Research Results in Pharmacology

Research Results in Pharmacology 5(3): 67–77
UDC: 615.065+616.053.2
DOI 10.3897/rrpharmacology.5.39521

Research Article

Search for new pharmacological targets for increasing the efficiency of correction of cardiovascular diseases

Liliya V. Korokina[1], Ivan V. Golubev[2], Olga N. Pokopejko[3], Anastasia V. Zagrebelnaya[1], Sergey A. Demchenko[1]

1 *Belgorod State National Research University, 85 Pobedy St., Belgorod 308015, Russia*

2 *Kursk City Clinical Maternity Hospital, 10 Pirogova St., Kursk, 305016, Russia*

3 *I.M. Sechenov First Moscow State Medical University, 2 B. Pirogovskaya St., Moscow, 19435, Russia*

Corresponding author: *Liliya V. Korokina* (korokina@bsu.edu.ru)

Academic editor: *Tatyana Pokrovskaya* ♦ **Received** 1 August 2019 ♦ **Accepted** 2 September 2019 ♦ **Published** 30 September 2019

Citation: Korokina LV, Golubev IV, Pokopejko ON, Zagrebelnaya AV, Demchenko SA (2019) Search for new pharmacological targets for increasing the efficiency of correction of cardiovascular diseases. Research Results in Pharmacology 5(3): 67–77. https://doi.org/10.3897/rrpharmacology.5.39521

Abstract

Introduction: Cardiovascular disease (CVD) is the leading cause of death worldwide: no other reason causes as many deaths a year as CVD. An estimated 17.9 million people died of CVD in 2016, accounting for 31% of all deaths in the world. People with CVD or at high risk for these diseases (due to one or more risk factors, such as high blood pressure, diabetes, hyperlipidemia, or an already developed disease) need early detection and assistance through counseling and, if necessary, taking medication.

Ways to find new targets for the correction of endothelium-associated pathology: The basis of the modern therapy for arterial hypertension and other cardiovascular diseases is the postulate of the need to correct endothelial dysfunction as an indication of the adequacy of antihypertensive and other types of treatment. Lowering blood pressure (BP) without normalizing endothelial function cannot be considered a successfully resolved clinical task. Currently, there are no drugs for specific pharmacological correction of endothelial dysfunction in cardiovascular diseases, and the search for new targets for pharmacological correction of endothelial dysfunction is one of the main tasks of pharmacology.

Keywords

endothelial dysfunction, pharmacological correction, target, nitric oxide.

Introduction

Cardiovascular diseases continue to be the leading cause of death and disability, which necessitates the need for an in-depth study of the pathogenesis and the development of treatment and prevention measures.

In the modern world, especially in developed countries, arterial hypertension (AH) is one of the main factors that increase disability and mortality due to such severe complications as myocardial infarction, heart failure and stroke. In Russia, the prevalence of hypertension among the adult population exceeds 30%, which makes the country a leader in Europe in mortality from strokes and coronary artery disease. One of the main causes of

hypertension is endothelial dysfunction (ED), in the first place – impaired relaxation properties of the endothelium.

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 a decrease in BP without normalizing endothelial function cannot be considered a successfully solved clinical task (Kyoung and Woo 2012).

"ADMA-eNOS" as a pharmacological target is of undoubted interest and has been the object of pharmacological studies (Gumanova et al. 2007, Jiang et al. 2016, Korokina et al. 2009, Korokin et al. 2011, Kostina et al. 2016, Pokrovskii et al. 2006, Pokrovskii et al. 2008, Pokrovskii et al. 2011, Pokrovskii et al. 2012, Pokrovskaya et al. 2007, Voronkov et al. 2008, Tsepeleva et al. 2011, Tyurenkov and Voronkov 2008, Tyurenkov et al 2009, Shabelnikova et al. 2015, Shakhno et al. 2017, Yakushev and Pokrovskii 2016). The 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 showed that preventing the accumulation and overcoming of inhibition of eNOS by an already established ADMA can be one of the ways to pharmacologically correct endothelial dysfunction. Endothelium protective properties realized by reducing the inhibitory effect of ADMA on eNOS agents, such as L-arginine (Pokrovskii et al. 2008), tetrahydrobiopterin, non-selective arginase inhibitor L-norvaline, and selective arginase II inhibitors (Yakushev and Pokrovskii 2016), etc., were experimentally confirmed.

It has also been established that systemic inflammatory diseases, by increasing the level of oxidative stress, lead to the formation of oxidized forms of high-density circulating lipoprotein. The accumulation of lipid peroxidation products may adversely affect the function of endothelial cells, but the basic mechanisms of this state, as well as the possibilities for its correction, remain unclear (Pérez et al. 2019).

Along with the accumulation of knowledge about the pathways of metabolic disorders of nitric oxide, the methods for assessing endothelial dysfunction also change. Numerous *in vivo* or *in vitro*, as well as invasive or non-invasive methods are used to study endothelial function. A number of methods widely used in clinical studies cannot be used for diagnostic purposes because of their invasiveness, high cost, and complex standardization. A number of authors propose further research and investment in this area to make these methods applicable in clinical practice and, therefore, to minimize the public health problems associated with cardiovascular diseases by early diagnosis of endothelial dysfunction and early correction of nitric oxide metabolism (Steppan et al. 2013).

However, currently there are no drugs for specific pharmacological correction of the metabolic pathway L-arginine–eNOS–nitric oxide, whose disorders are a universal and fundamental trigger mechanism for initiating a cascade of metabolic disorders leading to arterial hypertension and other diseases of the cardiovascular system.

Methods to improve and restore endothelial dysfunction in various pathological conditions are being intensively studied. The data from clinical studies of drugs that improve endothelial dysfunction are shown in Table 1.

This article is aimed at making the case for possible ways to search for new targets for the pharmacological correction of endothelial dysfunction.

The role of NOX1 and NOX2 oxidases in the pharmacological correction of endothelial dysfunction

The study of endothelium-dependent vasodilation began with the observation that the removal of endothelial cells from isolated arteries prevented the vasodilatory response to the introduction of acetylcholine. Various vasoactive substances, such as ligands of the G-protein-conjugated receptors, exhibit a similar inducing effect on vasodilation. Endothelium-dependent vasodilation is caused by the release of prostacyclin PGI_2 and nitrogen monoxide (NO) or endothelial hyperpolarization factors (for example, H_2O_2) (Kvietys and Granger 2012). Dilation of isolated arteries caused by agonists or the bloodstream is blocked by catalase. The enzymatic destruction of glycocalyx, lining the surface of endothelial cells, significantly weakens the bloodstream-induced shear vasodilation (Kvietys and Granger 2012). Thus, the pharmacological stabilization of the microfilament / microtubule network of the endothelial cytoskeleton can contribute to vasodilation induced by the bloodstream.

NADPH oxidases (NOX) are enzymes that produce reactive oxygen species (ROS) and are involved in the pathogenesis of a number of endothelium-associated diseases, such as hypertension and stroke (Drummond and Sobey 2015).

An interesting fact is that studies have shown a decrease in the level of NO and an increase in the activity of arginase under the action of NOX2, which directly indicates the participation of NOX2 in the pathogenesis of endothelial dysfunction. Inhibition of NOX2 activity in endothelial cells in mice significantly prevents premature aging of endotheliocytes by limiting an increase in expression and arginase activity (Rojas et al. 2017).

The main source of reactive oxygen species (ROS) in vascular oxidative stress, which is one of the main causes of cardiovascular diseases, is NOX1 and NOX2 oxidases (Drummond et al. 2011). These NADPH•H oxidase isoforms are expressed by endothelial cells, and a balanced decrease in their activity with the help of selective inhibitors can be a rational approach to the therapy, including that of endothelial dysfunction. Vessel walls are exposed to direct destruction under the action

of excess ROS, which trigger a number of redox-sensitive transcriptional pathways (Drummond et al. 2011). Low-density lipoproteins (LDL) are trapped and oxidized in the vascular endothelium, and the oxidized form of LDL causes further oxidative stress in endothelial cells, smooth muscle cells, and foamy cells, which aggravates atherogenesis. ROSs produced in the vessel wall are highly reactive particles and do not force stable by-products into the bloodstream, which complicates the diagnosis of atherogenesis (Lee et al. 2012). Studies on the role of NADPH-H oxidases in endothelial homeostasis provide strong evidence that pharmacological inhibitors of this enzyme can be used as agents for the treatment of oxidative stress and vascular pathologies associated with it. Some inhibitors with an established NADPH-oxidase inhibitory activity are presented in Table 2 (Drummond et al. 2011).

Recently, a group of scientists developed and synthesized new photoactivatable analogs of NADPH. These compounds have one or two carboxymethyl groups at the 2'- and / and 3'-positions of the ribose in the adenosine fragment instead of the 2'-phosphate group and differ in nature by the electron donor in their photoactivatable chromophore (replacing the nicotinamide fragment). The dependence of eNOS on the formation of photoinduced NO was blocked using two NOS inhibitors (NS1 and L-NAME) aimed at the reductase and oxygenase domains, respectively. It has been established that two compounds that have one carboxymethyl group at the 3'-position of the ribose colocalize with the help of the Golgi apparatus (the main intracellular location of eNOS) and demonstrate a high intracellular two-photon brightness. In addition, eNOS-dependent photo-oxidation was observed only for these two compounds, which is accompanied by a significant intracellular production of NO, which takes into account specific photo-cytotoxic effects. Thus, it is obvious that effective photoactivatable NADPH analogues targeting NOS can have important consequences for the generation of apoptosis in tumor cells or the modulation of NO-dependent physiological processes. Studies have also shown a role of a number of naturally occurring phenolic compounds, such as berberine, timoquinone, catechin, celastrol, apocynin, resveratrol, curcumin, hesperidin, G-hesperidin and quercetin in the inhibition of NOX (Yousefian et al. 2019).

NOX1 and NOX2 oxidases appear to be the main causes of vascular oxidative stress in cardiovascular diseases, which makes them promising targets; however, a rational development of highly selective inhibitors of these isoforms is required. NOX2 oxidase plays a decisive role in oxidative stress and vascular dysfunction caused by insulin resistance (Sukumar et al. 2013). Acute and chronic inhibition of NOX2 with the 18-amino acid peptide gp91ds-tat causes the restoration of vasomotor function and a decrease in oxidative stress in animal models of insulin resistance (Sukumar et al. 2013). In transgenic mice with endothelium insulin resistance and the deleted NOX2 genome, superoxide anion formation reduced and the vascular function improved, which also indicates the involvement of NOX2 oxidase in the pathogenesis of diabetic endothelial dysfunction (Sukumar et al. 2013). The activation of NOX2 oxidase was documented and confirmed in neurovascular endothelium and neurons in such neurodegenerative diseases,as mild cognitive impairment, Parkinsons disease, and Alzheimer's disease (Zhang et al. 2001). The survival rate of neurons is significantly reduced in the presence of β-amyloid, but the toxic effect of the latter is weakened in the presence of apocynin, which is a NOX2 inhibitor (Cahill-Smith and Li 2014). NADPH oxidases are also considered as therapeutic targets for new treatments for ischemic stroke, often accompanied by endothelial dysfunction of cerebral vessels (McCann and Roulston 2013). Such inhibitors of NOX2 oxidase, such as apocynin, diphenylene iodonium, gp91ds-tat, VAS2870 (Table 1) showed promising results in reducing damage and restoring ischemia-exposed laboratory animals (McCann and Roulston 2013).

The role of ACE2 in the pharmacological correction of endothelial dysfunction

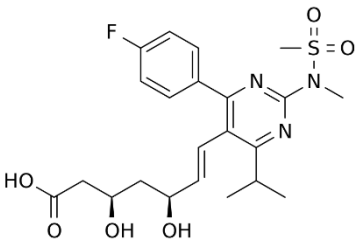
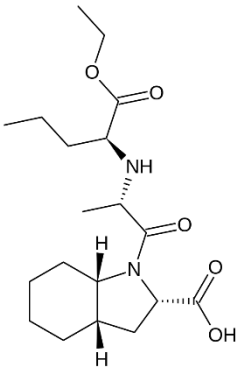
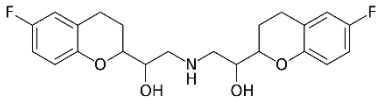
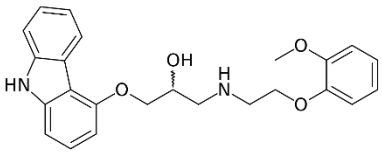
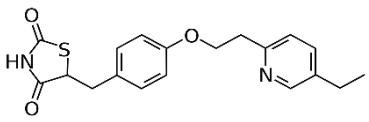
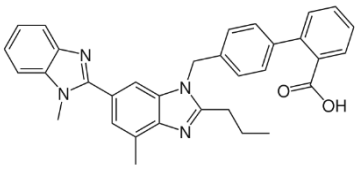
The next molecular target, the pharmacological effect on which can lead to an improvement in endothelial dysfunction, is agiotensin-converting enzyme 2 (ACE2). ACE2 catalyzes the transformation of peptides Ang I and Ang II into shorter peptides Ang-(1–9) and Ang-(1–7).

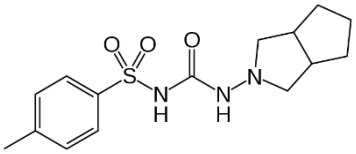
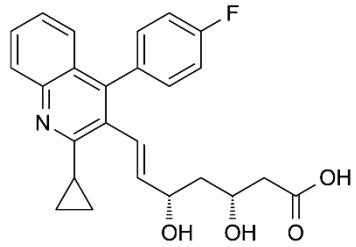
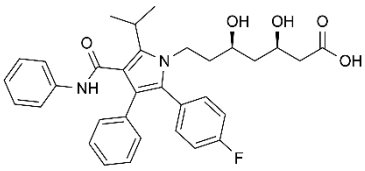
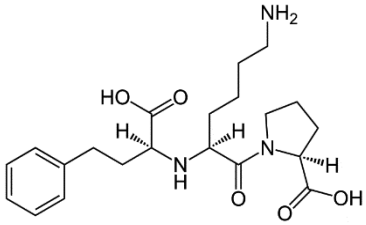
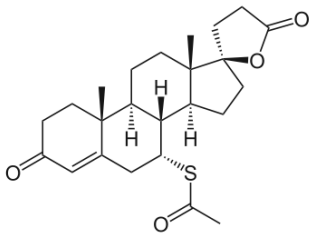
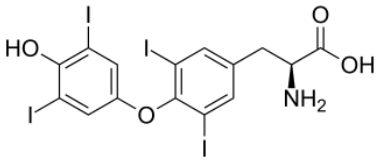
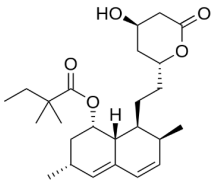
Numerous data suggest that the renin-angiotensin system (RAS) is the most important regulator of cardiovascular homeostasis and plays an important role in the pathogenesis of endothelial dysfunction and atherosclerosis (Zhang et al. 2015).

The low content of Ang-(1–7), caused by deactivation of ACE2, leads to vascular damage in hypertension, diabetes, atherosclerosis and kidney disease (Montezano et al. 2014). The effect of the low-molecular-weight compound XNT, which activates ACE2, on the endothelial function was studied in spontaneously hypertensive and diabetic rats (Fraga-Silva et al. 2013). The vasodilator responses of the aorta of the laboratory animals to acetylcholine and sodium nitroprusside increased significantly in XNT-treated (1 mg/kg per day for 4 weeks) animals with both pathologies; also a decrease in the level of ROS in the aorta was recorded (Fraga-Silva et al. 2013).

Currently, it has been demonstrated that overexpression of ACE2 and Ang-(1–7) protects the function of endothelial cells and inhibits atherosclerotic evolution. The mechanism of protection of endothelial cells and the anti-atherosclerotic action of ACE2 and Ang-(1–7) is due to the counterregulation of Ang II signaling, the inhibition of the inflammatory response and an increase in the ability of the antioxidant (Zhang et al. 2015).

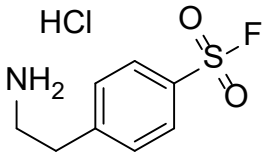
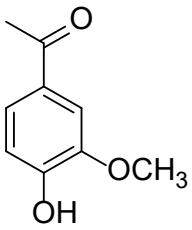
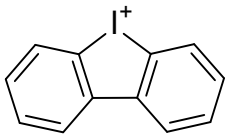
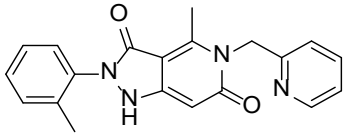
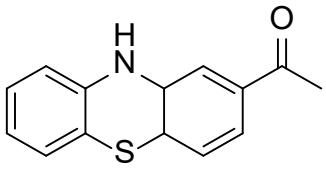
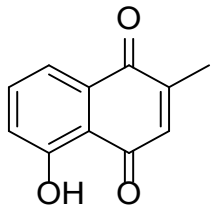
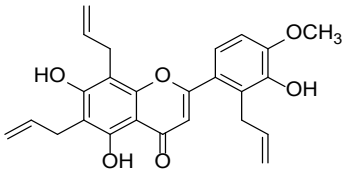
Table 1. Clinical studies of drugs that resulted in improved endothelial dysfunction.

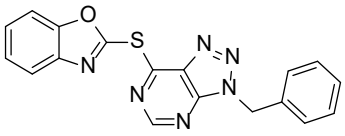
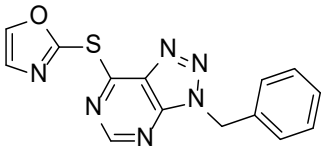
Pathological condition	Medicinal product	Structural formula	Main pharmacological effects
Chronic heart failure	Rosuvastatin (lipid-lowering drug of IV generation from the group of statins)		Oxidized LDL ↓ Lipid peroxidation ↓ Stem cells and progenitor cells ↑ POR ↑ (Erbs et al. 2011) EDVD ↑ Apoptosis ↓ SOD ↑ MDA ↓ (Geng et al. 2019, Radenković et al. 2013)
Acute coronary syndrome	Perindopril (angiotensin converting enzyme (ACE) inhibitor)		Apoptosis ↓ CD34 + mobilization ↑ VEGF ↑ TNF-α ↓ Bradykinin ↑ Angiotensin 2 ↓ eNOS mRNA ↑ (Cangiano et al. 2011)
Cardiac syndrome X	Nebivolol (cardioselective beta-blocker of III generation with vasodilating properties)		FMD ↔ High sensitivity to C-reactive protein ↓ Factor von Willebrand ↓ Fibrinogen ↓ MDA ↓ EDVD ↑ (Borghi et al. 2017)
Hypertensive left ventricular hypertrophy	Carvedilol (alpha and beta-blocker without internal sympathomimetic activity)		FMD ↑ Endothelin-1 ↓ NO ↑ (Xiaozhen et al. 2010) ENVD ↑ (Peller et al. 2015)
Coronary heart disease and impaired glucose tolerance	Pioglitazone (selective stimulant of PPAR-γ receptors and, to a lesser extent, of PPAR-α receptors)		FMD ↑ TNF-α ↓ Triglycerides ↓ high molecular weight adiponectin ↑ (Rizza et al. 2011)
Hypertension and impaired glucose tolerance / Peripheral artery disease	Telmisartan (angiotensin II receptor antagonist)		FMD ↑ Insulin resistance ↓ Glucose Tolerance ↑ (Perl et al. 2010) P38 MAPK NO ↑ Angiotensin 2 ↓ (Radenković et al. 2013) Maximum walking distance ↑ FMD ↑ (Zankl et al. 2010)

Pathological condition	Medicinal product	Structural formula	Main pharmacological effects
Type 2 diabetes	Gliclazide (insulin secretion stimulator by pancreatic beta cells)		FMD ↑ Endothelial progenitor cells ↑ (Chen et al. 2011) IL-6 ↓ ICAM-1 ↓ E-selectin ↓ (Erem et al. 2014)
Obesity	Pitavastatin (HMG-CoA reductase inhibitor)		FMD ↑ Triglycerides ↑ (Nagashima and Endo 2011) Apoptosis ↓ NO ↑ PGE2 ↓ eNOS ↑ (Haybar et al. 2019)
Behcet's disease	Atorvastatin (selective competitive inhibitor of HMG-CoA reductase)		
Behcet's disease	Lisinopril (an ACE inhibitor of prolonged action)		FMD ↑ (Inanc et al. 2010) Apoptosis ↓ CD34 + mobilization ↑ TNF-α ↓ Bradykinin ↑ Angiotensin 2 ↓ (Cangiano et al. 2010)
Polycystic ovary syndrome	Spironolactone (potassium-sparing diuretic, competitive antagonist of aldosterone and other mineralocorticoids)		FMD ↑ (Bajuk et al. 2011) Icam-1 ROS ↓ eNOS ↑ (Dutzmann et al. 2014)
Subclinical hypothyroidism	Levothyroxine		POR ↑ FT4 ↑ TSH ↓ Total cholesterol ↓
Ankylosing spondylitis	Infliximab (monoclonal antibodies to TNF-α)	–	FMD ↑ Serum nitrite ↓ Erythrocyte sedimentation rate ↓ C-reactive protein ↓ (Syngle et al. 2010)
Chronic hemodialysis	Simvastatin (active metabolite inhibits HMG-CoA reductase)		FMD ↑ Oxidized LDL ↓ VCAM-1 ↓ 8-epi-prostagandin F2 ↓ NO bioavailability (Kishimoto et al. 2019)

Note: LDL – low-density lipoproteins; SOD – superoxide dismutase; MDA – malonic dialdehyde; FMD – flow-mediated dilatation; VEGF – Vascular endothelial growth factor; TNF-α – tumor necrosis factor –α; TSH – thyroid-stimulating hormone; VCAM-1 – vascular cell adhesion molecule 1; ROS – reactive oxygen species; eNOS – endothelial nitric oxide synthase.

Table 2. NADPH inhibitors•H-oxidases (26).

Name	Structural formula	Mechanism of action related to NADP•H-oxidase	Other pharmacological effects
4- (2-aminoethyl) benzenesulfonylfluoride		Oxidase assembly inhibitor: inhibits the association of the NOX2 subunit and p47phox. Does not remove $O_2^{\bullet-}$ generated in systems that do not contain cells	Non-selective serine protease inhibitor
Apocynin		Oxidase assembly inhibitor: inhibits the association of p47phox and membrane-bound heterodimer	H_2O_2 absorber
Diphenyleneiodonium		Flavoprotein inhibitor	Inhibitor of NADPH•H-ubiquinone oxidoreductase, NADPH •H-dehydrogenase, xanthine oxidase, cytochrome-p450 oxidoreductase, NOS and bacterial nicotine oxidase
GK-136901		The claimed inhibitor of NOX1 and NOX4 oxidases. The mechanism of action is not established, but the structural similarity with NADP•H implies competitive substrate inhibition	–
ML171		Selective inhibitor of NOX1 oxidase (IC_{50} 0.25 μM) compared with other isoforms of NADPH-H oxidases (IC_{50}>3 μM). Does not absorb ROS generated by xanthine oxidase	–
Nox2ds-tat	(H)-RKKRRQRRRCSTRIRRQL-NH2	Oxidase assembly inhibitor: inhibits the association of the NOX2 subunit and p47phox. Does not remove $O_2^{\bullet-}$ generated in systems that do not contain cells	–
Plumbagin		Inhibits the NADPH-dependent $O_2^{\bullet-}$ formation in various cell lines expressing NOX4 oxidase; mechanism of action is unknown	Chemical structure can provide an ROS-absorbing effect.
PR-39	RRRPRPPYLPRPRPPPFFPPRLPPRIPPG FPPRFPPRFP	Binds to the SH3 domains of the p47phox subunit and prevents binding to the p22phox subunit	Appears to bind to other proteins containing SH3 domains.
S17834		Alleged direct inhibitor of NADPH oxidase, but the mechanism of action is not established.	–

Name	Structural formula	Mechanism of action related to NADP•H-oxidase	Other pharmacological effects
VAS2870		Unidentified action mechanism, does not bind $O_2^{\bullet-}$	–
VAS3947		Reduces ROS produced by NADPH-oxidase, has micromolar activity, regardless of the isoform, does not inhibit the formation of ROS by xanthine oxidase and eNOS activity	–

Note: eNOS – endothelial NO synthase; SH3 – Src homologous 3 domain.

Arginase II is a promising target for the creation of drugs aimed at the pharmacological correction of endothelium-associated pathology

The decrease in the bioavailability of NO, leading to endothelial dysfunction, can be caused by competition between endothelial NO synthase (eNOS) and arginase for a common substrate – L-arginine. The expression of arginase and its activity is increased in many cardiovascular diseases, including ischemic-reperfusion injuries, hypertension, atherosclerosis, and diabetes (Rabelo et al. 2015, Shakhno et al. 2017).

The studies conducted have provided evidence of the role of Arg-I in promoting endothelial cell aging, confirming that activation of both Arg-I and Arg-II have similar functions of inducing the uncoupling of eNOS from the substrate. The molecular basis for the existing subtle difference between Arg-I and Arg-II is yet to be further studied.

Arginase contributes to microvascular endothelial dysfunction in obesity. Its effect decreases with age due to a higher level of vascular oxidative stress. Obesity is accompanied by accelerated microvascular remodeling, the extent of which is related to the amount of arginase in the vascular wall (Masi et al. 2018).

In endothelial cells, arginase isoform II prevails. The structure of both human arginase isoforms was studied using an X-ray structural analysis, which made it possible to identify a binuclear manganese cluster in the active site of enzymes and the identical structure of the active sites. In the active site of the enzyme, ornithine and urea are formed as a result of the breakdown of the tetrahedral intermediate formed by adding a hydroxide anion coordinated by manganese ions to the guanidine group of arginine. The breakthrough step in the development of arginase inhibitors was the discovery of 2-(S)-amino-6-boronohexanoic acid (ABH) (Masi et al. 2018). A fragment of boronic acid ABH undergoes a nucleophilic attack by a hydroxide anion, as a result of which a tetrahedral boronate anion is formed, resembling an intermediate, resulting in the cleavage of arginine. The rational design of stronger inhibitors led to the understanding that substituents can be introduced into the α-position of the amino acid fragment, providing for additional interactions with arginase. The result of this work was the production of (R)-2-amino-6-borono-2-(2-(piperidin-1-yl) ethyl) hexanoic acid (1) (Fig. 1), which has sub-micromolar activity with respect to human arginase I and II (Van Zandt et al. 2013). Inhibitor 1 was also investigated in the *in vivo* model of myocardial ischemia / reperfusion injury and showed a significant reduction in the area of infarction (Van Zandt et al. 2013). It should be noted that the enantiomer of compound 1, which is distinguished by the absolute configuration of the stereogenic center, has neither an inhibitory activity against arginase, nor a therapeutic effect in myocardial ischemia.

The introduction of the piperidine and tropan fragments into the α-position of the ABH amino acid fragment resulted in arginase inhibitors 2 and 3, respectively (Fig. 1), having a nanomolar activity against human arginase (Golebiowski et al. 2013). Correction of endothelial dysfunction caused by obesity was achieved by introducing the inhibitor of arginase N^{ω}-hydroxy-nor-L-arginine (nor-NOHA) into the diet of the studied rats (Chung et al. 2014). In this case, a significant increase in the expression of eNOS and in the level of NO and a significant decrease in the content of the intercellular adhesion molecule 1 (ICAM-1) in plasma were recorded (Chung et al. 2014). Despite the curative effects of arginase inhibitors, its presence in macrophages should be taken into account, as it may entail inflammatory and other undesirable effects on the vessels and some organs (Steppan et al. 2013).

The possible role of the regulatory enzyme GSNO reductase (GSNOR) in the pharmacological correction of endothelial dysfunction

One of the main organs dependent on GSNOR is the heart and its surrounding vascular system. GSNOR is an attractive therapeutic goal. Inhibition of GSNOR increases the availa-

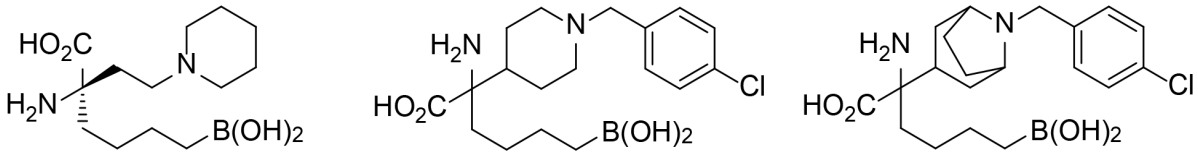


Figure 1. Structural formulas of arginase inhibitors, developed using rational design.

bility of NO in the cell and, in turn, normalizes NO-mediated signaling pathways. (Cahill-Smith and Li 2014)

One of the physiological forms of NO is S-nitrosoglutathione (GSNO) of cytoplasm. GSNO catabolism with the help of the GSNO reductase regulatory enzyme (GSNOR) reduces the amount of GSNO *in vivo*, which leads to an influence on the processes involving NO, including vasodilatation. The endothelial function was studied under the conditions of inhibiting GSNOR, and the vasodilating effect of the GSNOR inhibitor N6338 was established (Chen et al. 2014). In hypertensive rats, oral administration of N6338 for 14 days reduced blood pressure and vascular resistance index, and restored the initially impaired flow-mediated vasodilation to the level of the normal animals. There was no chemical formula of N6338 in either (Chen et al. 2014), or in cross references.

In addition, dozens of small molecules were identified that can inhibit GSNOR to varying degrees (Green et al. 2012, Jiang et al. 2016).

Two of them, N6022 (3-(5-(4-(1H-imidazol-1-yl) phenyl)-1-(4-carbamoyl-2-methylphenyl)-1H-pyrrol-2-yl) propionic acid) and N91115 from Nivalis Therapeutics (USA) are promising as potentially safe and effective GSNOR inhibitors that have been clinically tested for the treatment of asthma (clinictrials.gov – NCT01316315) and cystic fibrosis. In mice, it has been demonstrated that the ILD compound SPL-334 functions as a prophylactic and therapeutic agent for attenuating profibrotic cytokines and the accumulation of collagen in the lungs.

Thus, GSNOR can also be considered as a target for drugs that improve endothelial dysfunction.

Conclusion

From an enzymological point of view, NOS has a number of unique properties: NOS is the most regulated enzyme in biology. This is the only known enzyme that has 5 cofactors. As a result, the system, the generation of nitric oxide is the most sensitive system that responds to many changes that occur in the body.

The most recent studies showing the ubiquitous localization of arginase I and II make it possible not to dwell on this differentiation and not to mark any significant distinction between selective and non-selective inhibitors. In addition, all arginase inhibitors are divided into specific, acting directly on the enzyme itself and blocking its active center, and non-specific, acting on the enzyme indirectly.

NOX1 and NOX2 oxidases, as NADP•H oxidase isoforms, expressed by endothelial cells, agiotensin-converting enzyme 2 and GSNO reductase may be of interest in a targeted search for drugs for the pharmacological correction of endothelial dysfunction.

Conflicts of Interest

The authors have no conflict of interest to declare.

References

- Bajuk Studen K, Šebeštjen M, Pfeifer M, Preželj J (2011) Influence of spironolactone treatment on endothelial function in non-obese women with polycystic ovary syndrome. European Journal of Endocrinology 164(3): 389–395. https://doi.org/10.1530/EJE-10-0709 [PubMed]
- Borghi C, Acelajado M, Gupta Y, Jain S (2017) Role of nebivolol in the control and management of central aortic blood pressure in hypertensive patients. Journal of Human Hypertension 31(10): 605–610. https://doi.org/10.1038/jhh.2017.26 [PubMed]
- Cahill-Smith S, Li J-M (2014) Oxidative stress, redox signalling and endothelial dysfunction in ageing-related neurodegenerative diseases: a role of NADPH oxidase. British Journal of Clinical Pharmacology 78(3): 441–453. https://doi.org/10.1111/bcp.12357 [PubMed] [PMC]
- Cangiano E, Marchesini J, Campo G, Francolini G, Fortini C, Carrà G, Miccoli M, Ceconi C, Tavazzi L, Ferrari R (2011) ACE inhibition modulates endothelial apoptosis and renewal via endothelial progenitor cells in patients with acute coronary syndromes. American Journal of Cardiovascular Drugs 11(3): 189–198. https://doi.org/10.2165/11589400-000000000-00000 [PubMed]
- Chen Q, Sievers RE, Varga M, Kharait S, Haddad DJ, Patton AK, Delany CS, Mutka SC, Blonder JP, Dubé GP, Rosenthal GJ, Springer ML (2014) Pharmacological inhibition of S-nitrosoglutathione reductase improves endothelial vasodilatory function in rats in vivo. Journal of Applied. Physiology 114(6): 752–760. https://doi.org/10.1152/japplphysiol.01302.2012 [PubMed] [PMC]
- Chen LL, Yu F, Zeng TS, Liao YF, Li YM, Ding HC (2011) Effects of gliclazide on endothelial function in patients with newly diagnosed type 2 diabetes. European Journal of Pharmacology 659(2–3): 296–301. https://doi.org/10.1016/j.ejphar.2011.02.044 [PubMed]
- Chung JH, Moonb J, Lee YS, Chung HK, Lee SM, Shin MJ (2014) Arginase inhibition restores endothelial function in diet-induced obe-

sity. Biochemical and Biophysical Research Communications 451(2): 179–183. https://doi.org/10.1016/j.bbrc.2014.07.083 [PubMed]

- Drummond GR, Selemidis S, Griendling KK, Sobey CG (2011) Combating oxidative stress in vascular disease: NADPH oxidases as therapeutic targets. Nature Reviews Drug Discovery 10(6): 453–471. https://doi.org/10.1038/nrd3403 [PubMed] [PMC]
- Drummond GR, Sobey CG (2015) Endothelial NADPH oxidases: which NOX to target in vascular disease. Trends in Endocrinology and Metabolism 25(9): 452–463. https://doi.org/10.1016/j.tem.2014.06.012 [PubMed]
- Dutzmann J, Bauersachs J, Sedding DG (2014) Evidence for the use of mineralocorticoid receptor antagonists in the treatment of coronary artery disease and post-angioplasty restenosis. Vascular Pharmacology 107: 20–26. https://doi.org/10.1016/j.vph.2017.12.065 [PubMed]
- Erbs S, Beck EB, Linke A, Adams V, Gielen S, Kränkel N, Möbius-Winkler S, Höllriegel R, Thiele H, Hambrecht R, Schuler G (2011) High-dose rosuvastatin in chronic heart failure promotes vasculogenesis, corrects endothelial function, and improves cardiac remodeling – results from a randomized, double-blind, and placebo-controlled study. International Journal of Cardiology 146(1): 56–63. https://doi.org/10.1016/j.ijcard.2010.02.019 [PubMed]
- Erem C, Ozbas HM, Nuhoglu I, Deger O, Civan N, Ersoz HO (2014) Comparison of effects of gliclazide, metformin and pioglitazone monotherapies on glycemic control and cardiovascular risk factors in patients with newly diagnosed uncontrolled type 2 diabetes mellitus. Experimental and Clinical Endocrinology and Diabetes 122(5): 295–302. https://doi.org/10.1055/s-0034-1370989 [PubMed]
- Fraga-Silva RA, Costa-Fraga FP, Murça TM (2013) Angiotensin-converting enzyme 2 activation improves endothelial function. Hypertension 61(6): 1233–1238. https://doi.org/10.1161/HYPERTENSIONAHA.111.00627 [PubMed] [PMC]
- Geng J, Xu H, Yu X, Xu G, Cao H, Lin G, Sui D (2019) Rosuvastatin protects against oxidized low-density lipoprotein-induced endothelial cell injury of atherosclerosis in vitro. Molecular Medicine Reports 19(1): 432–440. https://doi.org/10.3892/mmr.2018.9666 [PubMed] [PMC]
- Green LS, Chun LE, Patton AK, Sun X, Rosenthal GJ, Richards JP (2012) Mechanism of inhibition for N6022, a first-in-class drug targeting S-nitrosoglutathione reductase. Biochemistry 51(10): 2157–2168. https://doi.org/10.1021/bi201785u [PubMed]
- Golebiowski A, Whitehouse D, Beckett RP, van Zandt M, Ji MK, Ryder TR, Jagdmann E, Andreoli M, Lee Y, Sheeler R, Conway B, Olczak J, Mazur M, Czestkowski W, Piotrowska W, Cousido-Siah A, Ruiz FX, Mitschler A, Podjarny A, Schroeter H (2013) Synthesis of quaternary α-amino acid-based arginase inhibitors via the Ugi reaction. Bioorganic and Medical Chemistry Letters 23(17): 4837–4841. https://doi.org/10.1016/j.bmcl.2013.06.092 [PubMed]
- Gumanova NG, Artyushkova YeB, Metelskaya VA, Kochkarov VI, Pokrovskaya TG, Danilenko LM, Korneev MM, Pokrovsky MV, Pashin YeN (2007) Effect of antioxidants Q510 and resveratrol on the regulatory function of the endothelium in rats with simulated arterial hypertension. Bulletin of Experimental Biology and Medicine 143(6): 678–681. https://doi.org/10.1007/s10517-007-0212-x [PubMed]
- Haybar H, Shahrabi S, Rezaeeyan H, Shirzad R, Saki N (2019) Endothelial cells: from dysfunction mechanism to pharmacological effect in cardiovascular disease. Cardiovascular Toxicology 19(1): 13–22. https://doi.org/10.1007/s12012-018-9493-8 [PubMed]
- Inanc MT, Kalay N, Heyit T, Ozdogru I, Kaya MG, Dogan A, Duran M, Kasapkara HA, Gunebakmaz O, Borlu M, Yarlıoglues M, Oguzhan A (2010) Effects of atorvastatin and lisinopril on endothelial dysfunction in patients with Behçet's disease. Echocardiography. 27(8): 997–1003. https://doi.org/10.1111/j.1540-8175.2010.01180.x [PubMed]
- Jiang H, Polhemus DJ, Islam KN, Torregrossa AC, Li Z, Potts A, Lefer DJ, Bryan NS (2016) Nebivolol acts as a S-nitrosoglutathione reductase inhibitor. Journal of Cardiovascular Pharmacology and Therapeutics 21(5): 478–485. https://doi.org/10.1177/1074248415626300 [PubMed]
- Kishimoto N, Hayashi T, Sakuma I, Kano-Hayashi H, Tsunekawa T, Osawa M, Ina K, Iguchi A (2009) A hydroxymethylglutaryl coenzyme A reductase inhibitor improves endothelial function within 7 days in patients with chronic hemodialysis. International Journal of Cardiology 145(1): 21–26. https://doi.org/10.1016/j.ijcard.2009.05.023 [PubMed]
- Korokina L, Kolesnik I, Pokrovsky M (2009) Pharmacological correction of L-name induced nitric oxide deficiency with recombinant erythropoietin. Kuban Scientific Medical Journal [Kubanskij Nauchnyj Medicinskij Vestnik] 9: 66–69. [in Russian]
- Korokin M, Pokrovsky M, Novikov O, Gudyrev O, Gureev V, Denisyuk T, Korokina L, Danilenko L, Ragulina V, Konovalova E, Belous A (2011) Model of hyperhomocysteine-induced endothelial dysfunction in rats. Bulletin of Experimental Biology and Medicine 152(2): 173–175. https://doi.org/10.1007/s10517-011-1491-9 [PubMed]
- Kostina DA, Pokrovskaya TG, Pokrovskii MV, Lazareva GA, Provotorov VY, Stepchenko AA (2016) Approaches to pharmacological correction of endothelial dysfunction associated with metabolic endotoxemia. International Journal of Pharmacy and Technology 8(2): 14291–14299.
- Kvietys PR, Granger DN (2012) Role of reactive oxygen and nitrogen species in the vascular responses to inflammation. Free Radical Biology and Medicine 52: 556–592. https://doi.org/10.1016/j.freeradbiomed.2011.11.002 [PubMed] [PMC]
- Lee R, Margaritis M, Channon K, Antoniades C (2012) Evaluating oxidative stress in human cardiovascular disease: methodological aspects and considerations. Current Medicinal Chemistry 19(16): 2504–2520. https://doi.org/10.2174/092986712800493057 [PubMed] [PMC]
- Masi S, Colucci R, Duranti E, Nannipieri M, Anselmino M, Ippolito C, Tirotta E, Georgiopoulos G, Garelli F, Nericcio A, Segnani C, Bernardini N, Blandizzi C, Taddei S, Virdis A (2018) Aging modulates the influence of arginase on endothelial dysfunction in obesity. Arteriosclerosis, Thrombosis, and Vascular Biology 38(10): 2474–2483. https://doi.org/10.1161/ATVBAHA.118.311074 [PubMed]
- McCann SK, Roulston CL (2013) NADPH Oxidase as a therapeutic target for neuroprotection against ischaemic stroke: future perspectives. Brain Sciences 3(2): 561–598. https://doi.org/10.3390/brainsci3020561 [PubMed] [PMC]
- Montezano AC, Cat AND, Rios FJ, Touyz RM (2014) Angiotensin II and vascular injury. Current Hypertension Report 16(6): 431. https://doi.org/10.1007/s11906-014-0431-2 [PubMed]
- Nagashima H, Endo M (2011) Pitavastatin prevents postprandial endothelial dysfunction via reduction of the serum triglyceride level in obese male subjects. Heart and Vessels 26(4): 428–434. https://doi.org/10.1007/s00380-010-0071-7 [PubMed]
- Peller M, Ozierański K, Balsam P, Grabowski M, Filipiak KJ, Opolski G (2015) Influence of beta-blockers on endothelial function: a

meta-analysis of randomized controlled trials. Cardiology Journal 22(6): 708–716. https://doi.org/10.5603/CJ.a2015.0042 [PubMed]

- Perl S, Schmölzer I, Sourij H, Pressl H, Eder M, Zweiker R, Wascher TC (2010) Telmisartan improves vascular function independently of metabolic and antihypertensive effects in hypertensive subjects with impaired glucose tolerance. International Journal of Cardiology 139(3): 289–296. https://doi.org/10.1016/j.ijcard.2008.10.048 [PubMed]
- Pérez L, Vallejos A, Echeverria C, Varela D, Cabello-Verrugio C, Simon F (2019) OxHDL controls LOX-1 expression and plasma membrane localization through a mechanism dependent on NOX/ROS/NF-κB pathway on endothelial cells. Laboratory Investigation 99(3): 421–437. https://doi.org/10.1038/s41374-018-0151-3 [PubMed]
- Pokrovskaya T, Kochkarov V, Pokrovsky M, Artyushkova E, Pashin E, Korokin M, Korokina L, Zaloznykh Y, Klyavs Y, Brusnik M, Korneev M, Chernomortseva E, Chulyukova T, Zelenkova T, Akhmetzyanova I, Smeshko N, Malykhin V (2007) Principles of pharmacological correction of endothelial dysfunction. Kuban Scientific Medical Journal [Kubanskij Nauchnyj Medicinskij Vestnik] 1–2: 146–149. [in Russian]
- Pokrovskii M, Kochkarov V, Pokrovskaja T, Gladchenko M, Artjushkova E, Pashin E, Brusnik M, Chulukova T, Kljavs J, Korneev M, Zelenkova T, Malyhin V, Belous A, Zaloznyh J, Majakov A (2006) Methodical approaches for the quantitative assessment of the development of endothelial dysfunction in the L-NAME-induced model of nitric oxide deficiency in the experiment. Kuban Scientific Medical Journal [Kubanskij Nauchnyj Medicinskij Vestnik] 10(91): 72–77. [in Russian]
- Pokrovskii M, Pokrovskaya T, Kochkarov V, Artyushkova E (2008) Endothelium protective effects of L-arginine in the simulation of nitric oxide deficiency. Experimental and Clinical Pharmacology 71(2): 29–31.
- Pokrovskii M, Pokrovskaya T, Gureev V, Barsuk A, Proskuryakova E, Korokin M, Belous A, Korokina L, Ragulina V, Gudyrev O, Levashova O, Korolev A, Maltseva N, Polyanskaya O, Terekhova Y, Babko A (2011) Pharmacological correction of ADMA-eNOS-associated targets in preeclampsia. Obstetrics and Gynecology 2: 16–20.
- Pokrovskii M, Pokrovskaya T, Gureev V, Barsuk A, Proskuryakova E, Korokin M, Gudyrev O, Belous A, Kochkarov V, Danilenko L, Levashova O, Maltseva N, Polyanskaya O (2012) Use of L-arginine in the correction of endothelial dysfunction in experimental preeclampsia. Experimental and Clinical Pharmacology 75(2): 14–16.
- Rabelo LA, Ferreira FO, Nunes-Souza V, da Fonseca LJSD, Goulart MO (2015) Arginase as a Critical Prooxidant Mediator in the Binomial Endothelial Dysfunction-Atherosclerosis. Oxidative Medicine and Cellular Longevity. Article ID 924860 (20). https://doi.org/10.1155/2015/924860 [PubMed] [PMC]
- Radenković M, Stojanović M, Potpara T, Prostran M (2013) Therapeutic approach in the improvement of endothelial dysfunction: the current state of the art. BioMed Research International 52: 556–592. https://doi.org/10.1155/2013/252158 [PubMed] [PMC]
- Rizza S, Cardellini M, Porzio O, Pecchioli C, Savo A, Cardolini I, Senese N, Lauro D, Sbraccia P, Lauro R, Federici M (2011) Pioglitazone improves endothelial and adipose tissue dysfunction in pre-diabetic CAD subjects. Atherosclerosis 215(1): 180–183. https://doi.org/10.1016/j.atherosclerosis.2010.12.021 [PubMed]
- Rojas M, Lemtalsi T, Toque HA, Xu Z, Fulton D, Caldwell RW, Caldwell RB (2017) NOX2-induced activation of arginase and diabetes-induced retinal endothelial cell senescence. Antioxidants 6(2): 43. https://doi.org/10.3390/antiox6020043 [PubMed] [PMC]
- Shabelnikova AS, Lutsenko VD, Pokrovskii MV, Peresipkina AA, Korokin MV, Gudyrev OS, Pokrovskaia TG, Beskhmelnitsyna EA, Hoshenko YA (2015) Protective effects of recombinant erythropoietin in ischemia of the retina: The role of mechanisms of preconditioning. Research Journal of Medical Sciences 9(4): 200–203.
- Shakhno EA, Savitskaya TA, Pokrovskaya TG, Yakushev VI, Grinshpan DD (2017) New dosage form of L-arginine based on its complex with cellulose acetate sulfate and activated carbon. Research Results in Pharmacology and Clinical Pharmacology 3(2): 101–111.
- Steppan J, Nyhan D, Berkowitz D (2013) Development of novel arginase inhibitors for therapy of endothelial dysfunction. Frontiers in Immunology 4: 278. https://doi.org/10.3389/fimmu.2013.00278 [PubMed] [PMC]
- Sukumar P, Viswambharan H, Imrie H, Cubbon RM, Yuldasheva N, Gage M, Galloway S, Skromna A, Kandavelu P, Santos CX, Gatenby VK, Smith J, Beech DJ, Wheatcroft SB, Channon KM, Shah AM, Kearney MT (2013) Nox2 NADPH oxidase has a critical role in insulin resistance–related endothelial cell dysfunction. Diabetes 62(6): 2130–2134. https://doi.org/10.2337/db12-1294 [PubMed] [PMC]
- Syngle A, Vohra K, Sharma A, Kaur L (2010) Endothelial dysfunction in ankylosing spondylitis improves after tumor necrosis factor-*α*blockade. Clinical Rheumatology 29(7): 763–770. https://doi.org/10.1007/s10067-010-1402-x [PubMed]
- Van Zandt MC, Whitehouse DL, Golebiowski A, Ji MK, Zhang M, Beckett RP, Jagdmann GE, Ryder TR, Sheeler R, Andreoli M, Conway B, Mahboubi K, D'Angelo G, Mitschler A, Cousido-Siah A, Ruiz FX, Howard EI, Podjarny AD, Schroeter H (2013) 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. Journal of Medicinal Chemistry 56(6): 2568–2580. https://doi.org/10.1021/jm400014c [PubMed]
- Voronkov A, Robertus A, Tyurenkov I (2008) Study of the "L-arginine paradox" for the assessment of endothelial function in health and pathology. Regional Blood Circulation and Microcirculation. 7(3): 54–57.
- Xiaozhen H, Yun Z, Mei Z, Yu S (2010) Effect of carvedilol on coronary flow reserve in patients with hypertensive left-ventricular hypertrophy. Blood Pressure 19(1): 40–47. https://doi.org/10.3109/08037050903450492 [PubMed]
- Yakushev VI, Pokrovskii MV (2016) Cardiovascular effects of an arginase II selective inhibitor. Research Results in Pharmacology and Clinical Pharmacology. 2(3): 28–46. https://doi.org/10.18413/2500-235X-2016-2-3-28-45
- Yousefian M, Shakour N, Hosseinzadeh H, Hayes AW, Hadizadeh F, Karimi G (2019) The natural phenolic compounds as modulators of NADPH oxidases in hypertension. Phytomedicine 55: 200–213. https://doi.org/10.1016/j.phymed.2018.08.002 [PubMed]
- Zhang C, Hein TW, Wang W, Chang CI, Kuo L (2001) Constitutive expression of arginase in microvascular endothelial cells counteracts nitric oxide-mediated vasodilatory function. The FASEB Journal 15(7): 1264–1266. https://doi.org/10.1096/fj.00-0681fje [PubMed]
- Zhang YH, Hao QQ, Zhou XM (2015) ACE2 and Ang-(1–7) protect endothelial cell function and prevent early atherosclerosis by inhibiting inflammatory response. Inflammation Research 64(3–4): 53–60. https://doi.org/10.1007/s00011-015-0805-1 [PubMed]
- Zankl AR, Ivandic B, Andrassy M (2010) Telmisartan improves absolute walking distance and endothelial function in patients with peripheral artery disease. Clinical Research in Cardiology 99(12): 787–794. https://doi.org/10.1007/s00392-010-0184-0 [PubMed]

Contribution of authors

- **Liliya V. Korokina,** PhD, Associate Professor, Department of Pharmacology and Clinical Pharmacology; e-mail: korokina@bsu.edu.ru. The author analyzed publications on modern research in the field of pharmacological correction of endothelial dysfunction, possible approaches to the search for new pharmacological targets and took part in the systematization and generalization of the material for the article.

- **Ivan V. Golubev,** obstetrician-gynecologist; e-mail: golubevvano@yandex.ru. The author analyzed the role of NOX1 and NOX2 oxidase in the pharmacological correction of endothelial dysfunction

- **Olga N. Pokopejko,** student, Medical Faculty; e-mail: OPokopejko@mail.ru. The author conducted an analysis of the role of APF2 in the pharmacological correction of endothelial dysfunction

- **Anastasia V. Zagrebelnaya,** postgraduate student, Department of Pharmacology and Clinical Pharmacology; e-mail: Zagrebelnaya@bsu.edu.ru. The author searched for data on the possible role of the regulatory enzyme GSNO reductase (GSNOR) in the pharmacological correction of endothelial dysfunction.

- **Sergey A. Demchenko,** postgraduate student, Department of Pharmacology and Clinical Pharmacology; e-mail: demchenkoserg@mail.ru. The author analyzed arginase 2 as a target for pharmacological correction of endothelial dysfunction.