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USE OF L-ARGININE IMMOBILISED ON ACTIVATED CARBON FOR PHARMACOLOGICAL CORRECTION OF ENDOTHELIAL DISFUNCTION

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Abstract. For the first time a complex of L-arginine sodium salt of sulfate of cellulose acetate on activated carbon. To investigate the processes of sorption-desorption of L-arginine in a model environment. In animal experiments it was shown that granulirovannye the form of a combined preparation in dosages of 30 mg/kg, 70 mg/kg and 200 mg/kg exhibits a pronounced anti-hypertensive, endothelioprotecive and cardioprotective activity in a dosage of 200 mg/kg.
Keywords: L-arginine, L-NAME, water-soluble cellulose derivative, activated carbon, immobilized complex, endothelial dysfunction.

Endothelial dysfunction is the main predictor of diseases of cardiovascular system, diabetes and their complications [1, 2, 3, 4, 5]. It was previously shown [6, 7, 8, 9, 10, 11], that L-arginine applied both in monotherapy and in combination with antihypertensive agents in the ADMA-(asymmetric dimethylarginine)-like model of L-NAME-(N^G-nitro-L-arginine methyl ester)-induced endothelial dysfunction increases effectively the activity of endothelial NO-synthase and the production of nitric oxide, and also prevents the development of endothelial dysfunction in experimental animals [12].

Currently, the main pharmaceutical form of L-arginine is a capsule, which upon dissolving in the gastric environment can affect the medical and biological properties of the drug. One of the promising methods for increasing biological activity is a modification of the original substance L-arginine with the natural polymers and the creation of new dosage forms based on the product of their interaction [13, 14].

Objective of this study was to obtain polymer complexes of L-arginine with cellulose anionic polyelectrolyte - cellulose acetate sulfate in the form of sodium salt (Na-CAS) immobilized on activated carbon, and to evaluate their endothelio- and cardioprotective activity in experimental animals under L-NAME-induced nitric oxide deficiency [15, 16, 17, 18].

Experimental part.

Content of the combined sulfuric acid in Na-CAS was 27.8 wt. % combined acetic acid – 21.4 wt. %, average molecular weight – $30 \cdot 10^3$ g/mol. L-arginine was used in the form of hydrochloride.

Complexation was carried out by mixing water solutions of the components at the amino acid-to-polymer weight ratio of 5:1 to 1:5. Isolation of the obtained soluble complexes was carried out through precipitation in ethanol with further re-precipitation. Twice re-precipitated complex was dried in a rotary evaporator. L-arginine content in the complex was determined by UV spectroscopy with complexation with ninhydrin according to the procedure [19]. UV-spectra

were recorded with a spectrophotometer Metertech UV/VIS SP 8001 in quartz cuvettes 1 cm thick.

The intrinsic viscosity of the polymer and the complex in aqueous solutions was determined by using an Ubbelohde viscometer in the 0.2 M NaCl solution at 25°C. The surface tension at the liquid-air boundary was measured by stalagmometric method

Immobilization of the complex was conducted on AUT-MI activated carbon from an aqueous solutions followed by granulation and drying of the resulting paste. The specific Gibbs adsorption of arginine and arginine-polymer complex from their aqueous solutions on activated carbon OU-A, TH-90G and AUT-MI was evaluated by the residual concentration of L-arginine in solution after extraction of coal [7].

To determine the amount of arginine, isolating from the resulting dosage form in the simulated internal environments, the pharmacopeial buffers pH 1.2 (0.1 M HCl), pH 6.2 (phosphate buffer) and pH 7.5 (phosphate buffer) were used.

Experiments were performed in male albino Wistar rats weighing 180-200. For simulation of endothelial dysfunction, L-NAME was administered intraperitoneally at a dose of 25 mg/kg/day for seven consecutive days [4, 5, 7, 8, 9]. Complex of L-arginine with polymer immobilized on activated carbon was administered intragastrically by gavage 30 minutes prior to administration of L-NAME at a dose of 30 mg/g, 70 mg/kg and 200 mg/kg based on the active substance once a day for 7 days. Intact animals were intragastrically administered an equivalent volume of saline for 7 days. On day 10 of the experiment, a catheter was inserted under anesthesia (chloral hydrate 300 mg/kg) into the left carotid artery to record blood pressure (BP). Bolus administration of pharmacological agents was into the femoral vein. Hemodynamic parameters: systolic blood pressure (SBP), diastolic blood pressure (DBP) and heart rate (HR) were measured continuously with the use of a TSD104A sensor and the MP150 hardware and software system (BiopacSystem, Inc., USA). In addition to blood pressure measurements, a series of functional tests was performed with subsequent evaluation of changes in hemodynamic parameters (SBP, DBP, HR) in response to endothelium-dependent vasodilatation caused by intravenous administration of solution of acetylcholine (AC) at a dose of 40 mg/kg at the rate of 0.1 ml per 100 g body weight of animal (EDVD), as well as changes in hemodynamic parameters in response to the intravenous administration of sodium nitroprusside (NP) at a dose of 30 mg/kg at the rate of 0.1 ml per 100 g body weight of animal [20, 21]. The degree of endothelial dysfunction in experimental animal, as well as the degree of its correction with the studied medications was assessed by the estimated coefficient of endothelial dysfunction (EDC), which represents the ratio of the area of a triangle above the BP recovery curve in response to the NP administration (EIVD) to the area of a triangle above the BP recovery curve in response to the AH administration (EDVD) [4, 5, 7, 8, 9].

Results and discussion:

Complexation of L-arginine with Na-CAS is possible by electrostatic interaction of the sulfate groups of the polymer and protonated amine groups of L-arginine with the formation of an amino acid polymeric salt. It was found that the L-arginine with Na-CAS forms a soluble complex of constant composition upon mixing the initial components in any proportions. Complexation is accompanied by a decrease in charge density of a polyelectrolyte chain and, as a consequence, the transition of Na-CAS macromolecules to a more convolute conformation, which is confirmed by a decrease in the intrinsic viscosity of the polymer in the solution in the presence of L-arginine: from 0.44 to 0.32 dl/g [22]. The complex composition corresponds to the molar ratio of Na-CAS cellobiose ring to L-arginine – 1,0:1,0.

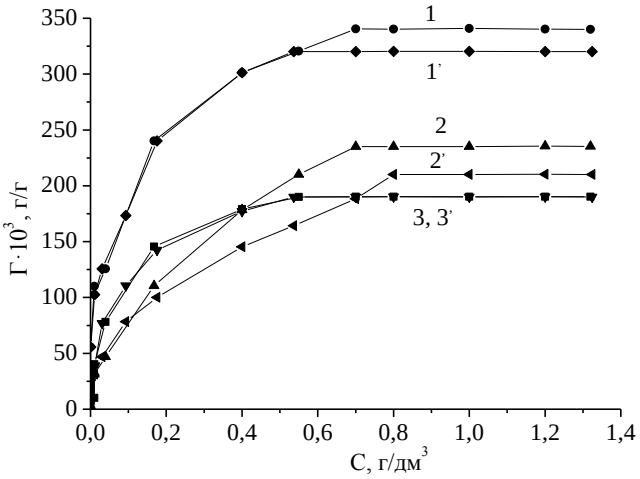

Figure 1. Adsorption isotherms of L-arginine and L-arginine -Na-SAC on activated carbons at T = 298 K. 1 – complex L-arginine – Na-SAC on AUT-MI; 1'– L-arginine on AUT-MI; 2 – complex L-arginine - Na-SAC on OU-A; 2'– L-arginine on OU-A; 3 – complex L-arginine - Na-SAC on TH-90G; 3' – L-arginine on TH-90G

Since the resulting complex is water-soluble, a water-insoluble carrier, namely, activated carbon (AC) was used to produce a solid dosage form. We may assume that the adsorption of L-arginine in AC occurs mainly in the micropores, since the values of maximum adsorption (Fig. 1) for AC with different

pore structures are correlated with the micropore volume (Table 1) [23].

Table 1

Maximum Gibbs adsorption value, specific surface area and pore structure characteristics of the applied activated carbons

Coal	Pore volume, cm^3/g			S_{spec}, m^2/g	$G_\infty \cdot 10^3$ (L-arginine), g/g	$G_\infty \cdot 10^3$ (complex), g/g
	V_{micro}	$V_{meso, macro}$	V_{total}			
AUT-MI (SvetlogorskKhimvolokno, JSC)	0.38	0.18	0.56	920	320.2	340.2
OU-A (sorbent, Perm)	0.24	0.31	0.55	750	210.1	235.1
TH-90G (Silicarbon, Germany)	0.22	0.25	0.47	800	190.1	190.2

According to the data from Fig. 1, the maximum adsorption of both individual and complex-bound L-arginine (expressed as L-arginine) is typical of the fibrous microporous coal AUT-MI. At the same time, complex-bound L-arginine is adsorbed to a greater extent that can be associated with an increase in its hydrophobic property resulting from complexation. Experimental evidence of hydrophobic properties of the L-arginine-Na-CAS complex can be its surface activity manifesting itself on the background of surface-indifferent properties of L-arginine and surface-inactive properties of Na-CAS (Fig. 2).

Desorption of L-arginine from the carbon carrier surface into pharmacopoeial buffers depends on pH. It was found that 10.0±0.1 wt. % of L-arginine is released in acidic gastric medium (pH 1.0). Further increase of the desorption time gives no release of L-arginine observed. In intestinal-simulating alkaline medium, a significantly higher amount of L-arginine is released during the same period of time – 96.3±0.5 wt. %.

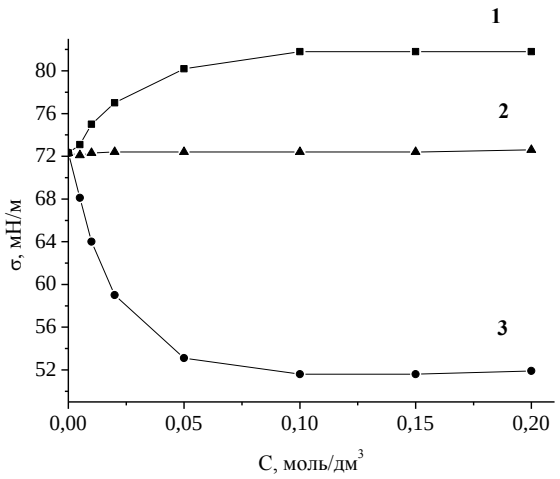

Figure 2. Surface tension isotherms of aqueous solutions at T = 298 K.
1 – Na-CAS; 2 – L-arginine; 3 – complex L-arginine-Na-CAS.

The influence of L-arginine in the form of a complex with Na-CAS immobilized on activated carbon at doses of 30 mg/kg, 70 mg/kg and 200 mg/kg on initial values of blood pressure and the coefficient of endothelial dysfunction was assessed *in vivo* in anesthetized rats upon modeling the L-NAME-induced pathology (Table 2) as compared to pure L-arginine [20].

Table 2

The effect of new dosage form of L-arginine on hemodynamics values and EDC in modeling of L-NAME-induced endothelium dysfunction (M±m n=10)

Groups of animals	Functional test	SBP, mm Hg	DBP, mm Hg	EDC
Intact	Reference	137.7±3.7	101.9±4.3	1,1±0,1
	AC	84.3±4.5	38.7±2.8	
	NP	83.0±3.7	42.1±4.4	
L-NAME (25 mg/kg)	Reference	190.3±6.7*	145.0±3.9*	5,4±0,6*
	AC	110.6±5.2	82.8±6.6*	
	NP	88.7±4.7	50.8±4.2	
L-NAME + L-arginine-Na-CAS (30 mg/kg)	Reference	180,8±8,8	141,4±9,0	3,0±0,2**^{X}
	AC	112,6±8,5	63,4±7,3	
	NP	89,4±4,3	57,6±5,8	
L-NAME + L-arginine (30 mg/kg)	Reference	188,5 ± 14,8*	137,3 ± 9,9*	3,6±0,3**
	AC	85,3±5,0**	53,1±3,4**	
	NP	97,0±6,2	66,7±4,3	
L-NAME + L-arginine-Na-CAS (70 мг/кг)	Reference	176,2 ± 6,0**^{X}	130,7±8,1**	2,3±0,2**^{X}
	AC	98,7±8,7	53,7±9,8	
	NP	96,5±11,5	50,7±3,4	
L-NAME+ L-arginine (70 мг/кг)	Reference	195,2±13,5	136,5 ± 4,8**	2,7±0,1**
	AC	116,8 ± 4,6*	85,1 ± 4,6*	
	NP	100,3 ± 6,7	58,9 ± 7,8**	
L-NAME + L-arginine-Na-CAS (200 мг/кг)	Reference	141,4±12,2**^{X}	118,7±9,8**	1,9±0,1**^{X}
	AC	86,3±10,3	47,1±8,7**	
	NP	94,6±7,1	42,3±6,4	
L-NAME + L-arginine (200 мг/кг)	Reference	177,6 ± 9,6*	120,1 ± 6,4*	2,5±0,1**
	AC	85,3 ± 5,0	51,3 ± 2,5	
	NP	102,0 ±3,8	44,1 ± 2,9	

Note: *– at p<0.05 as compared to control animals; **– at p<0.05 as compared to group receiving L-NAME; ^{X} – at p<0.05 as compared to group receiving to pure L-arginine in the appropriate dose

From table 2 that in all dosages of the new drug forms of L-arginine, the values of EDC were more close to the level of intact animals in comparison with pure form of L-arginine, additionally, a new pharmaceutical form of L-arginine at a dose of 200 mg/kg in the largest extent prevented the development of severe systolic hypertension.

Table 3

The effect of new dosage form of L-arginine on the left ventricle of rats' heart contractility (M±mn=10)

Groups of animals	Initial LVP, mm Hg	Adrenoreactivity, mm Hg	Myocardial reserve level, (%)
Intact	108,6±4,3	199,2±8,3	83,6±4,2
L-NAME (25 mg/kg)	167,8±5,6*	247,3±4,8*	66,0±3,4*
L-NAME + L-arginine-Na-CAS (30 mg/kg)	131,9±11,2**^{X}	222,7±16,6**^{X}	68,5±6,3*
L-NAME + L-arginine (30 mg/kg)	173,7±37,6*	253,2±10,8*	69,0±6,1*
L-NAME + L-arginine-Na-CAS (70 мг/кг)	136,0±5,0**^{X}	229,6±2,2**^{X}	83,1±3,7**^{X}
L-NAME+ L-arginine (70 мг/кг)	160,0±29,8*	250,8±12,3*	70,5±5,8*
L-NAME + L-arginine-Na-CAS (200 мг/кг)	127,9±11,5**^{X}	201,6±12,3****^{X}	84,0±4,7**
L-NAME + L-arginine (200 мг/кг)	142,1±8,3**	223,5±7,3**	84,3±4,5**

Note: *- at p<0.05 as compared to control animals; **- at p<0.05 as compared to group receiving L-NAME; ^{X} - at p<0.05 as compared to group receiving to pure L-arginine in the appropriate dose.

When carrying out a load of heart samples the initial contractility of the left ventricle in animals treated with L-NAME were significantly higher than the intact (table 3). Preparations of L-arginine immobilized in the form of significantly better prevented the increase in baseline left ventricular pressure, which indicates best negative inotropic action of this form of L-arginine in the simulation of hyperkinetic disorders of the myocardium [24].

The results of the study of the functional state of the myocardium during exercise testing revealed the benefits of cardioprotective action of a new form of L-arginine in all doses, expressed in preventing the increase in adrenoreactivity. A statistically significant advantage in preventing the fall of LVP the tests on the load resistance in the conventional form revealed only by the introduction of a new form of L-arginine at a dose of 200 mg/kg. L-arginine at doses of 30 mg/kg and 70 mg/kg of both forms, showed cardioprotective effect in lesser degree.

Thus, L-arginine in the form of a complex with Na- Na-CAS immobilized on activated carbon, at doses of 30 mg/kg, 70 mg/kg and 200 mg/kg showing the most pronounced antihypertensive and endothelioprotective activity. The most pronounced cardioprotective activity showed L-arginine in the form of a complex with Na-CAS, immobilized on charcoal, in the dose of 200 mg/kg.

References

1. Johnson, F.K., Johnson, R.A., Peyton, K.J., Durante, W. Arginase inhibition restores arteriolar endothelial function in Dahl rats with salt-induced hypertension. American Journal of Physiology-Regulatory, Integrative and Comparative Physiology. 288, 4 (2005): 1057-1062. [PubMed] [Full text]

2. Pokrovskiy M.V., Korokin M.V., Tsepeleva S.A. Arginase inhibitor in the pharmacological correction of endothelial dysfunction. International Journal of Hypertension. (2011) – art. no. 515047. [Scopus] [Full Text]

3. Savvidou, M.D., Hingorani, A.D., Tsikas, D., Frölich, J.C., Vallance, P., Nicolaides, K.H. Endothelial dysfunction and raised plasma concentrations of asymmetric dimethylarginine in pregnant women who subsequently develop pre-eclampsia. The Lancet. 361, 9368 (2003): 1511-1517. [PubMed]

4. Pokrovskii M.V., Artyushkova E.B., Pokrovskaya T.G. Methods of experimental modeling of endothelial dysfunction. Allergology and Immunology. Vol. 9, № 3 (2008): P. 327. [eLIBRARY]

5. Pokrovskii M.V., Kochkarov V.I., Pokrovskaya T.G. Comparative study of potential endothelioprotectors and impaza in modeled nitric oxide deficiency. Bulletin of Experimental Biology and Medicine. 148 (3) (2009): 514-517. [Scopus] [Full Text]

6. Kolesnik I.M., Pokrovskiy M.V., Pokrovskaya T.G. Pharmacological Preconditioning by Erythropoietin in Limb Ischemia. Biomedicine. 1(4) (2011): 90-92. [eLIBRARY] [Full Text]

7. Kochkarov V.I., Molchanova O.V., Pokrovskii M.V. Endothelium-protective action of thioctic acid and rosuvastatin combination at concomitant hypoestrogen and L-Name-induced deficit of nitric oxide. Research Journal of Pharmaceutical, Biological and Chemical Sciences. 5 (2014): 1054-1057. [Scopus] [Full Text]

8. Pokrovskii M.V., Pokrovskaya T.G., Gureev V.V. Correction of endothelial dysfunction by L-arginine under experimental pre-eclampsia conditions. Eksperimental'naya i Klinicheskaya Farmakologiya. 75 (2012): 14-16. [Scopus] [Full Text]

9. Pokrovsky M.V., Pokrovskaya T.G., Gureev V.V. Pharmacological Correction of ADMA-ENOS-associated targets in Preeclampsia. Obstetrics and Gynecology. 2 (2011): 16-20. [eLIBRARY] [Full Text]

10. Bredt, D.S., Snyder, S.H. Nitric oxide: a physiologic messenger molecule. Annual review of biochemistry. 63,1 (1994): 175-195. [PubMed]

11. Bagnost, T., Berthelot, A., Bouhaddi, M., Laurant, P., André, C., Guillaume, Y., Demougeot, C. Treatment with the arginase inhibitor N-hydroxy-nor-L-arginine improves vascular function and lowers blood pressure in adult spontaneously hypertensive rat. Journal of hypertension. 26,6 (2008): 1110-1118. [PubMed]

12. Filippenko N.G., Povetkin S.V., Pokrovsky M.V. Efficacy and tolerability of Renipril HT experimentally and clinically. Pharmateca. Vol.8 (2002): P.22 [eLIBRARY] [Abstract]

13. Eldin, M.S.M., Kamoun, E.A., Sofan, M.A., Elbayomi, S.M. L-Arginine grafted alginate hydrogel beads: A novel pH-sensitive system for specific protein delivery. Arabian Journal of Chemistry. 8,3 (2015): 355-365. [Full text]

14. Stepchenko A.A., Pribylova N.N., Filippenko N.G., Povetkin S.V., Grigorieva T.M. Depending efficiency of personalized pharmacological treatment of patients with peptic ulcer depending on phenotype of oxidizing metabolism. Kursk scientifiv and practical bulletin «Man and his health». № 2 (2010): P. 131-137 [eLIBRARY] [Full text]

15. Prokopenko L.G., Bystrova N.A. Immunomodulatory and antioxidant effects of magnetic laser exposure, coupled with the introduction of β-carotene, essentiale and riboxin during cold stress. Journal of Pathophysiology and Experimental Therapy. №4 (2000): P. 13 [eLIBRARY]

16. Uteshev B.S., Bystrova N.A., Brovkina I.L. et al. Immunomodulating and antioxidant effects of vitamins a and e in cases of air and immersion cooling. Experimental and Clinical Pharmacology. Vol.64, №1 (2001): P. 60-62. [eLIBRARY]

17. Nikolaev S.B., Bystrova N.A.Immunomodulating Effects of Mexicor at the Hypoxia. Journal of new Medical Technologies. Vol. 15, №1 (2008): P. 207-210. [eLIBRARY] [Full text]

18. Pribylova N.N., Osipova O.A. Neurohumoral mechanisms of chronic heart failure in patients with post-infarction cardiosclerosis. Journal of Heart Failure. Vol.10, №4 (2009): P. 196-198. [eLIBRARY]

19. Simonyan V.A., Salamotov, A.A., Pokrovskaya Y.S. The application of quantitative ninhydrin reaction for the determination of α-amino acids in various objects: guidelines. Volgograd State Medical University: Volgograd, 2007. 106. [Full text]

20. Pokrovsky, M.V., Kochkarov, V.I., Pokrovskaya, T.G. A new look at the correction of endothelial dysfunction. Russian Journal of Immunology. Vol.9 (2006): 60-61.

21. Lopin V.N., Lopina E.V., Povetkin S.V. The use of neural network classifiers for assessment of vascular remodeling in patients with arterial hypertension. Journal of new Medical Technologies. №1-2 (2003): P. 9 [eLIBRARY]

22. Solovskii, M.V., Eropkin, M.Y., Eropkina, E.M., Smirnova, M.Y., Gavrilova, I.I. Complexes of aminoglycoside antibiotics with copolymers of acrylamide with acrylic and methacrylic acids. Pharmaceutical chemistry journal. 44,6 (2010): 314-318. [OpenUrl]

23. Ivanets, M.G., Savitskaya, T. A., Nevar, T. N., Grinshpan, D.D. Adsorptive and Structural Characteristics of Carbon Sorbents. Inorganic Materials. 47,10 (2011): 1061-1065. [OpenUrl]

24. Pokrovsky M.V., Pokrovskaya T.G., Gureyev V.V., et al. Pharmacological correction of ADMA-ENOS-associated targets in preeclampsia. Obstetrics and Gynecology. №2 (2011). P: 16-20. [eLIBRARY] [Full text]