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Nitric oxide-dependent mechanism of endothelial dysfunction formation is a promising target link for pharmacological management

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Herein, we presented a review to show that the main mechanism underlying endothelial dysfunction is a decrease in the formation and bioavailability of NO against the background of inhibition of eNOS expression and reduced equivalents of the thiol-disulfide system with a simultaneous increase in the levels of cytotoxic forms of NO and the production of powerful vasoconstrictors. Based on the foregoing, the conjugated complex eNOS-L-arginine-NO/SH can be undoubtedly claimed in the near future as a promising target for the pharmacological correction of endothelial dysfunction. Since it is a nitrosative stress that plays the role in the development of endothelial dysfunction, it is topical to search for potential endothelial protectors in the series of S-substituted 1,2,4-triazole, which have the properties of antioxidanitts and NO scavengers. A striking representative of this cohort is Thiotriazolin which is a drug with cardioprotective, anti-ischemic and antioxidant properties, though does not possesses endothelial protective activity. As a result of chemical modification of the molecule of the latter, we obtained the compound (S)-2,6-diaminohexanoic acid 3-methyl-1,2,4-triazolyl-5-thioacetate (Angiolin), which manifests endothelium-protective properties against the background of cardioprotective, anti-ischemic and antioxidant activities.

K e y w o r d s: endothelial dysfunction, NO, nitrosative stress, reduced thiols, S-derivatives of 1,2,4-triazole

Introduction

According to the current concept of cardiovascular disease continuum, the endothelial dysfunction (ED) is one of early pathogenic factors that mediates a link of vascular damage

with incident cardiovascular diseases (CVD) [1–5]. The results of experimental and clinical studies of recent years have confirmed the concept of an important causal relationship between the endothelial dysfunction and the development and progression of hypertension, vascular encephalopathy, adverse cardiac remodeling, cardiomyopathy, heart failure, diabetes mellitus, chronic renal failure [5]. Despite the availability of a sufficient range of pharmaceuticals, an important role is assigned to the development of low-toxic and highly effective drugs on the basis of new original molecules [6–8]. Suffice it to mention that more than half of the original medicines created from 1981 to 2016 one way or another is associated with derivatives of 1,2,4-triazole, quinazoline, quinoline or imidazole [7, 9–11]. It is well known that these heterocyclic derivatives have a wide spectrum of biological activity which makes them promising in search for new drugs [11, 12]. Lately, the effects of 1,2,4-triazole derivatives on the vascular endothelium, myocardium and the brain have been studied; and the cardio-, endothelium — and neuroprotective properties have been discovered [9, 10, 12, 13].

We have developed a strategy and are implementing a program for the creation of new original drugs. The concept of creating cardio-, endothelium — and neuroprotective agents with five — and six-membered azaheterocycles is based on their chemical modification with the structures that mimic endogenous ligands of receptors, ion channels, or transcription factors.The NO system is considered as a promising target link in the pharmacological correction of ED. With ED, a deficiency of NO is recorded, and as a result, a decrease in its vasodilating properties due to the transformation into cytotoxic forms. All this determines the creation of molecules-modulators of the NO system as potential endothelioprotectors [4, 5, 8, 13].

The purpose of this review is to provide an up-to-date semantic analysis of our and the literature data on the medical and biological functions of the NO-dependent mechanism of the endothelial dysfunction formation that is a promising target link for pharmacological management.

Methods and Methodology

Research methods — bibliosemantic, analytical, logical, and generalization methods. We explored the bibliographic database of life science and biomedical information MEDLINE, EMBASE, Medline (PubMed), the Web of Science, and the Cochrane Central to search for English publications satisfying the keywords of this study. All authors independently selected articles, evaluated the quality of the data, presentation, and interpretation correspondence to the main idea of the study, and constructed the final list of the references.

Basic and alternative molecular mechanisms of endothelial dysfunction

The main mechanism for the formation of endothelial dysfunction is a decrease in the formation and bioavailability of NO, the appearance of its cytotoxic forms against the background of oxidative stress and a deficiency of low molecular weight reduced thiols [6, 8, 12, 13]. At the same time, the main reasons for NO deficiency in endothelial cells can be a reduced content of the precursor of nitric oxide L-arginine, a decrease in the ex-

pression or activity of endothelial NO synthase, a lack of cofactors for NO synthesis (especially tetrahydrobiopterin), increased levels of endogenous eNOS inhibitors asymmetric dimethylarginine and monomethyl-L-arginine, an increased production of reactive oxygen species (in particular, superoxide anion), as well as low density lipoproteins (especially their oxidized forms) [6, 8, 12]. It is known that during ischemia of the brain, myocardium, as well as diabetes in the vessels of the muscular type of target organs, there is a disproportion between the expression of endothelial and inducible forms of NOS with a decrease in the activity of total NOS — mRNAeNOS and eNOSdecrease, while mRNAiNOS and iNOS increase. We have established that under the above pathological conditions, the expression of eNOS and its activity are suppressed, and the cytokine-dependent expression of iNOS increases, and under the action of superoxide, a small amount of NO is converted into peroxynitrite [12, 13]. Similar reactions are enhanced with a deficiency of the NO-L-arginine precursor and a deficiency in reduced glutathione and SOD [13]. The molecular basis of vascular endothelial dysfunction is a complex and not fully understood problem. In this regard, the system "endothelial NOS-synthase-L-arginine-NO" can be certainly claimed as the leading link for the pharmacological management of endothelial dysfunction in the near future [6, 13].

A number of authors note the direct participation of NO in the process of cell death, including endothelial cells, under conditions of ischemia, atherosclerosis, alcohol intoxication, *etc.* [10, 11, 13]. More recently, it was established that such products of the NO conversion as peroxynitrite, nitrosonium, nitroxyl and diazotrioxide (N2O3) are the main substrates of nitrosative stress, resulting in a direct interaction of NO with metals (heme iron of hemoglobin, myoglobin, iron-containing enzymes, non-heme iron of iron-sulfur proteins and DNA, copper and zinc) as well as [in an] indirect interaction of NO+ (S-, N-, O-nitrosation) with thiol, phenol, hydroxyl and amino groups of proteins and DNA [6]. This interaction leads to desensitization of receptors, inhibition of the activity of mitochondrial enzymes and fragmentation of nucleic acids. An excess of NO and its cytotoxic forms inhibits the heme enzymes of the mitochondrial electron transport chain. NO+ is a potent nitrosylating agent that targets the nucleophilic groups of active thiols, amines, carboxyls, hydroxyls, and aromatic rings. NO+ is formed from NO excess with the participation of bivalent iron and oxygen. NO+ has restorative properties, produces positive inotropic, lusitropic action on the myocardium, and reduces the threshold of convulsive readiness. During ischemia under conditions of developing lactic acidosis, the destructive oxidative action of NO+ is the enhanced level of thiols and amines [6, 12].

The challenging role of thiol-disulfide system in NO toxicity

The thiol-disulfide system deserves special attention in the understanding of the mechanisms of NO cytotoxicity and cell death. The intermediates of the thiol-disulfide system have transport properties in relation to NO, thereby increasing its bioavailability; in addition, many thiols, such as glutathione, cysteine, methionine, can significantly limit the cytotoxicity of

NO and its derivatives, increasing the cell survival rates. NO transport occurs with the formation of N_2O_3, which nitrosylates thiols, and then NO is released due to the disulfide isomerase action. Another mechanism of NO release from S-nitrosoglutathione with the participation of glutamyl transpeptidase is also possible [6, 8, 9, 13]. A reduction in the NO bioavailability plays a central role in the development of endothelial dysfunction because NO produces diverse physiological effects, including vasodilation, antiplatelet, anti-inflammation, antiproliferation, and antimigration [6, 11, 13]. GSH, competitively binding to NO, forms a complex in the form of S-nitrosoglutathione as well as a depot of endogenous NO (the NO release is catalyzed by the thioredoxin system). The mutual regulation of the pool of endogenous NO and GSH has been established [8]. We have found that the formation of nitrosative stress *in vitro* causes a persistent GSH deficiency, which leads to an increase in the cytotoxicity of NO derivatives and their interaction with aliphatic and aromatic amines to form N-nitrosamines. This is confirmed by an increase in the level of nitrotyrosine in the cell suspension [8, 12]. RTD modulators (S-substituted 1,2,4-triazole-5-thiones) under conditions of nitrosative stress *in vitro* restore the GSH/GSSG ratio, increase the bioavailability of NO, and reduce the formation of nitrotyrosine. Depletion of GSH upon administration of BSO (L-butionine-sulfoximine) to animals enhances the manifestations of ED (decrease in NO, increase in nitrotyrosine, decrease in the density of proliferating endotheliocytes in muscle-type vessels in the brain [8]. This justifies the use of TDS modulators to correct ED.

Pharmacological approaches to ED management

Pharmacotherapy of vascular endothelial dysfunction is also of great practical interest in cardiovascular pathology, when reperfusion, antithrombotic and cardioprotective therapy will be combined with the drugs-correctors of endothelial dysfunctions [4,14]. In connection with the above, it was topical to study the endothelial protective properties of drugs with various pathogenic mechanisms of action, improving metabolism, having antioxidant properties, being natural donors of NO and activators of the enzyme NO-synthase, containing essential phospholipids, as well as affinity-purified polyclonal antibodies to endothelial NO-synthase in experimental models of cerebrovascular pathologies [4, 11, 13]. Experimental and clinical studies have shown that a variety of investigational and currently used drugs of such classes as organic nitrates, angiotensin-converting enzyme (ACE) inhibitors, angiotensin AT1 receptors blockers, antioxidants, beta-blockers, calcium channel blockers, endothelial NO synthase enhancers, phosphodiesterase 5 inhibitors and statins,. Thus, organic nitrates restore the deficiency of endogenous NO, ACE inhibitors, in addition to reducing the synthesis of angiotensin (AT)-II, prevent from the destruction of kinins; statins improve the barrier function of endothelial cells against oxidized LDL; calcium channel blockers inhibit the activity of AT II and endothelin in vascular smooth muscles, enhancing the vasodilator effect of NO; angiotensin II AT1 receptors blockers promote the accumulation of NO; inhibitors of endothelin-converting enzyme and antagonists of endothelin-1 receptors inhibit the activity of the peptide [11, 12, 22]. It is par-

ticularly noteworthy the specific effect aimed at improving NO synthesis, such as replacement therapy with L-arginine, which is a substrate for endothelial nitric oxide synthase (eNOS), as well as tetrahydrobiopterin, a cofactor of eNOS, which determines the activity of this enzyme [6, 15, 22]. Additionally, there is a scientific evidence confirming that the currently used cytoprotective agents of metabolic action Trimetazidine (Table 1) and Mildronate (Meldonium, Table 2) not only block beta-oxidation of fatty acids in mitochondria and, on an alternative basis, stimulate glucose oxidation (Tables 1 and 2), but also affect the induction of nitric oxide biosynthesis by increasing gamma-butyrobetaine levels [19–21].

There is an evidence for the influence of Mexidol on the preservation of the morphological and functional parameters of endothelial cells under conditions of oxidative stress. Mexidol (succinate salt of 2-ethyl-6-methyl-3-hydroxypyridine), a structural analogue of vitamin B6 and succinic acid, is a new generation antioxidant [12]. Since it is the oxidative and nitrosative stress that plays a leading role in the development of endothelial dysfunction, the urgent task of modern experimental pharmacology is the development of a new technology for preclinical assessment of the endotheliotropic activity of drugs with antioxidant action and the search for potential endothelial protectors in a broad range of S-substituted azaheterocycles. In this regard, the drug Thiotriazolin (morpholinium 3-methyl-1,2,4-triazolyl-5-thioacetate), which is widely used for the management of cardiovascular diseases and visual impairment, is of great interest (Table 3) [6–11].

However, all of the above metabolitotropic cardioprotective agents, antioxidants, antianginal and neuroprotective agents have no experimentally proven and clinically significant independent endothelial protective activity, which dictates the urgent need to create an original drug with the endothelium-protective action.

New original cardioprotective metabolic drugs

In 2006, by chemical modification of thiotriazolin, a new compound was obtained that

Table 1. **Clinical and pharmacological characteristics of Trimetazidine [12, 17–19].**

Preclinical study data	Clinical study data
Trimetazidine may inhibit mitochondrial 3-ketoacyl coenzyme A thiolase, decreasing long chain fatty acid β-oxidation but not glycolysis in the myocardium.	Twenty-three studies (1378 patients) met the inclusion criteria. Information on mortality, cardiovascular events, and quality of life was scarce. Trimetazidine, compared with placebo, reduced the number of weekly angina attacks.
Research with rodents has helped to demonstrate that trimetazinide (60 mg/kg) attenuates myocardial damage after exhausting exercise by inhibiting cell apoptosis and oxidative stress by regulating the Nrf2/NF-κB signaling pathway during exhausting exercise.	Trimetazidine has a significant protective effect against chronic ischemic heart failure. A meta-analysis (including 17 clinical trials, 955 patients) showed that trimetazidine dramatically increased the ejection fraction in patients with heart failure).
Trimetazidine also promotes the expression of NADPH oxidase 2 and reduces ROS. It participates in the modulation of cardiomyocyte autophagy by regulating AMPK.	

Table 2. **Clinical and pharmacological characteristics of Mildronate [12, 16, 19–21]**

Preclinical study data	Clinical study data
Mildronate reduces the level of l-carnitine due to the influence on the enzyme synthesis of l-carnitine γ-butyro-beta-hydroxylase and the carnitine/electric cation transporter type 2 (OCTN2), inhibits the oxidation of fatty acids, exhibits a mitoprotective effect, stimulates glucose metabolism and reduces metabolic concentration of l β-carnitines such as long chain acyl carnitines and trimethylamine N-oxide; increases the synthesis of ATP during ischemia; and reduces hyperenzymemia LDH in adrenal myocardial infarction in rats (150 mg/kg).	512 patients with chronic coronary heart disease who had ischemia as the limiting factor received Mildronate from 100 to 1000 mg for 12 weeks. All patients showed a noticeable increase in the mean change in total duration of physical activity, but the most pronounced result was at a dose of 500 mg (P = 0.002).
Mildronate causes a temporary increase in the concentration of nitric oxide (NO) in the blood and myocardium of rats.However, these properties of mildronate (250 mg/kg) were not confirmed in the model of chronic alcohol intoxication in rats.	Pre-intervention Mildronate therapy in patients with Functional Class II-III stable angina who underwent coronary artery bypass graft (CABG) surgery or percutaneous coronary intervention (PCI) was associated with decreased blood levels of lipid peroxidation products, due to the activation of antioxidant enzymes – superoxide dismutase (SOD) and glutathione peroxidase (GP)

Table 3. **Clinical and pharmacological characteristics of Thiotriazolin [12, 22, 24]**

Preclinical study data	Clinical study data
In rats with modeling isadrin-pituitrin myocardial infarction Thiotriazolin (50 mg/kg) stimulated LDH in the direction of the formation of pyruvate from lactate, which eliminated lactic acidosis and normalized intracellular pH and stimulated the Krebs cycle by increasing pyruvate. In the same experimental mode Thiotriazolin activated the malate-aspartate shunt in the myocardium in the acute period of myocardial infarction.	Thiotriazolin administration (600 mg/day) to 8298 patients with II–III class stable angina pectoris reduced the number of weekly angina attacks by 46.32 %; in the control group — by 33.24 % (p = 0.028), respectively (p = 0.031), and increased exercise tolerance. Thiotriazolin in patients with acute coronary syndrome significantly reduced mortality, the number of ventricular arrhythmias, and quickly restored the contractile function of the myocardium.
Thiotriazoline (10^{-5}–10^{-7} M) *in vitro* reduced the levels of superoxide radical and peroxynitrite due to the presence of a thiol group in its structure.	In the group of 60 patients with CAD, additionally receiving Thiotriazolin (600 mg/day), there was an increase in the expression of gamma-butyrobetaine hydroxylases and linoleyl-CoA desaturase that helped to reduce atherosclerotic processes in the vascular wall and cardiomyocytes.

combines in its molecule the structural fragments of thiotriazolin (thiazotate acid) and the amino acid lysine (L-lysine, converted to pipecolic acid, which increases GABA affinity and reduces glutamate excitotoxicity; increases the effectiveness of L-Arginine; participates in the formation of L-carnitine; regulates eNOS expression) — (S)-2,6-diaminohexanoic acid 3-methyl-1,2,4-triazolyl-5-thioacetate (Angiolin) [12, 23, 25]. Angiolin, combining fragments of thiotriazolin and L-lysine molecules in its structure, exhibits high endothelial protective, anti-ischemic, cardioprotective, and antioxidant activities. Noteworthy,

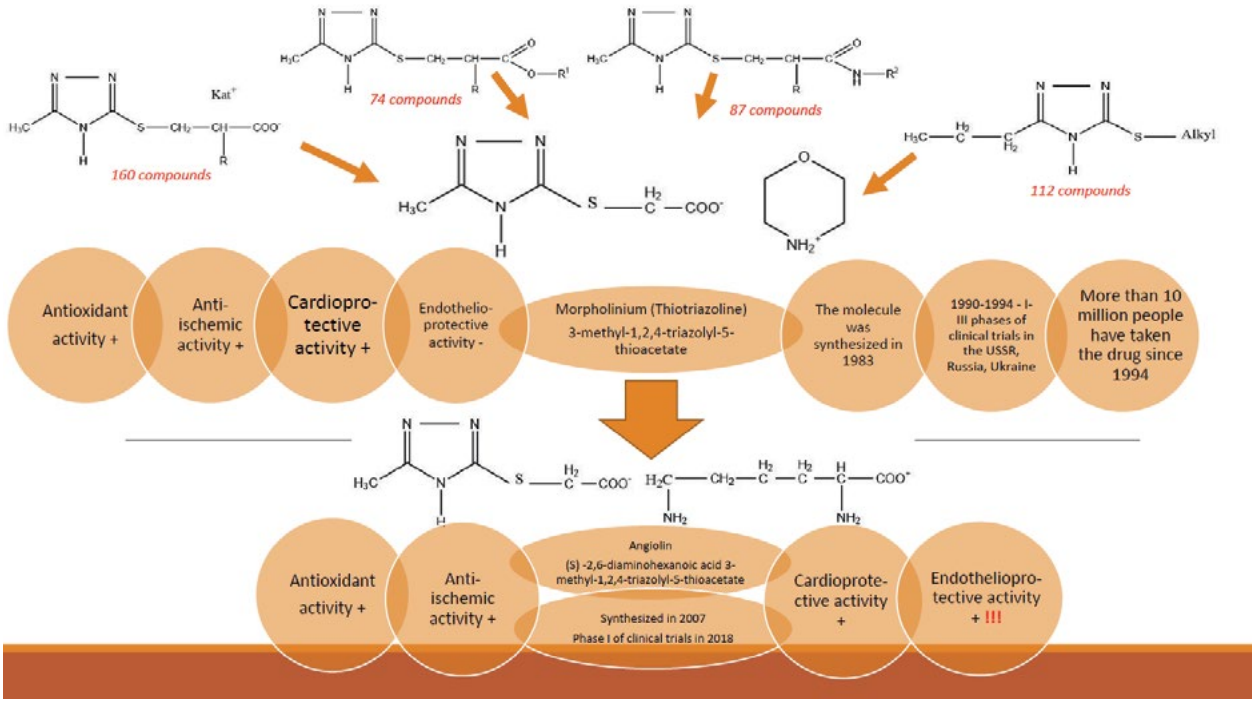

Fig. 1. Targeted Synthesis of a New Molecule with Endothelial Protective, Cardioprotective and Anti-Ischemic Activities.

Angiolin has a significantly higher safety profile compared to Thiotriazolin [12]. According to the concept of creating cationic-anionic drugs, Angiolin can be considered as "improved" or pharmacologically modified Thiotriazolin. The result of replacing morpholinium with L-lysine was that Angiolin not only preserves, but also improves the "proprietary" properties of Thiotriazolin — cardioprotective, antioxidant, anti-ischemic, and obtain fundamentally new — endothelial protective activity [8, 25].

To create cardioprotective properties based on S-substituted 1,2,4-triazolyl derivatives, the morpholinium molecule 3-methyl-1,2,4-triazolyl-5-thioacetate (Thiotriazolin), which exhibits anti-ischemic, cardioprotective, and antioxidant activities was selected. Then by chemical modification of thiotriazolin, a new original compound that combines in its molecule the structural fragments of thiotriazolin and the amino acid lysine (exhibiting an independent endotheliotropic activity, as well as strong anti-ischemic, cardioprotective and antioxidant activities) — (S)-2,6-diaminohexanoic acid 3-methyl-1,2,4-triazolyl-5-thioacetate (Angiolin) was obtained. Angiolin exhibits high endothelioprotective, anti-ischemic, cardioprotective, and antioxidant activities.The endothelial protective effect is due to the ability of Angiolin to increase the bioavailability of NO and improve its transport to target cells under conditions of dysfunction of the vascular endothelium.

In vitro experiments we found that Angiolin at concentrations of 10^{-3} M–101^{-7} M in the reaction of photoinduced oxidation of sodium nitroprusside (accompanied by the formation of NO, judging by the rate of ascorbate oxidation) reduced the level of NO. Angiolin also reduced the level of NO and its conversion into peroxynitrite (measured by nitrotyrosine levels) when incubated with excess iron(II) cysteine complex dinitrosol (DNIC) [8].

The Angiolin introduction in experimentally justified doses of 50–100 mg/kg reduced the progression of necrosis in the zone of ischemia, decreased the vascular endothelial cell loss (increased the density of vascular endothelial cells in the capillary network and myocardium), increased the RNA content in the nuclei of endothelial cells, increased the density of proliferating endothelial cells against the background of an increase in the concentration of vascular endothelial growth factor (VEGF) as well as improved myocardial energy metabolism, improved ECG parameters, decreased the levels of molecular and biochemical markers of injury (CPK-MV, ST, D-dimer, myoglobin), and oxidative stress (nitrothiozine, MDA, carbonylated proteins), characterizing the system of production and transport of nitric oxide and thiol-disulfide equilibrium, reducing the formation of endothelial dysfunction in experimental myocardial infarction and heart failure caused by Doxorubicin in rats and rabbits. Angiolin improved the cardio and systemic hemodynamics markers in acute myocardial ischemia: normalized systolic blood pressure, reduced the manifestations of ischemic left ventricular systolic dysfunction (*increasing left ventricular pressure, stroke work, ejection fraction and systolic index*), and reduced the total peripheral resistance after myocardial ischemia caused by occlusion of the descending coronary artery in rabbits [23, 25]. Angiolin had an endothelioprotective effect in chronic cerebral ischemia and chronic alcoholism in experimental animals [8, 25]. Angiolin according to the degree of influence on such indicators of endothelial protective action as the density of endotheliocytes, the density of proliferating endotheliocytes, RNA concentration, VEDF expression, GSH/nitrotyrosine ratio has a significant advantage over Mildronate, Mexidol, Piracetam and Thiotriazoline [8, 25].

Plausible molecular mechanisms of Angiolin actions

In our opinion, the mechanism of the endothelium-protective action of Angiolin is associated with its projective action with respect to NO as well as an increased bioavailability of this molecular messenger. It is generally known that NO is an unstable, short-lived radical, and it is envisaged such mechanisms as the formation of stable S-nitrosole complexes with thiocontaining low molecular compounds for its stabilization and subsequent transportation [6, 8].

Under conditions of a deficiency of thiol compounds, the NO transport is impaired, because it is attacked by such reactive oxygen species as superoxide radical with transformation into a cytotoxic metabolite — peroxynitrite [6, 8, 12, 25].

The protective effect of Angiolin with regard to NO is due to the peculiarities of its chemical structure suggesting that the molecule of the drug, when interacting with NO, plays the role of a “spin trap”. To prove this

hypothesis, we performed calculations of the quantum-mechanical energy descriptors of the boundary molecular orbitals: the energy of the highest occupied molecular orbital (E_{HOMO}) and the energy of the lowest unoccupied molecular orbital (E_{LUMO}) in the WinMopac software package (ver 7.2, descriptors — HOMOEnergy, LUMOEnergy AM1 method, with settings: Calculation = SinglePoint, WaveFunction = ClosedShell (RHF). The following characteristics were calculated as well: the size of the energy gap (the difference between the energies of HOMO and LUMO); absolute hardness — according to the formula: $\eta = -(E_{HOMO} - E_{LUMO})/2$ and absolute electronegativity – according to the formula: $\chi_0 = -(E_{HOMO} + E_{LUMO})/2$. The quantum-chemical parameters of the Angiolin molecule correlate with our earlier studies [8, 12] and show that the E_{HOMO} parameter (HOMOEnergy descriptor) has the greatest effect on the oxidative stress marker nitrotyrosine, which is directly proportional to the concentration of the degradation product NO-peroxynitrite. The mechanism of interaction between the Angiolin molecule and NO can be realized due to electron transfer from the highest occupied molecular orbital of the "spin trap" to the lowest unoccupied molecular orbital of the radical with the formation of a more stable radical complex. Thus, Angiolin can play the role of an NO transporter molecule that can be an important link in the mechanism of endothelial protection.

Conclusion

1. Literature analysis and our own research show that the main mechanism underlying endothelial dysfunction is a decrease in the formation and bioavailability of NO against

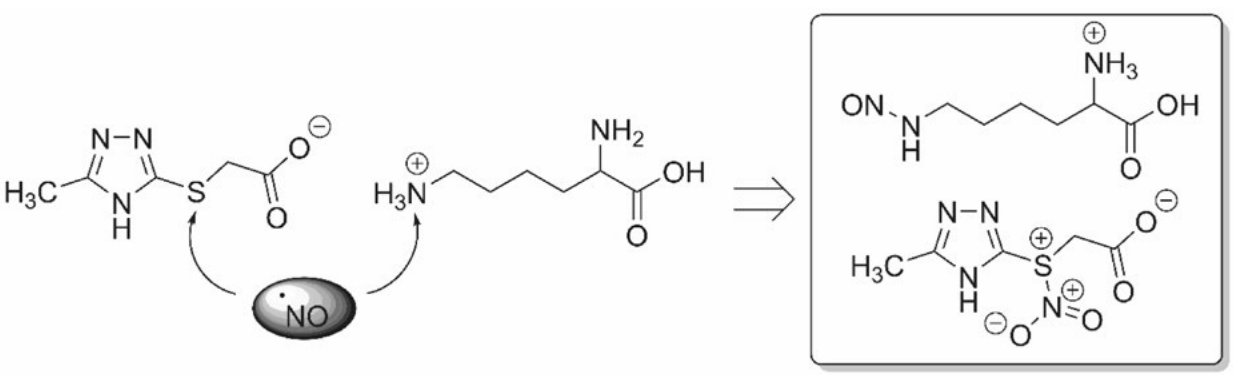

Fig. 2. Hypothetical mechanism of (S)-2,6-diaminohexanoic acid 3-methyl-1,2,4-triazolyl-5-thioacetate (Angiolin) interaction with NO

The NO-scavenging activity of studied compound is associated with the reactivity of both cationic and anionic part[s] of the molecule. Thus, lysine interacts with NO by ε-amino group what yielded corresponding N-nitroso derivative. At the same time, the anionic part of the studied compound molecule apparently forms the S-nitro derivatives similar to described elsewhere. Apparently, the NO-scavenging activities of anionic and cationic part are synergetic that explains the prominent level of the studied compound effect. Noteworthy, the obtained results are consistent with the computational results published previously.

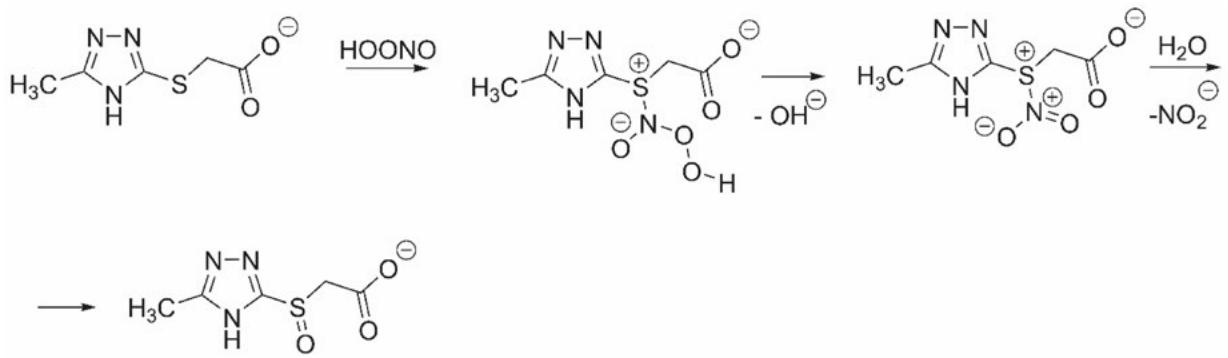

Fig. 3. Hypothetical mechanism of (S)-2,6-diaminohexanoic acid 3-methyl-1,2,4-triazolyl-5-thioacetate (Angiolin) interaction with Peroxynitrite

In the presence of peroxynitrite, the Sulfur atom of tiazotate anion interacts with positively charged nitrogen atom that yielded corresponding adduct. The latter eliminates hydroxyl anion to form S-nitro derivative that under hydrolysis resulted in sulfoxide and nitrite anion.

the background of inhibition of eNOS expression, and reduced equivalents of the thiol-disulfide system with a simultaneous increase in the levels of cytotoxic forms of NO and superoxidanion, and the production or release of powerful vasoconstrictors.

2. The main causes of NO deficiency in the endothelial cells may be: a decreased content of the precursor of NO — L-arginine, a decrease in the expression or activity of eNOS, a lack of cofactors for NO synthesis, an increase in the levels of endogenous eNOS inhibitors — asymmetric dimethylarginine, and increased formation of reactive oxygen species; a decrease in the bioavailability of NO and its conversion into peroxynitrite or other aggressive forms occur due to violations of TDS and its glutathione unit.

3. Based on the foregoing, the conjugated complex eNOS-L-arginine-NO/SH can be undoubtedly claimed in the near future as a promising target for the pharmacological correction of endothelial dysfunction.

4. RTD modulators and, especially, S-substituted of 1,2,4-triazole, are the basis for the creation of promising endothelial protectors. The prospect of searching in this series is Thiotriazoline, a drug with cardioprotective, anti-ischemic and antioxidant properties, but without endothelial protective activity.

5. As a result of chemical modification of the Thiotriazoline molecule the compound (S)-2,6-diaminohexanoic acid 3-methyl-1,2,4-triazolyl-5-thioacetate (Angiolin) was obtained, which possesses additional endothelial protective properties against the background of cardioprotective, anti-ischemic and antioxidant activities and has a high profile of safety and harmlessness.

6. The entire complex of preclinical studies was carried out for Angiolin, in accordance with the requirements of the State Expert Center of the Ministry of Health of Ukraine, and the dosage forms for parenteral use and tablets were developed. Angiolin, according to the decision of the State Expert Center of the

Ministry of Health of Ukraine, passed the 1st phase of clinical trial.

7. Thus, the confirmation of developed strategy and creation of new endothelioprotectors is a new original drug Angiolin.

Abbreviations:

ACE — angiotensin-converting enzyme
AT — angiotensin
CAD — coronary artery disease
CHD — coronary heart disease
CHF — congestive heart failure
CVD — cardiovascular disease
ED — endothelial dysfunction
E_{HOMO} — energy of the highest occupied molecular orbital
E_{LUMO} — energy of the lowest unoccupied molecular orbital
eNOS — endothelial nitric oxide synthase
GP — glutathione peroxidase
HOMO — highest occupied molecular orbital
iNOS — inducible nitric oxide synthase
LDH — Lactate dehydrogenase
LUMO — lowest unoccupied molecular orbital
NO — nitric oxide
NO^+ — nitrosonium cation
NO^- — nitroxyl ion
N_2O_3 — diazotrioxide
NOS — nitric oxide synthases
$ONOO^-$ — peroxynitrite
PCI — percutaneous coronary intervention
SOD — superoxide dismutase
VEGF — vascular endothelial growth factor

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All authors met the authorship criteria according to the latest ICMJE guidelines.

Conflict of interest

Authors do not have any conflicts of interest to declare.

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Ethics statement

None.

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Оксид азоту залежний механізм формування ендотеліальної дисфункції є перспективною цільовою ланкою для фармакологічного лікування

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У цьому огляді показано, що основним механізмом, що лежить в основі ендотеліальної дисфункції, є зниження продукції та біодоступності NO на тлі пригнічення експресії eNOS та знижених еквівалентів тіол-дисульфідної системи з одночасним підвищенням рівня цитотоксичної форми NO та продукції потужних вазоконстрів. Виходячи з вищевикладеного, кон'юговані системи eNOS-L-аргінін-NO/SH, безперечно, можуть претендувати в найближчому майбутньому на роль перспективної мішені для фармакологічної корекції ендотеліальної дисфункції. Оскільки саме нітрозативний стрес відіграє роль у розвитку ендотеліальної дисфункції, актуальним є пошук потенційних ендотеліопротекторів у ряді S-заміщених 1,2,4-триазолів, які мають властивості антиоксидантів та скавенджерів NO. Яскравим представником цієї когорти є Тіотриазолін — препарат, що володіє кардіопротекторними, антиішемічними та антиоксидантними властивостями, але не має ендотеліопротекторної дії. В результаті хімічної модифікації його молекули отримано сполуку (S)-2,6-діаміногексанової кислоти 3-метил-1,2,4-триазоліл-5-тіоацетат (Ангіолін), яка має ендотеліопретективну дію на тлі кардіопротекторної, антиішемічної та антиоксидантної активності

Ключові слова: ендотеліальна дисфункція, NO, нітрозативний стрес, відновлені тіоли, S-похідні 1,2,4-триазолу

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