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Research Article

ENDOTHELIAL PROTECTIVE ACTIVITY OF 2,6-DIISOBORNYL-4-METHYLPHENOL IN A MODEL OF MYOCARDIAL ISCHEMIA/REPERFUSION IN RATS

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ABSTRACT

Objective: Our research focuses on the endothelial protective effects of 2,6-diisobornyl-4-methylphenol. Its effect was revealed while studying rats experiencing myocardial ischemia/reperfusion. The research results demonstrated that there are significant disturbances in the vascular endothelium manifested by a decrease in the vasodilating activity and antiplatelet properties of 2,6-diisobornyl-4-methylphenol.

Methods: We designed our own model of myocardial ischemia/reperfusion and applied it to 52 adult outbred Wistar males. We employed some methods of hemostasiological research such as thromboelastography to determine the antiplatelet activity of the vascular wall, G. Born nephelometric method to study platelet aggregation, phase contrast microscopy to count platelet counts in blood plasma, measurement of intra-arterial pressure to study the endothelial vasodilating function, and calculated the endothelial dysfunction coefficient in rats.

Results: Preventive intragastric injection of 2,6-diisobornyl-4-methylphenol (100 mg/kg, 3 days before and 5 days after reproducing the myocardial ischemia/reperfusion model) increased the antiplatelet activity of the vascular endothelium in rats by 37% compared to the endothelium of the abdominal aorta segment of untreated animals. Moreover, 2,6-diisobornyl-4-methylphenol decreased the endothelial dysfunction coefficient by 43% in comparison with the value in the control group.

Conclusion: 2,6-diisobornyl-4-methylphenol has an endothelial protective effect proved by its ability to increase antiplatelet properties of the endothelium and decrease the endothelial dysfunction coefficient. The revealed endothelial protective properties of 2,6-diisobornyl-4-methylphenol can be regarded as one of the potential mechanisms of cardioprotective activity of the drug.

Keywords: 2,6-diisobornyl-4-methylphenol, Endothelial protective effect, Myocardial ischemia/reperfusion, Vascular endothelium.

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INTRODUCTION

Cardiovascular diseases (CVD) are one of the main causes of death around the world. According to the World Health Organization (WHO), 17.9 million people died from CVD in 2016, estimated 31% of all deaths worldwide. About 85% of all CVD deaths are due to heart attacks and strokes [1].

There is no doubt that the vascular endothelium dysfunction is one of the main pathophysiological mechanisms of the development and aggravation of CVD. It contributes to the development of atherosclerosis, coronary thrombosis, left ventricular remodeling, progression of heart failure, etc. Impaired local nitric oxide (NO) production and oxidative stress are the major causes of the endothelial dysfunction. As a result, excessive generation of endothelium-dependent superoxide inactivates NO molecules, damages the endothelial cell membranes with peroxynitrite and hydroxyl radicals. Thus, substances able to reduce the intensity of lipid peroxidation and/or protect NO from inactivation by reactive oxygen species can serve as effective endothelium protectors [2,3].

2,6-Diisobornyl-4-methylphenol is a promising drug currently undergoing the stage of preclinical trials. Its molecule represents a hindered terpenophenol with a proven potent antioxidant activity (Fig. 1a) [4].

The structural properties of 2,6-diisobornyl-4-methylphenol (for example, the presence of the hydroxyl group (OH) in the structure forming a conjugated system with aromatic ring bonds) determine its ability to easily interact with various free radicals (hydroxyl, peroxyl, and superoxide anion radical) neutralizing them and forming phenoxy radicals, which are of little reactivity with respect to other radicals and molecules. This probably is a major contribution to a high anti-ischemic activity of the drug (Fig. 1b).

Our previous studies demonstrated its hemorheological, antiplatelet, and antithrombogenic effects within the settings of the model of transient cerebral ischemia [4,5]. At present, we are carrying out a comprehensive study of the cardioprotective activity of the drug [6,7]. Our research focuses on the influence of 2,6-diisobornyl-4-methylphenol on the functional activity of the vascular endothelium under myocardial ischemia/reperfusion as one of the probable mechanisms of cardioprotective activity of the drug. Research on the application of the drug has never been done before and strives for expanding the existing understanding of mechanisms of its effect.

The research aims to study the influence of 2,6-diisobornyl-4-methylphenol on antiplatelet and vasodilating activity of the endothelium under the settings of the myocardial ischemia model with further reperfusion in rats.

METHODS

All the studies were conducted in accordance with good laboratory practice standards. Handling of rats was carried out in accordance

with the international guide for the care and use of laboratory animals. Animals were used for the study after securing the ethical clearance from the Institutional Animal Ethical Committee.

The experiments were performed on 52 adult outbred Wistar male rats of 250–290 g that were kept on a standard diet with free access to food and water. The study comprised two series of experiments.

Series 1 concerned the influence of 2,6-diisobornyl-4-methylphenol on the antiplatelet activity of the vascular wall; whereas in Series 2, the influence of the drug on the endothelium-dependent and endothelium-independent vasodilation was studied. In each series of the experiment rats were divided into three groups: Sham operated, control, and experimental group. The model of acute myocardial ischemia with subsequent recirculation was reproduced on the animals from the control and experimental groups. To do that, rats were anesthetized (sodium thiopental, 60 mg/kg intraperitoneally), then intubated and connected to Rodent Ventilator 7025 (Italy). After performing thoraco-pericardiotomy, the left coronary artery was occluded at the level of the auricula sinistra inferior margin without disturbing the cardiac topography in the chest according to professor Kogan's method [8]. The left coronary artery was occluded for 45 min. After that, the ligature was untied and post-ischemic reperfusion was performed. Electrocardiography monitoring in standard lead II was performed to verify the adequacy of coronary artery occlusion using the computerized electrocardiograph Poly-Spectrum-8/L (Russia). Sham-operated animals underwent a similar surgery but without ligation of the vessel.

On the 5th day of the experiment, a fragment of the abdominal aorta was removed from anesthetized rats (3.0±0.2 mg) to determine the influence of 2,6-diisobornyl-4-methylphenol on the antiplatelet activity of the vascular wall. The removed segment was washed from the blood and incubated for 3 min in standardized platelet-rich plasma obtained from donor rats. Then, aggregatograms were recorded on the automatic platelet aggregation analyzer AT-02 (Russia) with adenosine diphosphate (ADP) added to plasma at final concentration of 0.00004 M. To obtain platelet-rich plasma, seven intact male donor rats were used. Platelet aggregation in the platelet-rich plasma was determined by the G. Born nephelometric method [9]. Conventional methods were used to obtain platelet-rich and platelet-poor plasma and count the number of platelets [10]. Rich and poor-platelet forms of plasma were obtained by centrifugation at 400 g and 1800 g, respectively, on centrifuge PC-6 (Russia). The platelet count was measured with a microscopic method with phase contrast in the Neubauer counting chamber. Having counted the number of platelets in rich-platelet plasma, the platelet count was standardized by diluting poor-platelet plasma up to 400±30 thousand platelets/1 mm^3 sample.

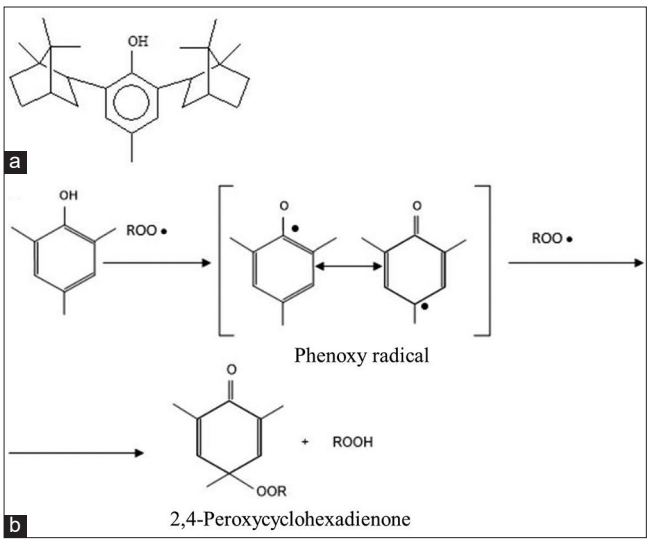

Fig. 1: (a) Structure of 2,6-diisobornyl-4-methylphenol (Mm 380.61); (b) scheme of redox of 2,6-diisobornyl-4-methylphenol (neutralization of free radicals)

To study the impact of 2,6-diisobornyl-4-methylphenol on the vasodilating function of the endothelium, anesthetized animals had their carotid artery catheterized to record blood pressure after intravenous administration of acetylcholine (5 μg/kg) and sodium nitroprusside (30 μg/kg), causing endothelium-dependent and endothelium-independent vasodilation, respectively. Intraarterial blood pressure was measured with TSD104A for Biopac MP150 (USA). The area above the curve of the reaction of blood pressure to the administration of acetylcholine and sodium nitroprusside was calculated. The endothelial dysfunction coefficient was determined as a ratio of the area above the curve under the administration of sodium nitroprusside to the value under the administration of acetylcholine [11].

In both series of experiments, the animals from the experimental group received 100 mg/kg of 2,6-diisobornyl-4-methylphenol intragastrically. About 1 ml of the suspension containing 2% of starch mucus was given once a day according to the prevention scheme: 3 days before and 5 days after myocardial ischemia/reperfusion. The rats of the control group and sham-operated animals received equivalent volumes of starch mucus intragastrically according to a similar pattern.

Statistica 12.5 was used for data processing. Average value and standard error were calculated. The non-parametric Mann–Whitney U-test was used to assess the validity of differences between groups. Statistically significant differences were considered at $p<0.05$.

RESULTS AND DISCUSSION

At the first stage, the influence of 2,6-diisobornyl-4-methylphenol on the antiplatelet activity of the vascular endothelium was studied in rats experiencing acute myocardial ischemia/reperfusion. It was established that original (without pre-incubation with vessel segment) amplitude of irreversible ADP-induced platelet aggregation in plasma of donor rats was 36%. Pre-incubation of the segments of the abdominal aorta of sham-operated animals in the donor plasma reduced the amplitude of platelet aggregation to 8%. After incubation of vascular segments of the animals with myocardial ischemia/reperfusion in rich-platelet plasma of donor rats, the amplitude of ADP-induced platelet aggregation made up 19%, which is 2.4 times higher than the value in the sham-operated animals (Table 1).

Consequently, the replication of myocardial ischemia/reperfusion in rats significantly reduced the antiplatelet properties of the vascular endothelium. Probably, one of the main causes of this effect is oxidative stress following ischemia and especially myocardial reperfusion, which is given a great importance in reducing antiadhesive and antiplatelet properties of the endothelium [5,12].

Table 1: The influence of 2,6-diisobornyl-4-methylphenol under preventive administration (100 mg/kg, intragastrically) on antiplatelet activity of the vascular wall in rats after myocardial ischemia/reperfusion

Group	Platelet aggregation amplitude, %
Before incubation with aorta segment	
Intact donor rats (n=7)	36±3
After incubation with aorta segment	
Sham operated (n=15)	8±1[#]
Myocardial infarction	
Control (n=15)	19±1[#*]
2,6-diisobornyl-4-methylphenol(n=15)	12±1[#+]

[#]$p<0.05$ compared to the values in intact rats; *$p<0.05$ compared to the values in sham operated animals; [+]$p<0.05$ compared to the values in the control group

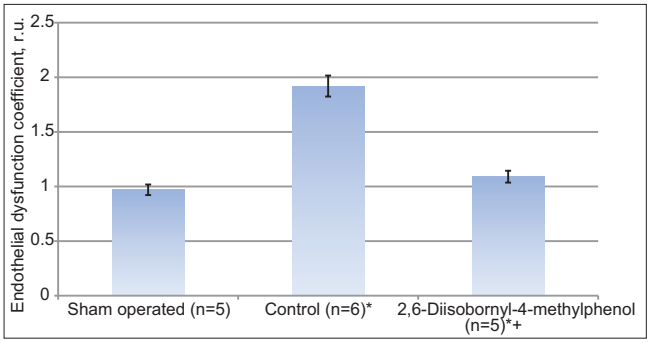

Fig. 2: Influence of 2,6-diisobornyl-4-methylphenol (100 mg/kg, intragastrically) under preventive administration on the endothelial dysfunction coefficient (r.u.) in rats after myocardial ischemia/reperfusion. *p<0.05 compared to the values in sham-operated rats; †p<0.05 compared to the values in control group; r.u: Relative unit

The amplitude of ADP-induced platelet aggregation of platelets after incubation of the abdominal aorta segment of rats with myocardial ischemia/reperfusion which received 2,6-diisobornyl-4-methylphenol according to the prevention scheme made up 12%, which was 37% lower than the value in the control group and close to the value in the group of sham-operated animals (Table 1).

Therefore, preventive administration of 2,6-diisobornyl-4-methylphenol prevents a decrease in the antiplatelet activity of the aortic endothelium caused by myocardial ischemia/reperfusion in rats.

Impaired endothelium-dependent vasodilation is an early criterion of the endothelial dysfunction and the first stage in CVD development. We recorded the reaction of arterial pressure to the administration of acetylcholine and sodium nitroprusside and calculated the endothelial dysfunction coefficient to study the influence of 2,6-diisobornyl-4-methylphenol on the vasodilating function of endothelium. Acetylcholine stimulates the release of NO by the endothelium; therefore, it is widely used in clinic and experiment to assess the endothelial dysfunction. However, in various pathological conditions, including myocardial infarction, the reaction of vascular smooth muscle to the action of other humoral and neurogenic factors may change. Therefore, sodium nitroprusside was used to evaluate endothelium-independent vasodilation.

In the group of sham-operated rats, the endothelial dysfunction coefficient made up 0.97±0.05, i.e., there were no significant changes in the reaction of arterial pressure to vasoactive agents (Fig. 2). In the control group, the endothelial dysfunction coefficient after acute myocardial ischemia/reperfusion made up 1.92±0.10, which is almost 2 times the value in the group of sham-operated rats (Fig. 2). In the group of rats that received 2,6-diisobornyl-4-methylphenol 3 days before and 5 days after the replication of the model of myocardial ischemia with reperfusion, the endothelium dysfunction made up 1.09±0.06. Consequently, 2,6-diisobornyl-4-methylphenol nearly recovers the vasodilating activity of the endothelium up to the value in the sham-operated animals (Fig. 2).

In accordance with modern concepts, the endothelium dysfunction is also of great importance in the pathogenesis of no-reflow after myocardial reperfusion [13]. The developing endothelial dysfunction disturbs the most important physiological functions such as balance of vasoconstriction and vasodilation, potentiation and inhibition of fibrinolysis factors, and platelet aggregation, as well as the production of pro- and anti-inflammatory factors [14]. Endothelial dysfunction is characterized by imbalance between vasoconstriction (thromboxane A_2, endothelin-1) and vasodilation (NO, prostaglandins) in favor of the latter. At the same time, endothelium-dependent vasodilation, anti-inflammatory, and anticoagulant properties of the vascular endothelium are disturbed [15].

Activation of lipid peroxidation in tissues with the release of active peroxide radicals into the system blood circulation may be one of the mechanisms for the development of endothelial dysfunction during ischemia/reperfusion [13].

High concentration of peroxides in blood accelerates NO degeneration – endothelial vasodilating factor with the formation of peroxynitrite – extremely cytotoxic compound. Accelerated decay of the endothelial NO stimulates an angiospasmus, whereas oxidation of exogenous NO, formed due to metabolism of consumed nitrate preparations, reduces their therapeutic efficiency. Furthermore, free radicals modify endothelial NO receptors decreasing their sensitivity and have a direct damaging effect on cardiomyocytes. The mentioned processes deteriorate ischemia, have an arrhythmogenic effect, increase the area of necrosis, and damage myocardium [16]. Dysfunction of the vascular endothelium is one of the main pathophysiological mechanisms of CVD since it contributes to the development of atherosclerosis, coronary thrombosis, left ventricular remodeling, progression of heart failure, etc. [14].

Our model of acute myocardial ischemia with reperfusion is characterized by retention of reduced blood circulation in heart during ischemia and, consequently, inflow of substrates and oxygen, which contributes to the activation of lipid peroxidation [17]. 2,6-Diisobornyl-4-methylphenol is a powerful antioxidant and is more likely to display its endothelial protective activity primarily due to inhibition of metabolic NO oxidation leading to the increase in its bioavailability and therefore, strengthening its antiplatelet and vasodilating effect [18].

CONCLUSION

In the model of myocardial ischemia/reperfusion in rats, 2,6-diisobornyl-4-methylphenol is able to prevent disturbances of the functional activity of the vascular endothelium, which is manifested in the increase in its antiplatelet activity and recovery of endothelium-dependent vasodilation. The revealed endothelial protective properties of 2,6-diisobornyl-4-methylphenol can be regarded as one of the potential mechanisms of cardioprotective activity of the drug.

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AUTHORS' CONTRIBUTIONS

All authors have equally contributed to making this article successful.

CONFLICTS OF INTEREST

All authors of this study declare that there are no financial or commercial conflicts of interest.

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