 6047 (82.3 %). Compounds 2486, 6043, 8402 and 6286 were characterized by moderate effect 78.2 %, 69.0 %, 57.0 % and 51.7 %, respectively. Compound 8403 had a slight effect (31.7 %). The comparison drug atorvastatin had a value of 63.0 %.

Analyzing the dependence of lipid profile on the structure of the tested derivatives, we were able to draw some preliminary conclusions. O-methyl-substituted derivatives of 3-methylxanthine (compounds 2439, 2486) and theophylline (compound 6047) showed the highest activity. Moreover, the amide (compound 2439) was more active than acids (derivatives 2486 and 6047). Elongation of the carbon chain in the theoilactic acid residue at position 8 of the xanthine molecule practically led to loss of hypocholesterolemic and hypolipidemic effect (compound 2487). Similar effects occurred when the methyl was replaced by the methoxy group (compounds 5705 and 6042). The significant decrease in hypolipidemic activity was observed in a large aromatic substitution (compounds 8402, 8403). For the more detailed analysis of the dependence in the series "structure-action", it is necessary to expand significantly the chemical library of the tested compounds.

Discussion

In recent years, heterocyclic compounds, including xanthine derivatives, have received considerable attention due to their importance in pharmacological research. In particular, the data obtained by us are confirmed by the research results of D. H. Ivanchenko and N. Singh: the use of 3,7-dimethylxanthine (theobromine) is associated with a reduced risk of cardiovascular disease. The main rationale for this is that theobromine has a beneficial effect on fastening serum lipids. It can be associated with inhibition of...
phosphodiesterases, which, by destroying cellular cyclic adenosine monophosphate (cAMP), increase the activity of ABCA1 (a gene encoding the protein that regulates cellular cholesterol and phospholipid homeostasis), which plays a role in a removal of cholesterol from macrophage foam cells to apolipoprotein A-I (the main apolipoprotein of HDL-cholesterol). Therefore, theobromine can increase HDL-cholesterol levels and have a cardioprotective effect [12–15].

Another generalized study that significantly confirms our results was that the compound KMUP-1 (7-β-[4-(2-chlorophenyl)piperazinyl]ethyl]-1,3-dimethylxanthine), by inhibiting phosphodiesterase, modulates G-protein-coupled receptors (GPCR) to reduce hyperlipidemia and lose weight. KMUP-1 reduced the accumulation of triglycerides, indicating inhibition of adipogenesis in cells, and the expression of mitogen-activated protein kinase (MAPK) and immunoreactivity at the stage of adipogenesis. Appropriate biochemical reactions contributed to the inhibition of adipocyte differentiation. These data suggest that KMUP-1 may inhibit hyperadiposis in adipocytes [16].

In addition, literature data confirms that KMUP-1 can cause the decrease in liver fat. However, the mechanisms of KMUP-1 action in obesity-induced steatohpatitis remain unclear. Prolonged administration of KMUP-1 to mice on a high-fat diet reduced body weight, triglyceride and glucose levels, which is completely correlated with our data. Furthermore, KMUP-1 reduced the amount of MMP-9 (matrix metalloproteinase 9 — extracellular zinc-dependent endopeptidase capable of destroying extracellular matrix protein) and reactive oxygen species (ROS), and increased the content of anti-inflammatory cytokine IL-10 in the liver of mice on the high-fat diet. Thus, it was shown that KMUP-1 reduces the accumulation of lipids in liver tissue, which is the promising aspect in the fatty liver disease treatment [17–19].

What is more, according to X. Zhu, it was shown that incubation of preadipocytes with a solution containing xanthines significantly reduced the incorporation of triglycerides during adipogenesis without affecting cell viability. Finally, the active study of xanthine derivatives with hypolipidemic activity continues [16].

Conclusions

1. The studies provided the data on the hypolipidemic activity of 7-β-hydroxy-γ-aryloxypropylxanthinyl-8-thioalane acid derivatives. The highest hypolipidemic activity (TC) relative to the control group was shown by compounds 6043 (27.5 %), 6047 (25.8 %) and 6048 (24.3 %). The most effective derivatives to increase the HDL-cholesterol levels were: 8402 (67.1 %), 6286 (54.4 %), 6042 (50.6 %).

2. The best results in LDL-cholesterol lowering relative to the control group were shown by derivatives: 8402 (45.0 %), 6043 (42.5 %), 6286 (36.3 %) and 2439 (34.3 %). The most effective hypotriglyceridemic activity was shown by the compounds 2439 (27.9 %), 2486 (26.9 %), 6047 (25.0 %).

3. When assessing the CAI, the most active compounds, relative to the control group, were 8402 (82.1 %), 6049 (69.3 %), 6043 (64.4 %), 2439 (63.2 %), 2486 (60.4 %), 2487 (53.4 %). By the generalized value of lipid metabolism indicators – EI Σ, the best hypolipidemic effect, relative to the control group, had compounds 2439 (87.5 %), 6047 (82.3 %).

4. The results of experimental studies clearly indicate the feasibility and prospects for further search on original lipid-lowering drugs among xanthine derivatives.

The prospect for further research is the study of 7-β-hydroxy-γ-aryloxypropylxanthinyl-8-thioalane acid derivatives on the ability to enhance a hypolipidemic effect and to influence the values of oxidative stress in various models of hyperlipidemia.

Conflicts of interest: authors have no conflict of interest to declare.

References

[1] Mach, F., Ballantyne, C., Catapano, A. L., Koskinas, K. C., Casula, M., Badimon, L., Chapman, M. J., De Backer, G. G., Delgado, V., Ferencz, B. A., Graham, I. M., Halliday, A., Landmesser, U., Mihaylova, B., Pedersen, T. R., Riccardi, G., Richter, D. J., Sabatine, M. S., Taskinen, M. R., Tokgozoglu, L., ... ESC Scientific Document Group. (2020). 2019 ESC/EAS Guidelines for the management of dyslipidaemias: lipid modification to reduce cardiovascular risk. European Heart Journal, 41(1), 111-188. https://doi.org/10.1093/eurheartj/ehz455

[2] Steffens, D., Braunlage, P., Scheeff, C., Kaisner, M., Hassanein, A., Friel, J., & Rauch-Köhler, U. (2020). PCSK9 inhibitors and cardiovascular outcomes. Expert Opinion on Biological Therapy, 20(1), 35-47. https://doi.org/10.1080/14712598.2020.1877604

Филиатова И. Р., д-р мед. наук, професор каф. біологічної фармацевтичної хімії, Запорізький державний медичний університет, Україна.
Atherosclerosis in mice.

Liu, L., Nagai, I., Gao, Y., Matsushima, Y., Kawai, Y., & Saya
s13065-017-0294-0

Chemistry Central Journal, 11

cils: pyrimidodiazepines, lumazines, triazolouracil and xanthines.

Article 1345.

in Obese Mice.

Improves Steatohepatitis via MMP-9/IL-10 and Lipolysis via HSL/p-HSL

Chai, C. Y., Dai, Z. K., & Chen, I. J. (2016). Theophylline-Based KMUP-1

Wu, B. N., Kuo, K. K., Chen, Y. H., Chang, C. T., Huang, H. T,

doi.org/10.3892/etm.2016.3975

therapies.

therapy with catechins and caffeine inhibits fat accumulation in 3T3-L1

Zhu, X., Yang, L., Xu, F., Lin, L., & Zheng, G. (2017). Combination

jacc.2018.04.060

of 22 September 2010 on the protection of animals used for scientific

Directive 2010/63/EU of the European Parliament and of the Council

European Parliament, & European Council. (2010, October 20).

https://doi.org/10.14739/2409-2932.2017.1.93430

issues in pharmacy and medicine: science and practice, 10(1), 14-19.

https://doi.org/10.1077/a0126-017-0259-7

Basu, S., Barakwar, D. A., Ramdas, V., Waman, Y., Patel, M., Pan

mand, A., Kumar, S., Tharad, S., Bonagiri, R., Jadhav, D., Mukho

padhyay, P, Prasad, V, Reddy, B. S., Goswami, A., Chaturvedi, S.,

Menon, S., Quraishi, A., Ghosh, I., Dusange, S., Palwai, S., ... Moorkhar, K. A. (2017). A2B adenosine receptor antagonists: Design,
synthesis and biological evaluation of novel xanthine derivatives.

European Journal of Medicinal Chemistry, 127, 386-396. https://doi.

org/10.1016/j.ejmech.2016.11.007

Mikhailchenko, E. K., Aleksandrova, K. V., Levich, S. V., & Sinchenko,

D. M. (2017). Synthesis and physical-chemical properties of 3-β-nordihydroxy-γ-(7-acetic and its derivatives. Current

issues in pharmacy and medicine: science and practice, 10(1), 14-19.

https://doi.org/10.14739/2409-2932.2017.1.93430

Mironov, A. N. (Ed.). (2012). Grif i K. [in Russian].

Ivanchenko, D. G., Romanenko, N. I., & Kornienko, V. I. (2018). Synthesis and Antioxidant Activity of 8-bromo-7-(2-hydroxy-3-aryloxyprop-1-yl)

eksperymentalnii hiperlipidemii

nykh 7-β-hidroksy-γ-(mono- ta dykhlorfenoksy)propilksantyniv pry

non-clinical studies of pharmaceuticals. Part one

Mukhalchenko, E. K., Aleksandrova, K. V., Levich, S. V., & Sinchenko,

D. M. (2017). Synthesis and physical-chemical properties of 3-β-nordihydroxy-γ-(7-acetic and its derivatives. Current

issues in pharmacy and medicine: science and practice, 10(1), 14-19.

https://doi.org/10.14739/2409-2932.2017.1.93430

Ivanchenko, D. G., Romanenko, N. I., & Komienko, V. I. (2018). Synthesis

and Antioxidant Activity of 8-bromo-7-(2-hydroxy-3-aryloxyprop-1-yl) theophyllines. Chemistry of Natural Compounds, 54(3), 532-534. https://doi.

org/10.1007/s40256-017-0259-7

Singh, N., Shreshtha, A. K., Thakur, M., & Patra, S. (2018). Xanthine

scaffold: scope and potential in drug development. Heliyon, 4(10),

Article e00829. https://doi.org/10.1016/heliyon.2018.e00829

Cook, N. R., Mora, S., & Ridker, P. M. (2018). Lipoprotein(a) and Cardiovascular Risk Prediction Among Women.

Cook, N. R., Mora, S., & Ridker, P. M. (2018). Lipoprotein(a) and Cardiovascular Risk Prediction Among Women. Journal of the American College of Cardiology, 72(3), 287-296. https://doi.org/10.1016/j.jacc.2018.04.090

Zhu, X., Yang, L., Xu, F., Lin, L., & Zheng, G. (2017). Combination

therapy with catechins and caffeine inhibits fat accumulation in 3T3-L1 cells. Experimental and Therapeutic Medicine, 13(2), 688-694. https://doi.

org/10.3829/jetr.2016.3975

Wu, B. N., Kuo, K. K., Chen, Y. H., Chang, C. T., Huang, H. T,

Chai, C. Y., Dai, Z. K., & Chen, I. J. (2016). Theophylline-Based KMUP-1

Improves Steatohepatitis via MMP-9/IL-10 and Lipolysis via HSL/p-HSL in Obese Mice. International Journal of Molecular Sciences, 17(8),

Article 1345. https://doi.org/10.3390/ijms17081345

El-Kalayoubi, S. A., Fayad, E. A., & Abdel-Hakeem, A. S., (2017). One

pot synthesis, antimicrobial and antioxidant activities of fused uracils: pyrimidoazepines, lumazines, triazolouracil and xanthisnes. Chemistry Central Journal, 11(1), Article 66. https://doi.org/10.1186/s13065-017-0294-4

Liu, L., Nagai, I., Gao, Y., Matsushima, Y., Kawai, Y., & Saya

ma, K. (2017). Effects of catechins and caffeine on the development of atherosclerosis in mice. Bioscience, Biotechnology, and Biochemistry,

81(10), 1948-1955. https://doi.org/10.1080/09168451.2017.1336418