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USE OF SELECTIVE INHIBITORS OF ARGINASE 2 AND TADALAFIL IN COMBINED COMPENSATION OF HOMOCYSTEINE-INDUCED ENDOTHELIAL DYSFUNCTION

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Annotation. Use of selective inhibitors of arginase 2 in combination with tadalafil on in the course of modeling of homocysteine-induced endothelial dysfunction provided endotelio- and cardioprotective effects manifested in preventing of SED increase, adrenoreactivity, maintaining myocardial reserve and normalization of the values of biochemical markers (Total NO, expression of eNOS).
Keywords: endothelial dysfunction, selective inhibitors of arginase 2, tadalafil.

Introduction: Hyperhomocisteinemia may function as an independent factor of endothelial dysfunction progress or intensify already existing endothelial lesions [1]. Known factors of hyperhomocisteinemia correction are quite scarce and would rather include only exogenous administration of vitamin B_6 and folic acid. In clinical investigations use of folic acid or 5-methyltetrahydrofolate (5-MTHF, active and circulating form of folic acid) was the most frequently studied subject of research since such agents may have potentially favorable influence on metabolism of tetrahydrobioptherine (BH4).

At the same time occurrence in the recent years of the notions of «endogenous inhibition» of endothelial NO-synthase (eNOS) and «eNOS uncoupling» resulted in intensification of the studies aimed at prevention of these factors as key elements in endothelial dysfunction correction. Methylated equivalents of L-arginine, namely asymmetric dimethylarginine (ADMA) and monomethylarginine (L-NMMA) are endogenic inhibitors of endothelial nitric oxide synthase (eNOS). [2-6] «eNOS uncoupling» is change-over of eNOS enzyme activity from nitric oxide production to superoxide production.

Among other things limited nitric oxide formation may be conditioned by high arginase activity. It becomes apparent that in order to reduce risks and frequency of cardiac and vascular diseases development it is necessary to suppress high arginase activity. To that effect it is possible to use inhibitors of arginase 2 [7].

The earlier studies demonstrated that L-arginine efficiently increased activity of endothelial NO-synthase and nitric oxide production as well as prevented endothelial dysfunction development in experiment both in the course of monotherapy and in combination with antihypertensive agents with use of ADMA-like model of L-NAME-induced endothelial dysfunction [2-6].

Methods: Simulation of homocysteine-induced endothelial dysfunction was made by means of daily oral administration of methionine in a dose of 3000 mg/kg over the period of 7 days [1]. The following selective inhibitors of arginase 2 were used: compounds with laboratory codes C239-0844, L207-0208, L207-0210, L207-0322, L207-0404, L207-0525, L327-0346 in a dose of 1 mg/kg and tadalafil in a dose of 0.1 mg/kg. During the 8th day of the experiments the following parameters were evaluated: the level of endothelial dysfunction on the basis of a design endothelial dysfunction coefficient (EDC), the results of functional tests for adrenoreactivity and myocardial reserve exhaustion, as well as changes in the values of TotalNO and eNOS expression [8, 9].

Validity of the absolute parameters variation was determined by a differential method of variation statistics involving determination of mean shift values (M), arithmetical mean (±m) and likely error probability (p) with use of the Student's tables. The differences were considered to be significant if $p<0.05$. Statistical calculations were performed with aid of Microsoft Excel 7.0 program.

Results: Inhibitors of arginase 2 produced moderate reduction effect on arterial pressure and EDC. The highest activity was observed for the compounds L207-0525, L327-0346 in a dose of 1 mg/kg where the values of EDC made 1.5±0.3 c.u. and

1.9±0.4 c.u. correspondingly while the controls showed 3.5±0.4 c.u. (Table 1). At the same time the compounds demonstrated cardioprotective effect by preventing adrenoreactivity increase and myocardial reserve exhaustion as well as negative growth of the values of the final nitric oxide metabolites, i.e. NOx and NOS expression (Tables 2, 3). Combined use of preparations intensified protective effect in regard to prevention of EDC increase and arterial pressure decline, as well as in regard to the final nitric oxide metabolites NOx and eNOS expression, nevertheless cardioprotective effect remained unchanged (Tables 1-3).

Discussion of the obtained results: As Figure 1 shows arginase activity growth may result in use of L-arginine necessary for NO production by eNOS and consequently in endothelial dysfunction progress. Given that decrease of bioavailability of NO in case of endothelial dysfunction is pathogenetically involved in the whole set of cardiovascular diseases intensification of arginase 2 activity may serve an important initiating factor of development of such diseases.

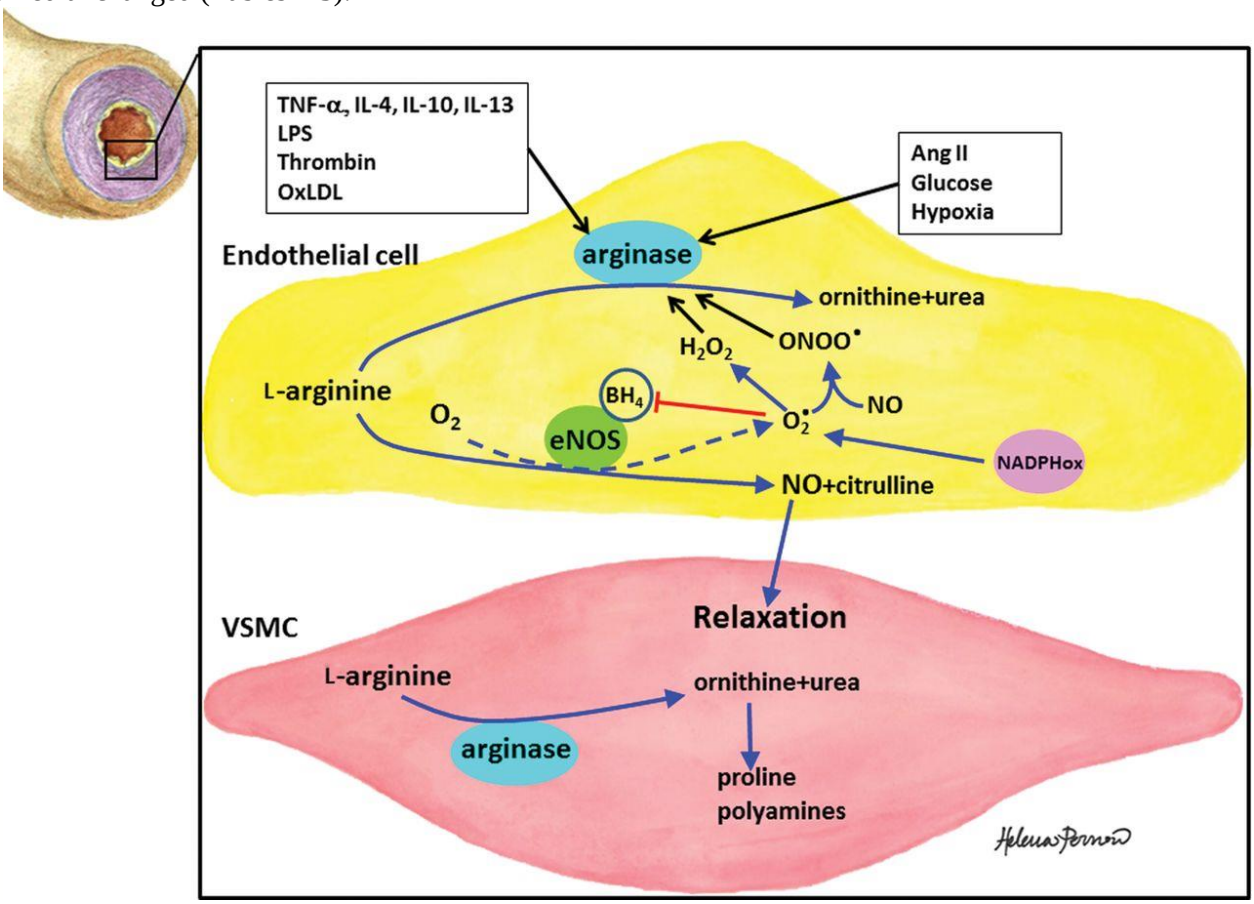

Figure 1. Schematic diagram of the effect of arginase in regulation of NO bioavailability and of function of vascular smooth muscle elements.Hyperactivity of arginase assisted by hydrolysis of L-arginine, ornithine and urea reduces L-arginine availability for NO-synthase (NOS), thus suppressing NO production. Absence of L-arginine will also result in eNOS «uncoupling» so that the enzyme will produce superoxide instead of NO. Generation of superoxide uncoupled with eNOS as well as of NADPH oxidase and peroxynitrite from superoxide and NO will cause the further arginase activity growth. Such changes in the aggregate will reduce NO bioavailability and promote endothelial dysfunction. In smooth muscle vascular cells ornithine favors more intensive formation of L-proline and polyamines which stimulate cells proliferation. Ang II-angiotensin II; BH4, tetrahydrobiopterin; OxLDL, oxidized low-density lipoprotein; LPS, lipopolysaccharide; NADPHox, nicotineamide-adenine dinucleotide phosphate oxidase; NO, nitric oxide; ONOO-peroxynitrite; VSMC, vascular smooth muscle cell.

A number of studies using experimental models of hypertension [10], atherosclerosis [11], diabetes [12] and ageing [13] demonstratively approved that arginase activity growth results in progress of endothelial dysfunction. Moreover increase of cytoplasmic level of arginase II accompanied by hypoxia acts as «eNOS decoupling» factors [14]. Finally arginase may also inhibit L-arginine

delivery in endothelial cells which causes the further reduction of availability of substrate for eNOS [14].

As was stated above arginase inhibition may potentially have favorable effect in case of a number of pathological cardiovascular diseases. A therapeutic effect of arginase inhibition was studied in a set of experimental models of cardiovascular diseases, the studies showed positive results. Clinical research data are very limited. Proof-of-principle clinical trials were performed with local use of arginase inhibitors by means of intracutaneous microdialysis in patients with coronary disease and type 2 diabetes mellitus [15], cardiac failure [16] and hypertension [17]. These observations suggest that arginase activity inhibition is of great importance in the process of human cardiovascular diseases progression Therefore more large clinical trials involving systematical prescription of arginase inhibitors have high potential.

To our opinion the results of arginase inhibitors use in case of pulmonary hypertension including simultaneous phosphodiesterase 5 inhibitors administration may be of the greatest interest. The results of our studies provide strong evidence of such combination efficiency for endothelial dysfunction correction.

Several pharmacological inhibitors are available for experimental studies. They belong to two classes, i.e. boric acid and equivalents of *N*ω–hydroxy L-arginine [13, 18]. It is important that the arginase inhibitors available presently have weak or no selectivity for arginase 2, thus their use is limited. For this reason it remains unclear which isoforms should be used for obtaining the most favorable effect. Since both isoforms of arginase I and II have effect on vascular system arginase inhibitors of the 2nd isoform are necessary in order to give comprehensive biological evaluation of these ywo isoforms.

In this connection the compounds with new chemical structure studied by us are of undoubted interest for further investigations.

In theory arginase inhibition may involve side effects particularly if to speak of the role of arginase in urea cycle. Nevertheless arginase expression and activity in liver is severalfold more intensive than in a vessel wall therefore it is unlikely that clinically significant doses may inhibit hepatic arginase insomuch that will disturb urea cycle [13, 18]. This statement is confirmed by absence of poisonous effects in case of prolonged administration of arginase inhibitors in animal models of hypertension [19] and atherosclerosis [20]. Moreover continued arginase inhibition does not apparently result in compensatory increase of the enzyme activity [19].

Conclusion: The results of studies are indicative of development of an additive effect of combined use of selective arginase 2 inhibitors and small doses of phosphodiesterase 5 inhibitor in respect of homocysteine-induced endothelial dysfunction progression.

Table 1

The effect of selective inhibitors of arginase 2 and tadalafil on dynamics of hemodynamic parameters in animal models of homocysteine-induced endothelial dysfunction (M±m, n=10).

Animal groups	SBP	DBP	EDC
Intact	129.5±2.2	89.1± 1.1	1.1 ± 0.1
Homocysteine-induced endothelial dysfunction (HIED) (n=10)	119.4±2.3*	84.3±2.2	3.5 ± 0.4*
HIED + C239-0844 1 mg/kg (n=10)	117.4±2.2	79.9±2.1	2.9±0.3*
HIED L207-0208 1 mg/kg (n=10)	123.3±2.4	85.3±1.9	2.3±0.5*
HIED + L207-0210 1 mg/kg(n=10)	120.3±3.0	85.9±2.4	2.8±0.3*
HIED + L207-0322 1 mg/kg (n=1)	125.3±3.2	83.7±2.3	2.7±0.5*
HIED + L207-0404 1 mg/kg (n=10)	125.6±3.3	84.7±2.4	2.5±0.2*
HIED + L207-0525 1 mg/kg (n=10)	122.4±3.3	84.8±2.3	1.5±0.3#
HIED + L327-0346 1 mg/kg(n=10)	127.6±3.1	87.4±2.3	1.9±0.4#
HIED + tadalafil 0.1 mg/kg (n=1)	116.9±3.0	80.3±2.4	1.9±0.4#
HIED + L327-0525 1 mg/kg + tadalafil 0.1 mg/kg (n=10)	124.4±3.6	84.3±2.4	1.2±0.2#

Remark: SBP - systolic blood pressure (mm hg), DBP - diastolic blood pressure (mm hg), EDC – endothelial dysfunction coefficient (c.u.), * – significant difference with intact animals group ($p<0.05$); # - significant difference with homocysteine-induced endothelial dysfunction (HIED) group ($p<0.05$).

Table 2

The effect of selective inhibitors of arginase 2 and tadalafil on dynamics of contractility parameters in the course of exercise testing in animal models of homocysteine-induced endothelial dysfunction (M±m, n=10).

Animal groups	Adrenoreactivity (mm hg)	Myocardial reserve exhaustion (%)
Intact	189.4±9.1	87.4±10.9
Homocysteine-induced endothelial dysfunction (HIED) (n=10)	239.2±8.6*	69.1±3.9*
HIED + C239-0844 1 mg/kg (n=10)	241.5±7.9*	72.0±4.7*
HIED L207-0208 1 mg/kg (n=10)	239.1±8.8*	77.1±4.2*
HIED + L207-0210 1 mg/kg(n=10)	227.9±8.4*	78.0±4.7*
HIED + L207-0322 1 mg/kg (n=1)	229.4±8.5*	79.4±5.0*
HIED + L207-0404 1 mg/kg (n=10)	239.1±8.7*	79.6±5.3*
HIED + L207-0525 1 mg/kg (n=10)	201.4±6.5#	99.0±4.9#
HIED + L327-0346 1 mg/kg(n=10)	201.0±6.3#	97.5±4.5#
HIED + tadalafil 0.1 mg/kg (n=1)	197.7±5.9#	98.1±4.7#
HIED + L327-0525 1 mg/kg + tadalafil 0.1 mg/kg (n=10)	196.8±5.8#	100.1±5.1#

Remark: * – significant difference with intact animals group ($p<0.05$); # - significant difference with homocysteine-induced endothelial dysfunction (HIED) group ($p<0.05$).

Table 3

The effect of selective inhibitors of arginase 2 and tadalafil on dynamics of biochemical markers value (TotalNO, eNOS expression) in animal models of homocysteine-induced endothelial dysfunction (M±m, n=10).

Animal groups	NOx	eNOS expression
Intact	121.5±10.4	5.4±0.21
Homocysteine-induced endothelial dysfunction (HIED) (n=10)	82.1±9.4*	2.05±0.21*
HIED + C239-0844 1 mg/kg (n=10)	82.4±9.3*	2.11±0.22*
HIED L207-0208 1 mg/kg (n=10)	92.0±8.9*	1.99±0.32*
HIED + L207-0210 1 mg/kg(n=10)	90.0±9.9*	2.17±0.41*
HIED + L207-0322 1 mg/kg (n=1)	92.1±9.7*	2.84±0.45*
HIED + L207-0404 1 mg/kg (n=10)	92.6±8.3*	2.01±0.66*
HIED + L207-0525 1 mg/kg (n=10)	121.7±9.5#	4.17±0.66#
HIED + L327-0346 1 mg/kg(n=10)	122.8±9.4#	4.25±0.67#
HIED + tadalafil 0.1 mg/kg (n=1)	129.6±9.3#	4.97±0.73#
HIED + L327-0525 1 mg/kg + tadalafil 0.1 mg/kg (n=10)	137.1±10.0#	5.92±0.87#

Remark: NOx – final metabolites of NO (micromole/l); eNOS expression (%);* – significant difference with intact animals group ($p<0.05$); # – significant difference with homocysteine-induced endothelial dysfunction (HIED) group ($p<0.05$).

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