Mechanisms of Endothelial Protection by 4-Hydroxy-3,5-Di-Tert-Butyl Cinnamic Acid in Cerebral Ischemia: Experimental Insights

Elena V. Kuznetsova, Ivan A. Petrov, Sergey V. Kostarev

Elena V. Kuznetsova and Ivan A. Petrov, Department of Pharmacology, Kazan Federal University, Kazan, Russia; Sergey V. Kostarev, Institute of Experimental Medicine, Russian Academy of Sciences, St. Petersburg, Russia

Citation: Elena V. Kuznetsova, Ivan A. Petrov, Sergey V. Kostarev (2025). Mechanisms of Endothelial Protection by 4-Hydroxy-3,5-Di-Tert-Butyl Cinnamic Acid in Cerebral Ischemia: Experimental Insights. Psychiatria, 17(12), 5-10. https://doi.org/10.5281/zenodo.19096693

ABSTRACT

Conducted research devoted to the study of potential mechanisms of action implementation endotelioprotection 4-hydroxy3,5-di-tretbutyl cinnamic acid in experimental conditions simulated cerebral ischemia in rats. The research found that the cerebral ischemia in rats is accompanied by the development of endothelial dysfunction, expressed in increasing the concentration of asymmetric dimethylarginine, nNOS, iNOS and PKC, as well as reducing the concentration of eNOS. Introduction of sulodexide (to a greater extent compared with thioctic acid and mexidol restores endothelial function in conditions of brain ischemia in rats. The use of 4-hydroxy-3,5-di-tretbutyl cinnamic acid helped reduce the concentration of ADMA, nNOS, iNOS and PKC on 83.9% ($p < 0.05$), 60.6% ($p < 0.05$), 61. 9% ($p < 0.05$), 108.6% ($p < 0.05$), respectively, as well as improve the content of eNOS at 114.2% ($p < 0.05$), thereby contributing to the correction that has developed in conditions cerebral ischemia, endothelium dysfunction.

Keywords: cerebral ischemia, endothelial dysfunction, endotelioprotection, immunoassay.

INTRODUCTION

Vascular endothelial dysfunction (ED) is one of the universal pathophysiological mechanisms underlying ischemic stroke [2, 8]. Normally, the vascular endothelium, through the secretion of a number of biologically active compounds, supports vascular homeostasis, ensuring the integrity of the vascular wall, the optimal level of microcirculatory blood flow and the rheological properties of blood [8]. However, under conditions of cerebral ischemia, vascular endothelium damage is observed, which leads to worsening of cerebral hemodynamics, hyperaggregation and hypercoagulation, activation of proliferation processes, and inflammation of the vascular wall, which in turn can aggravate the course of ischemic stroke [1, 2, 8, 9]. Separation of NO-synthase system activity (increased iNOS activity, nNOS and decreased eNOS activity), an increase in the concentration of asymmetric dimethylarginine (ADMA) and protein kinase C (PKC) play a special role in the development of ED under conditions of cerebral ischemia [3,8,9]. In conditions of ischemic brain damage, there is a mechanism for early expression of eNOS, which can contribute to a significant NO release and delay neuronal death, and hence to reduce the necrotic focus. However, this effect is shortterm (≈1h.) And further there is persistent inhibition of endothelial NO synthase activity, accompanied by an increase in the activity of nNOS and iNOS, which provides the second wave of synthesis of nitric oxide [5, 8, 9]. This process develops several hours after the ischemic attack and lasts up to 4-7 days. As a result, a significant amount of NO is formed, the excess of which is immediately oxidized into peroxonitrite, which mediates the cytotoxicity of nitric oxide [8, 10]. At the same time, the increased concentration of ADMA and PKC provides «superinhibition» of eNOS, and also mediates (by different mechanisms) direct cytotoxicity [3, 7]. Thus, effects on the enzymatic system of NO synthesis (changes in the activity of NOS isozymes), a decrease in the concentration of ADMA and activity of PKC can be considered as the "targets" for correcting ED in conditions of cerebral circulatory insufficiency. In order to correct ED, a relatively new group of pharmacological agents is used - endothelioprotectors, including drugs with different mechanisms of action [4]. However, for the vast majority of them, the endothelial effect is additional, and there are no "true" endothelioprotectors at the present time [4]. Earlier, the ability to normalize the vasodilating and antithrombotic functions of the vascular endothelium against the background of ischemic brain damage was established for the 4-hydroxy-3,5-di-tretbutyl cinnamic acid [1], suggesting the presence of endothelioprotective activity in this compound, and, thus, The question of elucidating the potential mechanisms of realizing the endothelioprotective action of 4-hydroxy-3,5-di-tret-butyl cinnamic acid can be considered relevant.

Purpose of the study

To study possible mechanisms for the realization of the endothelioprotective action of 4-hydroxy-3,5-di-tretbutyl cinnamic acid under conditions of cerebral ischemia in rats in the experiment.

**Author for Correspondence: pozdnyackow.dmitry@yandex.ru*

MATERIALS AND METHODS

The study was performed on 90 male rats of the Wistar line. The first group consisted of pseudo-operated animals (L/O) (n = 15). Subsequent groups of rats were reproduced with ischemic brain damage by the method of irreversible right-sided occlusion of the middle cerebral artery. In this case, the second group of animals (n = 15) did not receive pharmacological support - negative control (NC). The third, fourth and fifth groups of rats (n = 15) were administered mexidol (Aethylmethylhydroxypyridini succinas, FARMASOFT, Russia) at a dose of 30 mg / kg, thioctic
acid (Oktolipen, Pharmstandard - Leksredstva, Russia) at a dose of 50 mg / kg and sulodexide (Wessel DF, Alfa Wasserman, Italy) at a dose of 30 UL (units of lipoprotein lipase release), respectively. The sixth group of animals received the test compound 4-hydroxy-3,5-di-tretbutyl cinnamic acid (laboratory code - ATACL) at a dose of 100 mg / kg [1]. Comparison drugs and test compound were administered per os immediately after ischemia and for 3 days. At the end of this time, the animals were collected blood for the subsequent production of serum and determination of the concentration of isoenzymes of NO synthase (eNOS, nNOS, iNOS), protein kinase C (PKC), asymmetric dimethylarginine (ADMA). The measurements were performed by solid-phase enzymelinked immunosorbent assay using the Infinite F50 microtiter plate reader system (Tecan, Austria) and species-specific reagent kits of Cloud Clone Corp. (USA). The results of the experiments were processed using the variational statistics method using the STATISTICA 6.0 application software package (StatSoft, Inc., USA for the Windows operating system). The obtained data were checked for the normality of the distribution using the
Shapiro-Wilk test. In the case of a normal distribution of data, the Student's t-test was used to compare the averages. With an abnormal distribution of experimental results, further statistical processing of the data was carried out using the nonparametric Mann-Whitney U- test.

RESULTS AND DISCUSSION

In falsely operated animals, the concentration of NOS isoenzymes (eNOS, nNOS, iNOS) was 77.9 ± 0.009 pg / ml, 13 ± 0.008 ng / ml and 16.7 ± 0.013 g / ml, respectively (Fig. 1, Fig. 2). At the same time, the concentration of ADMA and PKC in L/O animals was 34.9 ± 0.029 ng / ml and 49.08 ± 9.794 ng / ml, respectively (Fig. 1). In conditions of focal cerebral ischemia, rats showed an increase in the concentration of ADMA and PKC in comparison with the L/O group of animals by 96% ($p < 0.05$) and 179.5% ($p < 0.05$) respectively (Fig. 1). It should be noted that the increase in the concentration of ADMA and PKC correlated with the dissociation of NOsynthase system activity, which is manifested by an increase in the concentration of nNOS and iNOS, as well as a decrease in the concentration of eNOS in a group of negative control rats with respect to the L/O group of animals by 63.5% ($P < 0.05$), 30.8% ($p < 0.05$), and 65.4% ($p < 0.05$), respectively.

The use of mexidol in experimental cerebral ischemia contributed to a decrease in the concentrations of PKC, ADMA and iNOS in rats by 109.5% ($p < 0.05$), 40% ($p < 0.05$) and 39.3% ($p < 0.05$). Respectively, against the background of the use of mexidol, the concentration of eNOS and nNOS did not statistically significantly differ from the similar parameters in the NC group of rats. In animals treated with thiectic acid, under conditions of focal cerebral ischemia relative to the NC group of rats, the concentration of ADMA, nNOS and iNOS decreased by 41.6% ($p < 0.05$), 48.3% ($p < 0.05$), and 26.9% ($p < 0.05$), respectively. Also, against the background of the introduction of thioctic acid, the concentration of PKC

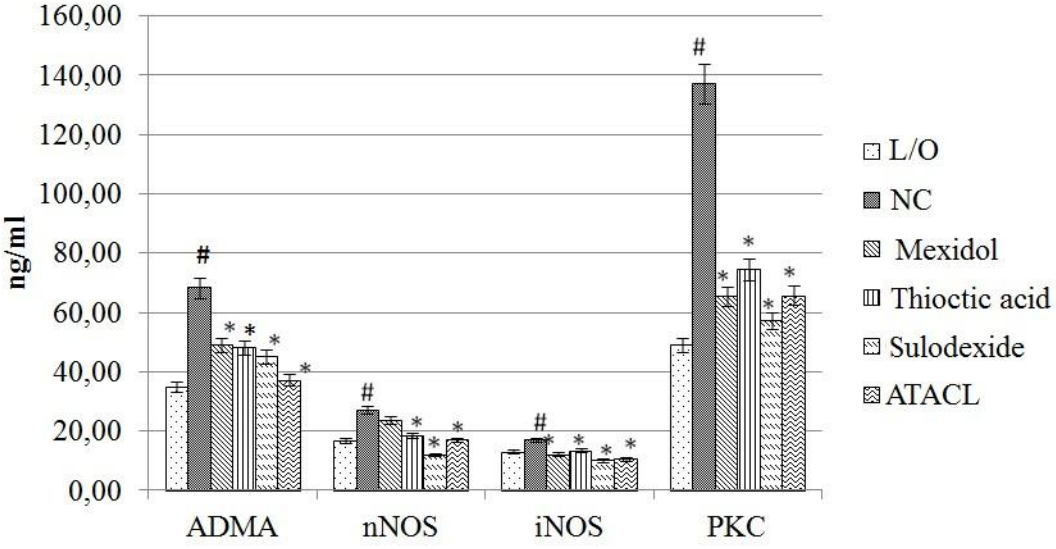


Figure 1: Effect of ATACL Compound and Comparison Drugs on Changes in the Concentration of ADMA, nNOS, iNOS and PKC in Rats in the Background of Focal Brain Ischemia

Note: # - statistically significant (U - Mann-Whitney test) for the L/O group of animals ($p < 0.05$); * - statistically significant (U - Mann-Whitney test) relative to the NC of the group of animals ($p < 0.05$).

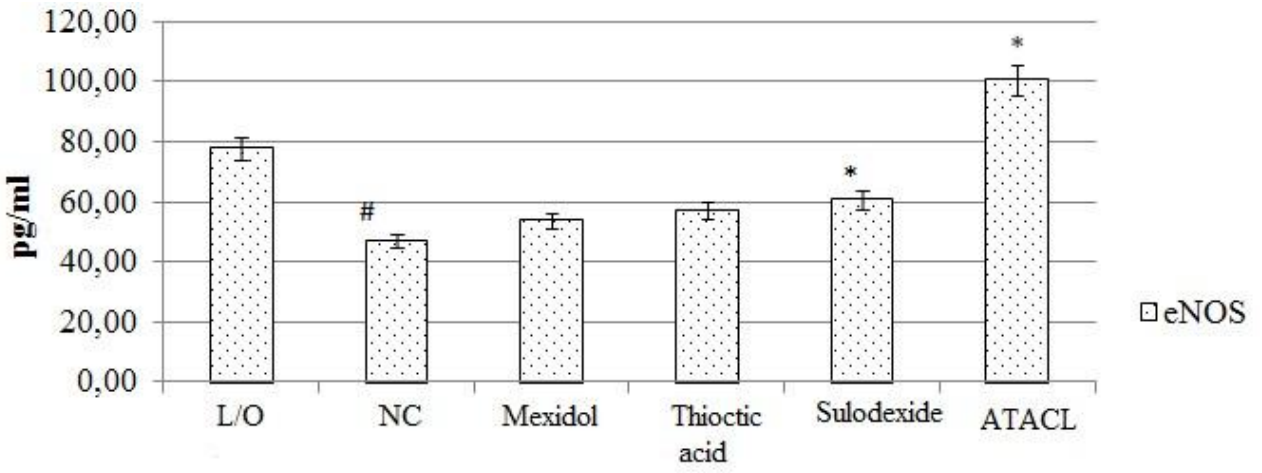


Figure 2: Effect of the ATACL compound and comparators on the change in eNOS concentration in rats against a background of focal cerebral ischemia

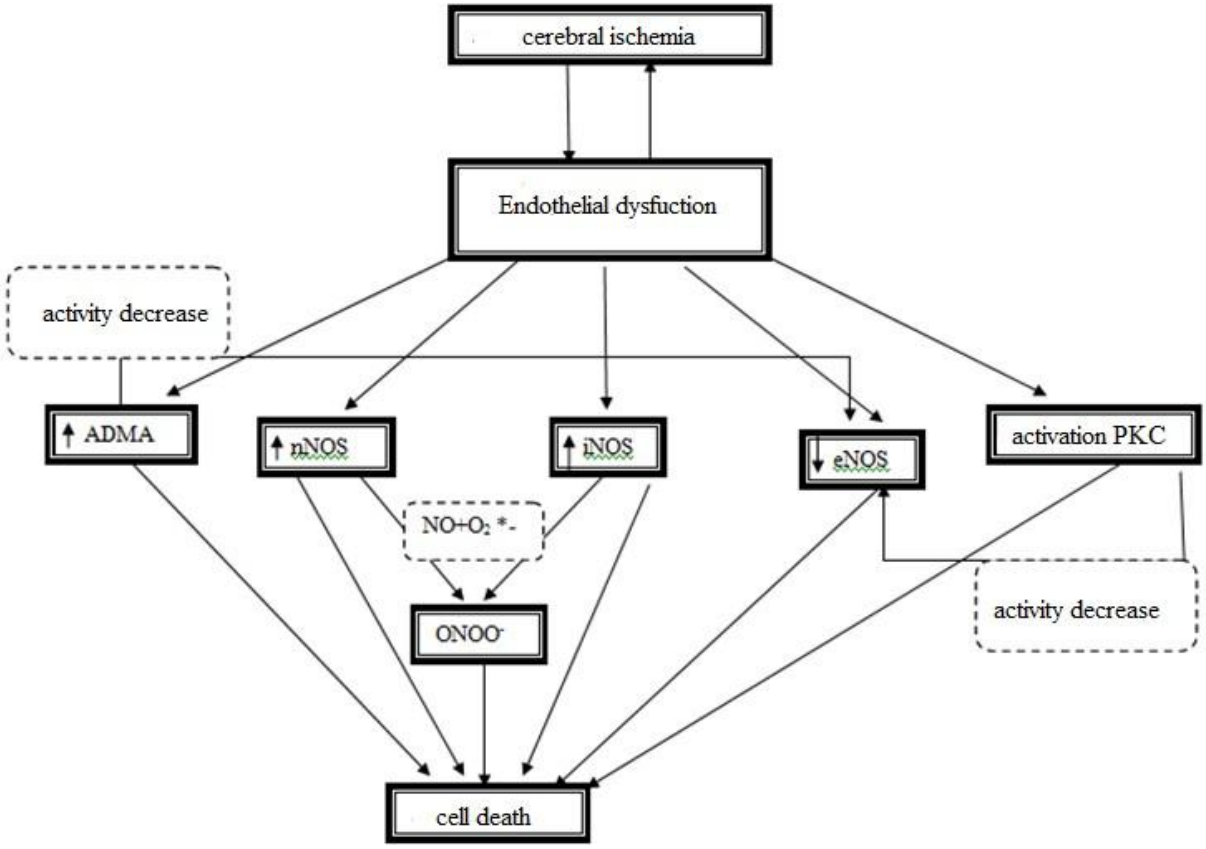
Note: # - statistically significant (U - Mann-Whitney test) for the N / O group of animals ($p < 0.05$); * - statistically significant (U - Mann-Whitney test) relative to the NC of the group of animals ($p < 0.05$).

decreased compared to the group of negative control rats by 83.8% ($p < 0.05$).

Against the backdrop of administration of sulodexide in rats, the decrease in ADMA concentration by 53.1% ($p < 0.05$), as well as nNOS and iNOS by 127.5% ($p < 0.05$) and 68.3% ($P < 0.05$), respectively (Fig. 1). In addition, in rats receiving sulodexide, an increase in the concentration of eNOS was observed (Fig. 2) compared to the group of negative control animals by 28.9% ($p < 0.05$). Also, against the background of the administration of sulodexide in rats, relative to the animals of the NK group, the PKC concentration decreased by 139.3% ($p < 0.05$). n rats receiving the ATACL compound, the concentration of ADMA decreased by 83.9% ($p < 0.05$) in relation to the group of negative control animals. Also in rats against the background of the ATACL compound, the concentration of nNOS and iNOS decreased by 60.6% ($p < 0.05$) and 61.9% ($p < 0.05$), respectively (Fig. 1). The content of eNOS (Fig. 2) in this group of animals, on the other hand, increased by 114.2% relative to the NC of the group of rats ($p < 0.05$). The concentration of PKC in rats upon administration of ATACL decreased by 108.6% in relation to the NC group of animals ($p < 0.05$). Thus, as a result of the study, it was established that experimentally modeled cerebral ischemia in rats is accompanied by an increase in the concentration of asymmetric dimethylarginine, nNOS, iNOS, PKC, and a decrease in the content of eNOS, which is consistent with literature sources [3,7-10]. Together, the changes observed in the NC of a group of rats observed during the study can be displayed by the following scheme: The use of comparative drugs allowed to correct the changes that occurred, the most pronounced pharmacological effect was observed with the use of sulodexide, which helped to reduce the concentration of ADMA, PKC, and also eliminated the NO-synthase system (the concentration of eNOS increased and selodexide decreased Content of nNOS, and iNOS). The use of the studied 4-hydroxy-3,5-di-tretbutyl cinnamic acid compound almost equals to the sulodexide of the degree contributed to the restoration of the endothelial function, which is reflected in a decrease in the concentration of ADMA, PKC, nNOS and iNOS, as well as an increase in eNOS compared to animals not Receiving pharmacological support. Moreover, these changes can underlie potentially possible mechanisms for the realization of the endothelioprotective action of 4hydroxy-3,5-di-tretbutyl cinnamic acid. Apparently, an increase in eNOS activity contributes to the elimination of brain hypoperfusion in conditions of its ischemia (due to the stabilization of NO production), vascular remodeling, and also the phenomena of hyperaggregation and hypercoagulability [9]. It is also important that when using 4-hydroxy-3,5- tretbutyl cinnamic acid, stabilizing the synthesis of nitric oxide (restoration of eNOS activity) could be accompanied by a decrease in its oxidative modification (by reducing the concentration of iNOS and nNOS) into peroxonitrite, a strong pro-oxidant, Which in turn helps to reduce cell damage (including endothelial cells) by the type of lipid peroxidation [5,8,10]. In addition, a decrease in iNOS concentration may help maintain the integrity of the blood-brain barrier, providing an anti-inflammatory effect of this compound [3,8], and reducing the content of nNOS by eliminating one of the main mechanisms of cell death in conditions of cerebral ischemia: NMDA - nNOS - AMPK pathway [5]. Reducing the concentration of ADMA - an endogenous inhibitor of eNOS, promotes recovery of the activity of this isoenzyme NOS, and also reduces apoptosis of cells [3]. Elimination of the inhibitory effect of PKC on the activity of endothelial NO synthase [6] may also underlie the endothelioprotective activity of 4-hydroxy-3,5-di-tertbutyl cinnamic acid.

CONCLUSIONS

1. Under conditions of experimentally induced cerebral ischemia, endothelial dysfunction develops in rats accompanied by a decrease in eNOS concentration (by 65.4% ($p < 0.05$)) and an increase in the content of nNOS, iNOS, ADMA and PKC (by 63.5% $P < 0.05$), 30.8% (p



Scheme 1: Basic mechanisms of endothelial dysfunction development in conditions of cerebral circulation insufficiency.

<0.05), 96% (p <0.05), and 179.5% (p <0.05), respectively.

2. Of the comparative drugs, the most pronounced endothelium-positive effect was the introduction of sulodexide, against the background of which normalization of all studied parameters was noted (an increase in the eNOS concentration by 28.9% (p <0.05), accompanied by a decrease in the content of ADMA, nNOS, iNOS and RKS by 53.1% (p <0.05), 127.5% (p <0.05) 68.3% (p <0.05), and 139.3% (p <0.05), respectively) .

3. The use of the studied ATACL compound under conditions of cerebral ischemia in rats facilitated (almost equal to the sulodexide degree) elimination of NOsynthase system disintegration, decrease in ADMA and PCC concentration.

4. At the base of endothelioprotective action, ATACL compounds can be affected by the following mechanisms: decrease in the concentration of ADMA, nNOS and iNOS, inhibition of PKC, and activation of eNOS.

REFERENCES

1. Voronkov A. V., Pozdnyakov D. I., Mamleev A. V. comparative evaluation of the effect of ATACL, mexidol and thioctic acid on the antithrombotic endothelial function, and some indices of peripheral blood of experimental animals on the background of focal cerebral ischemia. Modern problems of science and education. 2016; 2.; URL: https://scienceeducation.ru/ru/article/view?id=24398
2. Nechipurenko N.I., Paszkowski I. D., Musienko J. I.Basic pathophysiological mechanisms of cerebral ischemia. *Medical news.* 2008; 1: 7-13.
3. Chen S, Li N, Deb-Chatterji M, et al. Asymmetric Dimethyarginine as Marker and Mediator in Ischemic Stroke. *International Journal of Molecular Sciences*. 2012;13(12):15983-16004. doi:10.3390/ijms131215983.
4. Su JB. Vascular endothelial dysfunction and pharmacological treatment. *World Journal of Cardiology*. 2015;7(11):719-741. doi:10.4330/wjc.v7.i11.719.
5. Khan M, Dhammu TS, Matsuda F, Singh AK, Singh I. Blocking a vicious cycle nNOS/peroxynitrite/AMPK by S-nitrosoglutathione: implication for stroke therapy. *BMC Neuroscience*. 2015;16:42. doi:10.1186/s12868-015-0179-x.
6. Lucke-Wold BP, Turner RC, Logsdon AF, et al. Common Mechanisms of Alzheimer's Disease and Ischemic Stroke: The Role of Protein Kinase C in the Progression of Age-Related Neurodegeneration. *Journal of Alzheimer's disease : JAD*. 2015;43(3):711-724. doi:10.3233/JAD-141422.
7. Payne GA, Borbouse L, Kumar S, et al. Epicardial perivascular adipose-derived leptin exacerbates coronary endothelial dysfunction in metabolic syndrome via a PKC-β pathway. *Arteriosclerosis, thrombosis, and vascular biology*. 2010;30(9):17111717. doi:10.1161/ATVBAHA.110.210070.

8. Poggesi A, Pasi M, Pescini F, Pantoni L, Inzitari D. Circulating biologic markers of endothelial dysfunction in cerebral small vessel disease: A review. *Journal of Cerebral Blood Flow & Metabolism*. 2016;36(1):72-94. doi:10.1038/jcbfm.2015.116.
9. Tan X-L, Xue Y-Q, Ma T, et al. Partial eNOS deficiency causes spontaneous thrombotic cerebral infarction, amyloid angiopathy and cognitive impairment. *Molecular Neurodegeneration*. 2015;10:24. doi:10.1186/s13024-015-0020-0.
10. Terpolilli NA, Moskowitz MA, Plesnila N. Nitric oxide: considerations for the treatment of ischemic stroke. *Journal of Cerebral Blood Flow & Metabolism*. 2012;32(7):1332-1346. doi:10.1038/jcbfm.2012.12.