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# Cardioprotective Activity of 2,6-Diisobornyl-4-Methylphenol in Acute Myocardial Ischemia/Reperfusion in Rats

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We studied the cardioprotective effect of 2,6-diisobornyl-4-methylphenol under conditions of myocardial ischemia/reperfusion in rats. Daily administration of 2,6-diisobornyl-4-methylphenol (100 mg/kg intragastrically) over 3 days before and 5 days after modeling of myocardial ischemia/reperfusion prevented the increase in the infarction area by almost 2 times in comparison with the control by day 5 after recirculation. The type and severity of pathological changes in ECG parameters reflecting necrotic changes in the myocardium under the action of the compound significantly decreased by day 35 of the experiment. Animal survival rate during the first 24 h after ischemia/reperfusion modeling in the experimental group was by 29% higher than in the control group.

**Key Words:** *2,6-diisobornyl-4-methylphenol; rats; myocardial ischemia/reperfusion; infarction zone; ECG*

Despite significant progress in the treatment of coronary heart disease, the search for new drugs capable of protecting cardiomyocytes from damage caused by ischemia and reperfusion is still in progress [13,14]. Sterically hindered phenols based on terpenophenols are a promising group for the development of new drugs [10]. Synthesis of the original compounds of this group was performed at the Institute of Chemistry [10]. Compounds of this series exhibit antioxidant, antihypoxic, and hemorheological activities [5,7]. Pharmacokinetic studies demonstrated accumulation of one of the compounds of this series (2,6-diisobornyl-4-methylphenol) in the heart [9].

We studied the cardioprotective effects of 2,6-diisobornyl-4-methylphenol under conditions of acute myocardial ischemia/reperfusion (IR) in rats.

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## MATERIALS AND METHODS

The experiments were performed on outbred Wistar male rats ($n$=49) weighing 230-300 g. The animals were obtained from the vivarium of the Department of Experimental Biological Models, E. D. Goldberg Research Institute of Pharmacology and Regenerative Medicine. The experimental conditions complied with the regulations of the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes (1986), the principles of Good Laboratory Practice, and the protocol approved by the Animal Care and Use Committee of E. D. Goldberg Research Institute of Pharmacology and Regenerative Medicine (protocol No. 72052014).

2,6-Diisobornyl-4-methylphenol was synthesized at the Institute of Chemistry.

Myocardial IR was modeled as described elsewhere [1]; the rats were anesthetized with sodium thiopental (60 mg/kg intraperitoneally), intubated and connected to Rodent Ventilator 7025. A ligature was applied to the left coronary artery; the duration occlusion was 45 min. For blood flow resumption, the ligature was loosened without, but not removed, and the

wound was sutured layer-by-layer. In view of high animal mortality in the control group [8], the rats were divided into the control ($n$=33) and experimental ($n$=16) groups so that the number of survivors in both groups was comparable. 2,6-Diisobornyl-4-methylphenol was administered intragastrically in a dose of 100 mg/kg (in 1 ml 2% starch mucilage) by the therapeutic-and-preventive regimen: for 3 days before IR (last administration 15 min before surgery) and for 5 days after it. Controls received starch mucilage in the same regime.

In control rats and animals treated with 2,6-diisobornyl-4-methylphenol, ECG in standard lead II was recorded on days 1, 3, and 5 after IR modeling was recorded using a Poly Spectrum-8/L electrocardiograph and animal survival was assessed. The baseline ECG was recorded in anesthetized animals prior to IR modeling. The amplitude of *Q*, *R*, *T* waves and duration of *PQ* and *QT* intervals were measured.

On day 5 of the experiment, after ECG registration, artificial ventilation was started and after thoracotomy, the ligature on the left coronary artery was tightened. To identify hypoperfusion zones, bolus injection of 0.2 ml 5% solution of patent blue violet dye (Sigma) into the femoral vein was performed and the heart was removed 10-20 sec later. Perfused myocardium was stained green, and non-perfused areas remained unstained. Transverse sections of the heart with a thickness of 300 μ were prepared on a MZ-2 freezing microtome. To assess the zone of hypoperfusion, the sections were left in native form, and for evaluation of the infarction zone, they were stained with NTZ (Sigma) in after Seligman and Rutenberg [4]. The sections were embedded in glycerol gelatin gel and scanned. The areas hypoperfusion and infarction zones were assessed using Adobe Photoshop CS6.

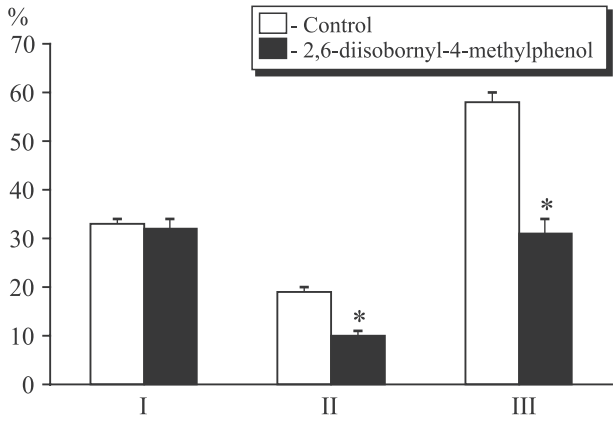


**Fig 1.** Effect of 2,6-diisobornyl-4-methylphenol (100 mg/kg, intragastrically) administered in the therapeutic-and-preventive regimen on the size of hypoperfusion and infarction zones by 5 day after IR. I: hypoperfusion area (% of total myocardial area), II: zone of infarction (% of the total myocardium area), III: infarction area (% of hypoperfusion area) *$p$<0.05 in comparison with the control.

Statistical processing of the results was performed using Statistica 9.0 software. The data are presented as the *M*±*SEM*. The normality of the distribution was assessed using Kolmogorov—Smirnov and Shapiro—Wilk tests. Significance of differences ($p$<0.05) between the series was determined using Student's *t* test

**TABLE 1.** Effect of 2,6-Diisobornyl-4-Methylphenol (100 mg/kg intragastrically) Administered in the Therapeutic-and-Preventive Regimen on ECG Parameters after Myocardial IR in Rats (*M*±*SEM*)

| Parameter | Initial values | After IR | | |
|---|---|---|---|---|
| | | day 1 | day 3 | day 5 |
| Control | | | | |
| HR, bpm | 439±15 | 441±15 | 431±12 | 420±12 |
| *PQ*, msec | 42.1±0.6 | 44.0±1.0 | 44.2±1.5 | 41.8±1.5 |
| *QT*, msec | 82.5±1.8 | 80.6±2.0 | 78.6±2.3 | 81.6±2.1 |
| *Q*, μV | 0±0 | 28.6±2.9* | 20.9±2.3* | 7.6±1.4* |
| *R*, μV | 310.2±14.7 | 174.1±14.2* | 186.3±8.9* | 228.3±12.3* |
| *T*, μV | 243.8±19.1 | 17.7±1.3* | 19.7±3.0* | 75.4±7.8* |
| 2,6-Diisobornyl-4-Methylphenol | | | | |
| HR, bpm | 439±11 | 441±13 | 419±12 | 439±11 |
| *PQ*, msec | 37.8±2.4 | 44.2±1.0 | 42.5±2.3 | 39.6±1.9 |
| *QT*, msec | 81.5±1.4 | 78.9±4.1 | 77.5±2.1 | 79.2±1.7 |
| *Q*, μV | 0±0 | 25.7±2.4* | 15.6±1.8* | 4.5±1.6*+ |
| *R*, μV | 316.2±21.5 | 192.3±20.0* | 240.0±24.7*+ | 274.6±27.6 |
| *T*, μV | 293.9±17.4 | 51.3±11.3*+ | 76.7±5.5*+ | 147.4±12.8*+ |

**Note.** $p$<0.05 in comparison with *initial values, +control.

(ECG parameters), Mann—Whitney test (size of infarction and hypoperfusion zones), and Fisher's exact test (animal mortality).

## RESULTS

In the animals of the control group, necrotic changes in the myocardium were revealed on days 1-5 after reperfusion (appearance of pathological *Q* wave and decrease in of *T* and *R* wave amplitude [2]; Table 1), which was consistent with previous results obtained on the model of myocardial infarction [3]. Ischemia led to hypoperfusion and formation of infarction zones. In animals of the control group, the area of hypoperfusion in the myocardium occupied 32.1±1.9% of the total myocardial section area. The infarction zone occupied 19.0±0.8% of the total area of myocardial sections and 57.9±2.4% of hypoperfusion zone (Fig. 1). The leading cause of the formation of the infarction zone in the myocardium is reperfusion damage; the probability of favorable prognosis decreases with increasing the area of affected myocardium [11]. The death of the animals in control group was observed during first day in the acute phase of ischemic and reperfusion damage of the myocardium and constituted 48% (17 of 33 rats).

In the group of animals receiving 2,6-diisobornyl-4-methylphenol in the treatment-and-preventive regimen, the size of hypoperfusion zone did not differ from that in the control group, while infarction zone by day 5 of observation was almost 2-fold lower than in the control (Fig. 1). The death of experimental animals was also observed during first day after IR modeling and constituted 19% (3/16 rats), which was significantly lower than in control group (Table 1).

Changes in ECG parameters were a result of a decrease in the area of damage zone in the left ventricle: by day 3 after IR modeling, the frequency and amplitude of *Q* wave tended to decrease, and by day 5, this parameter significantly differed from the control. In addition, the decrease in the amplitude of *T* and *R* waves was less pronounced than in the control group (Table 1). Time parameters of ECG (*PQ* and *QT* intervals) in both groups were stable throughout the experiment.

These results confirm the cardioprotective effect of 2,6-diisobornyl-4-methylphenol that can be attributed to its antioxidant effect [7] reducing the intensity of oxidative stress during reflow and, as a consequence, reperfusion damages [12]. In addition, the protection of the myocardium by 2,6-diisobornyl-4-methylphenol can be due to its ability to improve hemorheological parameters and microcirculation and produce antiplatelet and antithrombogenic effect [6,7]. These effects probably contribute to reduction of the severity of the phenomenon of "no-reflow" and therefore, limit zone of infarct after IR of the myocardium in rats.

Thus, course administration of 2,6-diisobornyl-4-methylphenol to rats in a model of myocardial IR prevents the increase in the infarction area in the left ventricle, promotes normalization of ECG parameters by days 3-5 after reperfusion, and improves animal survival.

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