The Cardio- and Endothelial Protective Effects of Ethyl Methyl Hydroxyl Pyridine Malate in Modeling L-Name Induced Nitric Oxide Deficiency

Pavel D. Kolesnichenko[1], Dmitry V. Shcheblykin[1], Alexey N. Demidenko[1], Olesya V. Shcheblykina[2]*, Konstantin M. Reznikov[1], Alhamzah Muhi Aldeen Azeez[1], Maxim A. Zhuchenko[1] and Sergey A. Demchenko[1]

1. Department of Pharmacology and Clinical Pharmacology, Medical Institute, Belgorod State University, 85 Pobedy St., Belgorod, 308015, Russia.

2. Postgraduate Student of the Department of Pharmacology and Clinical Pharmacology of the National Research University "BelSU", 85 Pobedy St., Belgorod, 308015, Russia.

Correspondence author: Olesya V. Shcheblykina, e-mail: sheolvi31@gmail.com

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Abstract

Currently, endothelial dysfunction is considered as a predictor of a number of pathologies, including arterial hypertension, ischemic heart disease, chronic heart failure, and is also a pathogenetic component of organ damage in diabetes, hypo estrogenic and other conditions. The purpose of the study is an experimental study of the cardio and vasoprotective effects of etoxidol under conditions of endothelial dysfunction.

Materials and methods: The study was performed on Wistar rats. The pharmacological properties of ethyl methyl hydroxyl pyridine malate (etoxidol) were studied on the model of endothelial dysfunction caused by the blockade of NO synthase on the background of the seven-day administration of L-NAME (25 mg / kg). To evaluate the effect of etoxidol on endothelial function, a calculated index of functional vascular samples — the coefficient of endothelial dysfunction — was used, and the morphological picture of the myocardium (determined the diameter of cardiomyocytes), carotid arteries (intima / media ratio) and adrenal mass was studied.

Results: The seven-day administration of L-NAME led to the development of severe endothelial dysfunction. The use of etoxidol in a dose of 50 mg / kg against a background of relatively high blood pressure values reduced QED by 30%, and by 49% when using a dose of 90 mg / kg. With a relatively high diameter of cardiomyocytes in all studied groups receiving L-NAME, a significant ($p \leq 0.05$) difference with the control was obtained with a dose of 90 mg / kg. When studying the intima / media ratio in the carotid artery, a significant decrease in the ratio to the control was obtained in the group receiving etoxidol at a dose of 90 mg / kg.

Conclusion: Etoxidol has a pronounced endothelial-, vaso- and cardio protective activity. The effectiveness of the drug is dose-dependent: the best endothelium and cardio protective effects are achieved with a dose of 90 mg / kg.

Keywords: Ethyl Methyl Hydroxyl Pyridine Malate, L-Name, Endothelial Dysfunction, Cardio Protection.

Introduction

Despite significant advances in medical science in the field of cardiology, the number of patients suffering from cardiovascular diseases is growing steadily, and a universal tool for the prevention and treatment of this ailment has not yet been developed.

In recent years, many experimental and clinical studies have been published on the role of endothelial dysfunction (ED) in the onset and progression of a number of cardiovascular diseases (atherosclerosis, hypertension, ischemic heart disease, myocardial infarction, and others) [1-3].

Endothelial dysfunction is understood as an imbalance between the formation of vasodilating, atrombogenic, antiproliferative factors, on the one hand, and vasoconstrictive, prothrombotic and proliferative substances that the endothelium synthesizes, on the other.

Hemodynamic causes, age-related changes, free radicaldamage dyslipoproteinemia, hypercytokinemia, hyperhomocysteinemia, exogenous and endogenous intoxications can contribute to the formation of endothelial dysfunction [4,5]. Endothelial dysfunction can lead to structural damage in the body: acceleration of apoptosis, necrosis, and desquamation of endotheliocytes [6]. However, functional changes in the endothelium, as a rule, precede morphological changes in the vascular wall [7, 8].

In this regard, the search and study of compounds with the ability to improve endothelial function, is one of the main tasks of modern pharmacology. One of the fundamentally significant approaches to the correction of endothelial dysfunction is the use of drugs with antioxidant and cytoprotective activity [9-13]. Experimental work on the modeling of arterial hypertension in animals has shown that an increase in reactive oxygen species leads to endothelial dysfunction, which is confirmed by an improvement in endothelium-dependent relaxation with the use of

antioxidants [14]. The development of endothelial dysfunction with excessive formation of reactive oxygen species has been confirmed in experimental models of type 2 diabetes and chronic renal failure in animals [15].

One of the drugs with an antioxidant orientation of pharmacological action is a derivative of 3-hydroxypyridine – ethyl methyl hydroxyl pyridine malate (etoxidol), the establishment of the ability of which to positively influence the myocardium and blood vessels was the main objective of the study.

Materials and Methods

The study was performed on white rats, males, Wistar line, weighing 200-220g., Obtained from the nursery "Stolbovaya" (Pushchino, Moscow region) and passed the 14-day quarantine regime. The maintenance of animals complied with the rules of laboratory practice when conducting preclinical studies in the Russian Federation (GOST Z 51000.3-96 and 51000.4-96) and the Order of the Ministry of Health of the Russian Federation No. 267 dated June 19, 2003 “On Approval of Laboratory Practice Rules” (GLP) in compliance with the International conventions for the protection of vertebrate animals used in experimental studies (1997).

Endothelial dysfunction was modeled by daily administration of nitro-L-methyl ether (L-NAME) at a dose of 25 mg / kg 1 time per day intraperitoneally for 7 days.

The animals were divided into several groups (n = 10): 1 - intact, which were injected intraperitoneally with saline 1 ml / kg 1 time per day for 7 days, then filtered intragastrically filtered water 1 ml / kg for 7 days (n = 10); 2 - control, with the introduction of L-NAME and saline intragastrically (1 ml / kg, 1 time per day); 3 - etoxidol 50 mg / kg 2 times a day during the administration of L-NAME; 4 - etoxidol 90 mg / kg 2 times a day on the background of the introduction of L-NAME. The study drug was administered through an intragastric probe daily for 7 days.

On the 8th day, experimental animals fixed on a heated surface under general anesthesia (chloral hydrate 150 mg / kg + zoletil 60 mg / kg intraperitoneally) were recorded using a carotid artery catheter connected to a TDS-144A sensor (Biopac, USA) cardiohemodynamic indicators - systolic blood pressure (SBP), diastolic blood pressure (DBP), heart rate (HR). Vasodilator vascular samples: endothelium-dependent (acetylcholine, 40 μg / kg) and endothelium-independent (sodium nitroprusside 30 μg / kg) sample, was made by bolus administration of vasodilators into the left femoral vein. To objectively assess the severity of endothelial dysfunction development in experimental animals, as well as the extent of its correction by the studied pharmacological agent, we used the calculated coefficient of endothelial dysfunction (QED) proposed in our laboratory [16], Characterizing the degree of endothelial dysfunction and reflecting the ratio of the area above the blood pressure recovery reaction in response to the introduction of sodium nitroprusside (NP) to the area above the blood pressure recovery reaction in response to the introduction of acetylcholine (AH) (Fig. 1, 2).

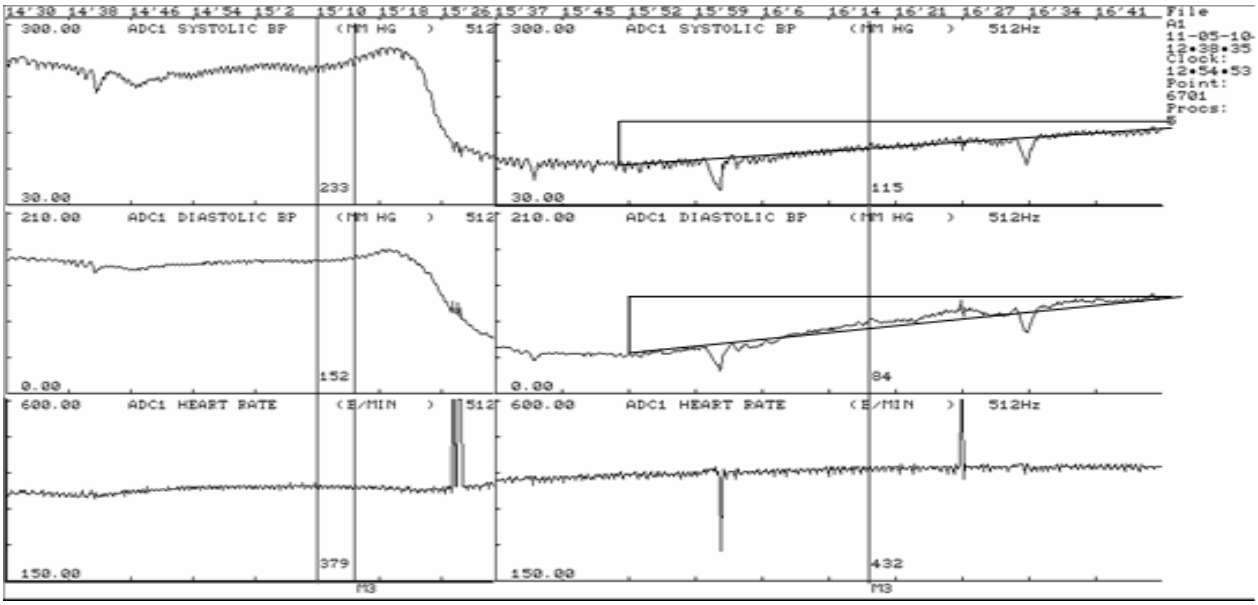

Figure -1: Curves of the indices of cardiohemodynamics during the functional test for endothelium-dependent vasodilation (intravenous administration of acetylcholine 40 μg / kg) in animals with L-NAME-induced deficiency of nitric oxide.

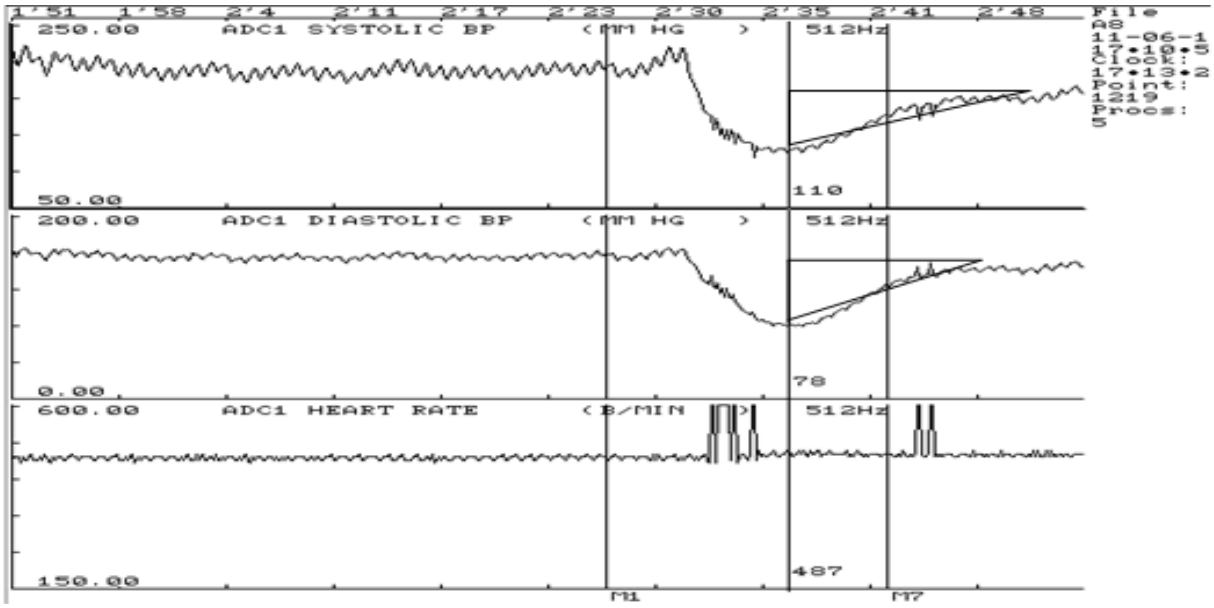

Figure-2: Curves of the indices of cardiohemodynamics during the functional test for endothelium-independent vasodilation (intravenous administration of nitroprusside 30 μg / kg) in animals with L-NAME-induced nitric oxide deficiency.

After carrying out vascular samples, animals were anesthetized (chloral hydrate 300 mg / kg intraperitoneally), for histological examination, the heart and carotid artery were removed, which were fixed in 10% neutral formalin and stained in a standard way using hematoxylin / eosin.

In addition, in order to determine the mass of the glands, the adrenal glands were removed from the animals.

The reliability of changes in the parameters observed during the action of the substance studied was determined by a differential method of descriptive statistics with finding the mean values of shifts (M), the average error of the arithmetic average (± m) and the probability of a possible error (p) calculated using T-test for groups with different dispersions. Differences were considered significant at $p < 0.05$. Statistical calculations were performed using the STATISTICA 10.0 program.

Results

Evaluation of endothelial function. The blockade of NO-synthase, caused by the seven-day administration of L-NAME, led to a significant increase in SBP, DBP and QED (Table 1). Experimental animals treated with the studied drug showed a positive dynamics of indicators - a decrease in the values of these parameters; however, there was no significant decrease in blood pressure and QED to the level of intact animals. So, when using etoxidol at a dose of 50 mg / kg against a background of relatively high blood pressure values, QED decreased by 30%, and by 49% when using the drug at a dose of 90 mg / kg.

Table -1: Systolic (SBP), diastolic (DBP) blood pressure and endothelial dysfunction coefficient (QED) in rats after administration of etoxidol (M ± m; n = 10).

experimental group	SBP	DBP	QED
intact	118.2±2.2	83.4±2.6	0.98±0.05
control	179.4±1.9*	132.5±4.5*	4.65±0.48*
L-NAME + etoxidol 50 mg / kg	173.8±4.7*	122.8±3.6*	3.22±0.30*#
L-NAME + etoxidol 90 мг/кг	171.3±3.9*	116.0±3.7*#	2.25±0.21*#

* - $p \leq 0.05$ confidence when compared with a group of intact animals; # - $p \leq 0.05$ when compared with the control group.

Histological examination. The morphological study of the myocardium also revealed a positive trend in the application of the studied drug (Fig. 3, 4, 5).

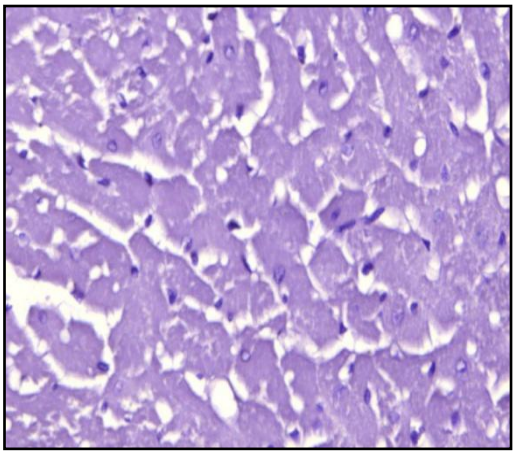

Figure - 3: Histological picture of the myocardium (left ventricular wall) of rats treated with L-NAME (25 mg / kg) for 7 days. Hematoxylin and eosin. × 400

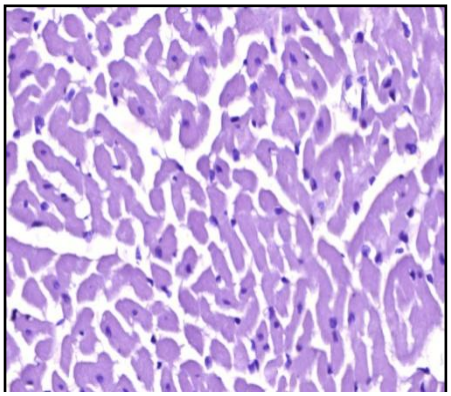

Figure- 4: Histological picture of the myocardium (left ventricular wall) of rats treated with etoxidol (50 mg / kg) for 7 days on the background of the introduction of L-NAME (25 mg / kg). Hematoxylin and eosin. × 400

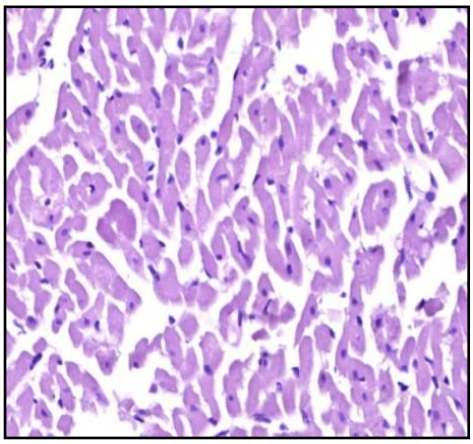

Figure -5: Histological picture of the myocardium (left ventricular wall) of rats treated with etoxidol (90 mg / kg) for 7 days against the background of the introduction of L-NAME (25 mg / kg). Hematoxylin and eosin. × 400

With a relatively large diameter of cardiomyocytes in all studied groups treated with L-NAME, a significant ($p \leq 0.05$) difference with the control was obtained with the administration of etoxidol at a dose of 90 mg / kg (Table 2).

Table -2: The average diameter of thecardiomyocytes of the left ventricular wall in animals with L-NAME-induced deficiency of nitric oxide after administration of etoxidol (M ± m, n = 10).

experimental group	diameter of cardiomyocytes, microns
intact	8.50±0.03
control	14.80±0.30*
L-NAME + etoxidol 50 mg / kg	14.28±0.03*
L-NAME + etoxidol 90 мг/кг	13.98±0.03*#

* - significant difference with the group of intact animals ($p \leq 0.05$); # - significant difference with the control group ($p \leq 0.05$).

When studying the ratio of intima / media in the carotid artery, a significant decrease in the ratio relative to the control in the group receiving a large dose of etoxidol was established (Table 3).

Table- 3: Effect of etoxidol on the intima / media ratio in animals with L-NAME-induced deficiency of nitric oxide (M ± m, n = 10).

experimental group	intima / media ratio
intact	0.228±0.013
control	0.379±0.028*
L-NAME + etoxidol 50 mg / kg	0.320±0.021*
L-NAME + etoxidol 90 мг/кг	0.290±0.034#

Note: * - significant difference with the group of intact animals ($p \leq 0.05$); # - significant difference with the group L-NAME ($p \leq 0.05$).

The mass of the adrenal glands. When studying the mass of the adrenal glands, no data were obtained indicating a significant change in their mass upon administration of etoxidol (Table 4).

This indicates the absence of a pronounced effect of etoxidol on the L-NAME-dependent adaptive response of the adrenal glands.

Table- 4: Effect of etoxidol on adrenal mass in animals with L-NAME-induced deficiency of nitric oxide (M ± m, n = 10).

experimental group	adrenal mass (g)
intact	0.019±0.004
control	0.021±0.004
L-NAME + etoxidol 50 mg / kg	0.021±0.002
L-NAME + etoxidol 90 мг/кг	0.022±0.004

Conclusion

A study of the activity of etoxidol in modeling L-NAME induced nitric oxide deficiency demonstrated its pronounced endothelium protective effect, which was reflected in the prevalence of endothelium-dependent vascular relaxation and a decrease in the rate of endothelial dysfunction (QED).

At the same time, the cardio protective effect of the drug was revealed, which is expressed in the ability to reduce the diameter of the cardiomyocytes with L-NAME-induced deficiency of nitric oxide.

Thus, the obtained results allow us to state that the study drug “Etoxidol” is a promising endothelium and cardio protective agent, which is consistent with the data of numerous studies [17-19].

The study of the histological picture of the vascular wall showed a decrease in the intima / media ratio under the influence of etoxidol, which indicates a decrease in the proliferative processes (remodeling) of blood vessels, which was not previously described in the scientific literature.

Conflict of interest

The authors declare no conflict of interest.

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