Study of the Selective Phenolic Inhibitors of Arginase 2 Acute Toxicity

Liliya V. Korokina[1]*, Mikhail V. Pokrovskii[2], Mikhail V. Korokin[2], Olga N. Pokopejko[2], Tatyana G. Pokrovskaia[2], Pavel G. Galenko-Yaroshevsky[2], Alexandr A. Dolzhikov[2], Lyudmila V. Reznichenko[2] and Elena G. Yakovleva[2]

1. PhD in Medicine, Researcher of the Department of Pharmacology and Clinical Pharmacology, Medical Institute, Belgorod State National Research University, 85, Pobedy St., Belgorod, 308015, Russia.
2. Department of Pharmacology, Medical Institute, Belgorod State University, 85, Pobedy St., Belgorod, 308015, Russia.
Correspondence author: Liliya V. Korokina, e-mail: korokina@bsu.edu.ru
Received: 17-05-2019, **Revised:** 13-06-2019, **Accepted:** 15-07-2019, **Published online:** 23-08-2019

How to cite this article: Liliya V. Korokina, Mikhail V. Pokrovskii, Mikhail V. Korokin, Olga N. Pokopejko, Tatyana G. Pokrovskaia, Pavel G. Galenko-Yaroshevsky, Alexandr A. Dolzhikov, Lyudmila V. Reznichenko and Elena G. Yakovleva (2019) Study of the Selective Phenolic Inhibitors of Arginase 2 Acute Toxicity, Journal of International Pharmaceutical Research *46*(4): 258-261

Abstract

Introduction: We have previously shown that the isolated arginase 2 inhibitors have a pronounced endothelioprotective level. A strategy is proposed for the targeted search for isolated arginase 2 inhibitors among compounds of a phenolic nature. Received 3 compounds, isolated arginase inhibitors 2. As a result of the study, the acute toxicity of the compounds obtained was studied.

Objectives of the study: to substantiate the choice of a phenolic compound, a selective arginase 2 inhibitor, which has the lowest acute toxicity among the compounds obtained.

Methods: The experiment was conducted on laboratory mice of both sexes. Substance administered intragastrically. Observation of the animals was carried out for 14 days.

Results: Summarizing the data obtained, it can be concluded that, after intragastric administration to mice, the investigated compounds of phenolic nature under laboratory ciphers KUD259, KUD972, KUD973 are slightly toxic.

Conclusion: As the main candidate for the drug, it is advisable to consider the compound KUD972, which showed the highest LD50 values, and therefore has the lowest toxicity.

Keywords: Acute Toxicity, Arginase Inhibitors 2, Compounds of Phenolic Nature.

Introduction

The basis of modern therapy of arterial hypertension and other cardiovascular diseases is the postulate of the need to correct endothelial dysfunction as an indicator of the adequacy of antihypertensive and other types of treatment. In fact, this means that lowering blood pressure (BP) without normalizing endothelial function cannot be considered a successfully solved clinical problem.

"ADMA-eNOS" as a pharmacological target is of undoubted interest and has been the object of research by pharmacologists [1-6]. Studies have revealed the role of methylated derivatives of L-arginine monomethylarginine (L-NMMA) and asymmetric dimethylarginine (ADMA) as endogenous inhibitors of endothelial NO synthase (eNOS).

Earlier studies have shown that preventing the accumulation and overcoming of inhibition of eNOS by an already established ADMA claims to be one of the ways to pharmacological correction endothelial dysfunction. Endothelium protective properties implemented by reducing the inhibitory effect of ADMA on eNOS agents such as L-arginine [7-8], tetrahydrobiopterin [9], non-selective arginase inhibitor L-norvaline [2, 5, 23], selective arginase II inhibitors [10-12, 25] and so forth, were experimentally confirmed.

Thus, the urgent task of pharmacology is to search for substances that selectively affect the metabolism of nitric oxide, namely, selectively inhibit the enzyme Arginase 2.

Currently, preclinical studies of toxicological safety, pharmacological activity, pharmacokinetics and bioequivalence are an integral part of the study of new drugs [13-22].

We have previously shown that selective arginase 2 inhibitors have a pronounced endothelioprotective effect [12, 24]. A strategy for targeted search for selective arginase 2 inhibitors among compounds of phenolic nature has been proposed. Using the methods of computer simulation and high-tech screening, we obtained 3 compounds, selective arginase inhibitor 2.

Objectives of the study: to substantiate the choice of a phenolic compound, a selective arginase 2 inhibitor, which has the lowest acute toxicity among the compounds obtained.

Materials and Methods

Experiments on the study of acute toxicity were performed on laboratory mice of both sexes. The

studied compounds of phenolic nature KUD259, KUD972, KUD973 were administered intragastrically once to the animals using metal probes with atraumatic smooth olives at the end in five doses. The individual volume of the injected dose for each animal was calculated based on the value of body weight.

Phenolic compounds under laboratory ciphers KUD259, KUD972, KUD973 were administered intragastrically once in doses of 500, 600, 700, 800 and 900 mg / kg. In the study of "acute" toxicity, the clinical observation of each animal was carried out during the first hour after administration of the drug, daily for the next 14 days. The number of dead animals was counted during the experiment.

Daily visually noted deviations in the consumption of food and water by animals in individual cells. At the opening of the dead animals visually inspected the internal organs, noted pathological changes in the color, size, location of organs.

After completion of the experiment according to the method of B.M. Shtabsky counted LD50.

Results

Studies have shown that after intragastric administration to mice of the studied compounds of phenolic nature, in the indicated dosages, the death of animals was recorded.

The death of animals occurred within 5-24 hours after priming. As a result of the study, the average lethal dose was calculated after intragastric administration of phenolic compounds to mice (Tables 1–4).

After the introduction of the studied compounds, the intensity of physical activity decreased on average after 1-2 hours, coordination of movements was disturbed, the skeletal muscle tone decreased, the reaction to tactile, painful, sonic and light stimuli. After 2-24 hours, the frequency of respiratory movements increased, and the depth decreased with a subsequent decrease in the frequency of respiratory movements. The state of the hair and skin patches, did not change during the entire observation period. The amount and consistency of fecal masses, frequency of urination and urine color did not change in all experimental groups during the entire observation period. The surviving animals remained sluggish and sedentary for 1-2 days hours after acute seeding.

Table-1: Data characterizing the toxicity of compounds KUD259 phenolic nature in the study of "acute" toxicity in white mice after intragastric administration

Group animal	Dose, mg / kg	Number of animals			LD_{16} mg/kg	LD_{50} mg/kg	LD_{84} mg/kg
		Total	Fallen	Survivors			
Control	0	10	0	10			
KUD259	500	10	0	10	677.3	813.3	949.3
	600	10	2	8			
	700	10	2	8			
	800	10	5	5			
	900	10	7	3			

Table-2: Data characterizing the toxicity of compounds KUD972 of phenolic nature in the study of "acute" toxicity in white mice after intragastric administration

Group animal	Dose, mg / kg	Number of animals			LD_{16} mg/kg	LD_{50} mg/kg	LD_{84} mg/kg
		Total	Fallen	Survivors			
Control	0	10	0	10			
KUD972	500	10	0	10	723.3	808.3	893.3
	600	10	0	10			
	700	10	3	7			
	800	10	4	6			
	900	10	7	3			

Table-3: Data characterizing the toxicity of compounds KUD973 phenolic nature in the study of "acute" toxicity in white mice after intragastric administration

Group animal	Dose, mg / kg	Number of animals			LD_{16} mg/kg	LD_{50} mg/kg	LD_{84} mg/kg
		Total	Fallen	Survivors			
Control	0	10	0	10			
KUD973	500	10	1	9	556.2	604.8	653.3
	600	10	5	5			
	700	10	8	2			
	800	10	10	0			
	900	10	10	0			

Upon the death of animals after the administration of the compounds under study, animals were dissected in all experimental groups for macroscopic assessment of the state of the internal organs.

Visual examination of the thoracic and abdominal cavities of the dead animals of all groups of macroscopically distinguishable changes was not detected. There were no pathological contents in the thoracic and abdominal cavities.

According to the results of the autopsy and macroscopic examination of the internal organs of the dead experimental animals that received the test compounds, no pathological changes were found:

TONGUE. The tongue is clear.

ESOPHAGUS. The mucosa of the esophagus gray, smooth, shiny.

STOMACH. Stomach with well pronounced folds of the mucous. Gray mucus-wet, shiny.

LIVER. The liver is dense with a smooth surface, on a section of red-brown color.

THICK AND SLIM INTESTINE. The contents of the intestine corresponds to each of its sections. Its mucosa forms transverse folds, moist, gray.

LYZY LITTLES, TRAHEAS, MAIN BRONCHES pink, smooth, moist, shiny.

A HEART. The heart is rounded, well cut. Under the epicardium, the usual venous pattern of the location of the coronary vessels. The flaps are thin, translucent. The endocardium is smooth and shiny. The aorta is elastic, its intima is clean, smooth.

KIDNEYS. The capsule of the kidneys is easily removed, under it the smooth surface of the kidneys, on the incision of the kidney of moderate blood supply with a clear division into layers. Mucous pelvis, bladder gray, moist, shiny.

SPLEEN. The spleen with a smooth capsule, in the cut gray-red, pulp scraping does not.

BRAIN. The brain shells are translucent, smooth, shiny. Brains of the brain are well defined. On a section the substance of the brain is wet, brilliant, with a symmetrical pattern of the structure. The vessels of the base of the brain are arranged symmetrically, with collapsed walls.

The organs of the thoracic and abdominal cavities are located correctly. Cavities are free from liquids and adhesions.

LUNGS. Light air, gray-pink color, covered with a thin pleura, lower lobes with uneven blood filling.

ADRENAL. The adrenal glands are round, with a clear division of the parenchyma into the cortical and cerebral zones.

Conclusion

Summarizing the data obtained, it can be concluded that, when administered intracellularly to mice, the test compounds of phenolic nature under laboratory ciphers KUD259, KUD972 and KUD973 are slightly toxic and belong to class IV toxicity.

The most toxic compound with the lowest mean lethal dose is a phenolic compound under the laboratory cipher KUD973 (LD50 - 604.8 mg / kg). The least toxic compound with the highest average lethal dose is a phenolic compound under the laboratory coding KUD972 (LD50 - 808.3 mg / kg).

Thus, as the main candidate for the drug, it is advisable to consider the compound KUD972, which showed the highest values of LD50, and therefore has the lowest toxicity.

References

1. Korokin, M. V., Pokrovsky, M. V., Novikov, O. O., Gureev, V. V., Denisyuk, T. A., Korokina, L. V.,... Belous, A. S. (2011). Effect of L-arginine, vitamin B6 and folic acid on parameters of endothelial dysfunction and microcirculation in the placenta in modeling of L-NAME-induced NO deficiency. *Bulletin of Experimental Biology and Medicine*, 152(1), 70-72.
2. Ivlitskaya I, Korokin M, Loktionov A (2016) Pharmacological efficiency of statins and L-norvalin at an endotoxin-induced endothelial dysfunction. *Research Results in Pharmacology* 2(2): 25-35.
3. Korokin, M. V., Pokrovskiy, M. V., Novikov, O. O., Gudyrev, O. S., Gureev, V. V., Denisyuk, T. A.,... Belous, A. S. (2011). A model of hyperhomocysteine-induced

endothelial dysfunction in rats. *Bulletin of Experimental Biology and Medicine*, 152(2), 213-215.
4. Pokrovskii, M. V., Pokrovskaya, T. G., Gureev, V. V., Barsuk, A. A., Proskuryakova, E. V., Korokin, M. V., . . . Polyanskaya, O. S. (2012). Correction of endothelial dysfunction by L-arginine under experimental pre-eclampsia conditions. *Eksperimental'Naya i Klinicheskaya Farmakologiya*, 75(2), 14-16.
5. Rajkumar DSR, Gudyrev O, Faitelson A, Stepchenko A, Dolzhikov A, Povetkin S (2016) Study of the influence of L-norvaline, rosuvastatin and their combination on the level of microcirculation in bone tissue in experimental osteoporosis and fractures on its background. *Research Results in Pharmacology* 2(1): 20-24.
6. Shakhno E, Savitskaya T, Pokrovskaya T, Yakushev V, Pokrovskii M, Grinshpan D (2016) Use of L-arginine immobilised on activated carbon for pharmacological correction of endothelial disfunction. *Research Results in Pharmacology* 2(1): 30-35.
7. Soumendra Mohan Saha, Sutapa Biswas Majee,Gopa Roy Biswas. "Effect of Polyox and Gum Karaya on The Release Kinetics of A Model Antihypertensive Drug From A Cellulose Derivative Based Buccal Patch." *International Journal of Pharmacy Research & Technology* 4.2 (2014), 34-38.
8. Ratnasari, R.T., Gunawan, S., Fauzi, R.M.Q., Septiarini, D.F.“ Patient intimacy and innovation development to improve health service performance”,(2018), *Journal of International Pharmaceutical Research*, 45, pp. 1-4.
9. Korokin, M. V., Pokrovskii, M. V., Kochkarov, V. I., Gudyrev, O. S., Korokina, L. V., Pokrovskaya, T. G., & Gureev, V. V. (2014). Endothelial and cardio protective effects of tetrahydrobiopterin, L-norvaline, L-arginine and their combinations by simulation of hyperhomo-cysteine induced endothelial dysfunction. *Research Journal of Pharmaceutical, Biological and Chemical Sciences*, 5(6), 1375-1379.
10. C. Zhang, T.W. Hein, W. Wang et al, Constitutive expression of arginase in microvascular endothelial cells counteracts nitric oxide–mediated vasodilatory function (2001), *Faseb J.*, 15, pp. 1264 –1266.
11. Yakushev V, Filippenko N, Kizilova I, Korokin M, Beskhmelnitsyna E, Litvinova A (2016) Research dose-dependent endothelio- and cardioprotective activity of selective arginase II inhibitor in hyperhomocysteine-induced endothelial dysfunction. *Research Results in Pharmacology* 2(1): 42-45.
12. Pokrovskii, M.V., Korokin, M.V., Kudryavtsev, K.V. et al., Study of Endothelial Protective Activity of Phenol-Derived Thrombin and Arginase-2 Inhibitors KUD-259 and KUD-974 (2017) *Bulletin of Experimental Biology and Medicine*, 163(4), pp. 436-438
13. Radenković M., Stojanović M., Potpara T., Prostran M. Therapeutic approach in the improvement of endothelial dysfunction: the current state of the art (2012), *BioMed Res. Int*, 52, pp. 556–592.
14. Fraga-Silva R. A., Costa-Fraga F. P., Murça T. M. et al., Angiotensin-converting enzyme 2 activation improves endothelial function (2013) *Hypertension*, 61, pp. 1233–1238.
15. Dzhimak, S.S., Basov, A.A., Volchenko, N.N., Samkov, A.A., Fedulova L.V., Baryshev, M.G., 2017. Changes in the functional activity of mitochondria isolated from the liver of rat that passed the preadaptation to ultra-low deuterium concentration. *Doklady Biochemistry and Biophysics* 476: 323–325.
16. Van Zandt M. C., Whitehouse D. L., Golebiowski A., Discovery of (R)-2-amino-6-borono-2-(2-(piperidin-1-yl)ethyl)hexanoic acid and congeners as highly potent inhibitors of human arginases I and II for treatment of myocardial reperfusion injury, (2013) *J. Med. Chem.*, 56, pp. 2568–2580.
17. Golebiowski A., Whitehouse D., Beckett R. P., et al., Synthesis of quaternary α-amino acid-based arginase inhibitors via the Ugi reaction (2013) *Bioorg. Med. Chem. Lett.*, 23, pp. 4837–4841.
18. Chung J. H., Moonb J., Lee Y. S., Chung H.-K., Lee S.-M., Shin M.-J. Arginase inhibition restores endothelial function in diet-induced obesity // *Biochem. Biophys. Res. Comm.* – 2014. – V. 451. – P. 179–183.
19. Buzov A, Kulikov A, Avtina T, Pokrovskii M, Osipova O (2016) Development and validation of methods of quantitative determination of the new antidiabetic drug in the blood plasma of rats by high performance liquid chromatography with mass spectrometric detection. *Research Results in Pharmacology* 2(1): 52-57.
20. Molchanova O, Pokrovskaya T, Povetkin S, Reznikov K (2016) Endothelioprotective property of the combination of the thioctic acid and rosuvastatin shown in the endothelial dysfunction models. Research Results in Pharmacology 2(1): 9-15.
21. Bassey U, Edoamodu O. In vivo Investigation of Haematological and Histological Effects of Leaves Extracted from some Herbals on Plasmodium berghei. *Medbiotech Journal.* 2018;02(01):35-41.
22. Krishtiana N, Kimatova L. Antibacterial Study and Green Preparation of Silver Nanoparticles through Some Plants. *Medbiotech Journal.* 2018;02(02):54-8.
23. Seydoglu S. Grafting of Aryl Group over Carbon Electrode Surface and Investigation of its Stability. *Medbiotech Journal.* 2017;01(01):48-51.
24. Abbasi, S., Moeini, M., Shahriari, M., Ebrahimi, M., & Khoozani, E. K. (2018). Designing and manufacturing of educational multimedia software for preventing coronary artery disease and-its effects on modifying the risk factors in patients with coronary artery disease. *European Journal of General Medicine*, 15(3).
25. Maral S, Albayrak M, öztürk ÇP. Current Treatment Approaches to Multiple Myeloma with New Agents. *J Clin Exp Invest.* 2018; 9(2):103-12.