PHARMACOLOGY AND TOXICOLOGY

Antithrombogenic and Antiplatelet Activity of Optho-Isobornyl Phenol Derivative

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We studied antithrombogenic and antiplatelet properties of 4-methyl-2,6-diisobornyl phenol, a new promising compound belonging to ortho-isobornyl phenol derivatives, under conditions of intravascular thrombosis and acute cerebral ischemia. It was found that 4-methyl-2,6-diisobornyl phenol prevents intravascular thrombus formation by reducing platelet aggregation and improving antiplatelet activity of the vascular wall.

Key Words: *thrombosis; ischemia; ortho-isobornyl phenol; sterically hindered phenols*

The causes of thrombus formation are still poorly understood [7,9,10] and the use of antiaggregants for the prophylaxis of thromboses is not pathogenetically substantiated [6]: various serious questions arise during treatment of these conditions. Recently, the resistance of patients to antiplatelet drugs became an important problem. The number of patients resistant to acetylsalicylic acid and clopidogrel is 24-35% and 15-40%, respectively [6].

The resistance to antiplatelet preparations prompted further search for new effective and safe compounds capable of preventing thrombus formation via modulation of various elements of the thrombotic process.

Here we studied antiplatelet and antithrombogenic activity of 4-methyl-2,6-diisobornyl phenol, an ortho-isobornyl phenol derivative, under conditions of intravascular thrombosis and ischemia of the brain.

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MATERIALS AND METHODS

The efficiency of 4-methyl-2,6-diisobornyl phenol was studied in three experimental series. In series 1, antithrombogenic activity of the test compound was evaluated on 15 male Wistar rats weighing 220-240 g and divided into 3 groups: control animals (group 1) received 1 ml 5% starch suspension, while experimental rats (groups 1 and 2) received pentoxyphylline (400 mg/kg) and 4-methyl-2,6-diisobornyl phenol (100 mg/kg) respectively. Starch suspension, pentoxyphylline, and the test drug in equivalent volumes were administered through a gastric tube once a day for 5 days.

One hour after administration of the last dose, the animals were narcotized (1 g/kg urethane, intraperitoneally), the left common carotid artery (CA) was separated, and intravascular thrombosis was induced by application of 10% $FeCl_2$ on the vessel [3]. A cuff-type sensor was applied to CA and the blood flow in the vessel was recorded using a MFV-1100 electromagnetic flow meter (Nihon Kohden). Blood flow was measured continuously (before, during 15-min application of $FeCl_2$, and for 90 min after washout), the time of complete blood flow

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arrest in the stenosed vessel and the degree of blood flow reduction during the experiment were determined. After that, the wound was treated with an antiseptic and sutured. After 1 day, the animals were euthanized and the vessel fragments treated with $FeCl_2$ were isolated. The vessel was cut, the thrombus was removed and weighed. In this and subsequent experiments, the animals were euthanized by urethane overdose.

In experimental series II, the antiplatelet effect of 4-methyl-2,6-diisobornyl phenol was evaluated. The experiments were carried out on 15 male rats weighing 300-360 g (3 groups, 5 animals per group). One hour before blood sampling, the animals of experimental groups 1 and 2 received pentoxyphylline and 4-methyl-2,6-diisobornyl phenol, respectively (the doses were the same as in series I). Control rats received starch suspension in the equivalent volume. The blood was taken from the common carotid artery under ether anesthesia and stabilized with sodium citrate (3.8%, 1:9 v/v). Platelet aggregation was measured nephelometrically [8]. Platelet-rich (PRP) and platelet-poor plasma (PPP) were obtained and platelets were counted routinely [1]. The number of platelet in PRP was adjusted to 400,000±30,000 per 1 mm^3 by adding PPP. The antiplatelet effect of the vascular wall was evaluated using PPP and PRP from intact animals. Platelet aggregation was assayed in the standardized plasma on an AT-02 aggregometer coupled with a Recorder 2210 writer.

In series III, the effect of 4-methyl-2,6-diisobornyl phenol on antiplatelet activity of the vascular wall was studied. Experimental ischemia of the brain was used as the model of endothelial dysfunction, because antiaggregant activity of the aorta is known to decrease during acute ischemia [4]. Ischemia of the brain was modeled under ether anesthesia by complete ligation of the left common CA and partial ligation of the right common CA (50% reduction of the blood flow compared to the initial level) [5].

Experiments were carried out on 15 male Wistar rats weighing 300-360 g. Sham-operated and control animals received starch suspension through a gastric tube; experimental animals received 4-methyl-2,6-diisobornyl phenol in a dose of 100 mg/kg. Starch suspension and 4-methyl-2,6-diisobornyl phenol were administered daily for 5 days. One hour after administration of the last dose, a fragment of the abdominal aorta (3.0±0.7 mg) was obtained under ether narcosis. The vessel was washed from blood and after 3-min incubation in standardized PRP obtained from donor rats the amplitude of platelet aggregation was measured.

The data were processed statistically using Statistica 6.0 software. The means and standard errors were calculated, the intergroup differences were evaluated using Student's *t* test.

RESULTS

In control animals, blood flow arrest in CA was observed 19±1 min after application of $FeCl_2$ on the vascular wall and thrombi formed in the lumen of stenosed vessels (Table 1).

After course intragastric treatment with pentoxyphylline, the blood flow decreased compared to the initial value after 90 min (Table 1). No thrombi in CA were found on the next day in these rats. Our findings are consistent with previous reports that pentoxyphylline reduced thrombus formation in stenosed vessels [11].

Course intragastric administration of 4-methyl-2,6-diisobornyl phenol prevented blood flow decrease after $FeCl_2$ application and completely inhibited thrombus formation in CA (Table. 1).

Thus, 4-methyl-2,6-diisobornyl phenol by its antithrombogenic activity is not inferior to pentoxyphylline.

In series II, the amplitude of ADP-induced platelet aggregation (4×10^{-6} M ADP) in the control group was 28±1%. In animals treated with pentoxyphylline, the amplitude of ADP-induced platelet aggregation was lower than in the control group. After treatment with 4-methyl-2,6-diisobornyl phenol, the amplitude of platelet aggregation was also appreciably below the corresponding values in the control group (Fig. 1).

Evaluation of antiplatelet activity of the vascular wall showed that in donor rats the amplitude

TABLE 1. Parameters of Intravascular Thrombus Formation during Course Intragastric Treatment with Pentoxyphylline and 4-Methyl-2,6-Diisobornyl Phenol (*M*±*m*)

Group	Initial blood flow, ml/min	Blood flow decrease over 90 min, %	Weight of thrombus after 1 day, mg
Control	5.0±0.5	100	1.2±0.2
Pentoxyphylline	6.3±0.6	31±12	0
4-Methyl-2,6-diisobornyl phenol	7.1±0.9	13±9	0

TABLE 2. Effect of Incubation of Abdominal Aortal Segment in Standardized PRP on Amplitude of ADP-Induced Platelet Aggregation (*M*±*m*)

Group	Initial platelet aggregation in donor plasma, %	Platelet aggregation in donor plasma after incubation, %
Sham-operated	41±2	7±1
Cerebral ischemia		
control	41±2	20±5*
4-methyl-2,6-diisobornyl phenol	41±2	10±2+

Note. $p<0.05$ compared to: *sham-operated animals, +control.

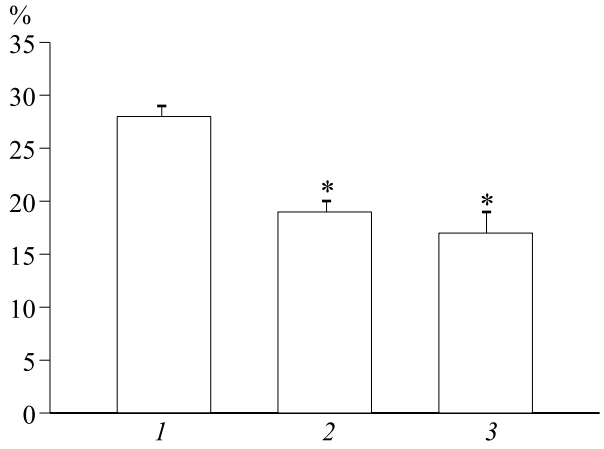

Fig. 1. Effect of pentoxyphylline and 4-methyl-2,6-diisobornyl phenol on amplitude of ADP-induced platelet aggregation. *1*) control; *2*) pentoxyphylline, *3*) 4-methyl-2,6-diisobornyl phenol. *$p<0.05$ compared to the control.

of platelet aggregation in PRP induced by ADP in a final concentration of 4×10^{-5} M (*i.e.* by one order of magnitude higher than in the previous experimental series) was 42±2% (Table 2). After incubation of donor PRP with a fragment of the abdominal aorta from sham-operated animals, the amplitude of platelet aggregation was by 83% lower than this parameter before incubation. After incubation of PRP with a fragment of the abdominal aorta from rats of the control group, the amplitude of platelet aggregation increased 3-fold, which attested to exhaustion of antiplatelet activity of the abdominal aorta in rats with cerebral ischemia (Table 2). In rats with cerebral ischemia receiving 4-methyl-2,6-diisobornyl phenol, this parameter was close to normal. This suggests that 4-methyl-2,6-diisobornyl phenol potentiates antiplatelet activity of aortal endothelium under conditions of cerebral ischemia.

Thus, 4-methyl-2,6-diisobornyl phenol is a new promising compound producing a pronounced effect on the thrombovascular homeostasis and characterized by low toxicity [2]. Our findings suggest that antithrombogenic activity of this agent can be determined by both antiplatelet and endothelium-protecting properties.

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