Hemorheological Effects of Ortho-Isobornyl Phenol Derivative under Conditions of Brain Ischemia in Rats

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Hemorheological activity of 4-methyl-2,6-di-isobornyl phenol, a new *o*-isobornyl phenol derivative, was studied under conditions of experimental prolonged partial cerebral ischemia. Brain ischemia is associated with hemorheological disorders which can be characterized as blood hyperviscosity syndrome: increased viscosity of the whole blood (within a wide range of shear rates), plasma viscosity, fibrinogen content in blood plasma, and platelet aggregation; deterioration of platelet deformability and reduced availability of oxygen for tissues. A course (5 days) of intragastric 4-methyl-2,6-di-isobornyl phenol (100 mg/kg) prevented the development of blood hyperviscosity syndrome by modulating blood macrorheology (reduction of plasma viscosity and fibrinogen content) and microrheology (reduction of erythrocyte aggregation and improvement of their deformability).

Key Words: *brain ischemia; blood rheology; blood hyperviscosity syndrome; o-isobornyl phenol*

Cerebral circulation disorders are associated with the formation of oxidative stress [1], which leads to disorders in blood rheology with the development of blood hyperviscosity syndrome [6,7,9]. The efficiency of correction of blood rheology in oxidative stress with drugs with antioxidant effects was reported [5,10,11].

We studied hemorheological activity of a new semisynthetic *o*-isobornyl phenol derivative 4-methyl-2,6-diisobornyl phenol (MD) under conditions of cerebral ischemia (CI) in rats. This compound exhibits high antioxidant activity *in vitro* and is characterized by low toxicity [4].

MATERIALS AND METHODS

Experiments were carried out on 30 male Wistar rats (220-240 g) divided into 3 groups, 10 animals per group. Controls and sham-operated (SO) rats received intragastrically 1 ml 5% starch gel, experimental animals received intragastrically MD (100 mg/kg). The animals received a daily dose of starch gel or MD suspension in starch gel for 5 days. The first dose was administered 1 h after CI modeling, the last one 1 h before blood collection.

The CI model was created under ether narcosis by complete occlusion of the left carotid artery and 50% limitation of the blood flow in the right carotid artery [4], which was monitored by an MFV-1100 electromagnetic flowmeter (Nihon Kohden). The intervention without vessel ligation was performed in SO animals.

The blood was collected from the common carotid artery under ether narcosis. Sodium citrate (3.8% solution) served as stabilizer (1:9 citrate/blood ratio). Whole blood viscosity, hematocrit, plasma fibrinogen content, plasma viscosity, erythrocyte aggregation and deformability were evaluated. Whole blood and plasma viscosities were recorded at shear rates of 3-300 sec^{-1} and 300 sec^{-1}, respectively, on an AKR-2 rotation

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viscosimeter. Hematocrit was evaluated by centrifugation in glass capillaries on an MGC-8 centrifuge. Plasma fibrinogen content was measured using Tech-Fibrinogen-Test kit on a Cormay KG-4 coagulometer. Spontaneous aggregation of erythrocytes was studied by syllectometry in our modification [2]. Half-time of erythrocyte aggregation served as the criterion of their aggregation activity. Erythrocyte deformability was evaluated by ektacytometry as described previously [8]; erythrocyte deformability index was calculated as (L-H)/(L+H) ratio, where L and H are the greater and lesser diameters of the first diffraction maximum, respectively. Oxygen availability for tissues was evaluated by hematocrit/blood viscosity ratio at high shear rates (50-300 sec^{-1}) [12].

The animals were sacrificed by ether overdosage.

The data were statistically processed using Statistica 6.0 software. The means±standard errors of the means were calculated. The differences between the groups were evaluated using Student's *t* test.

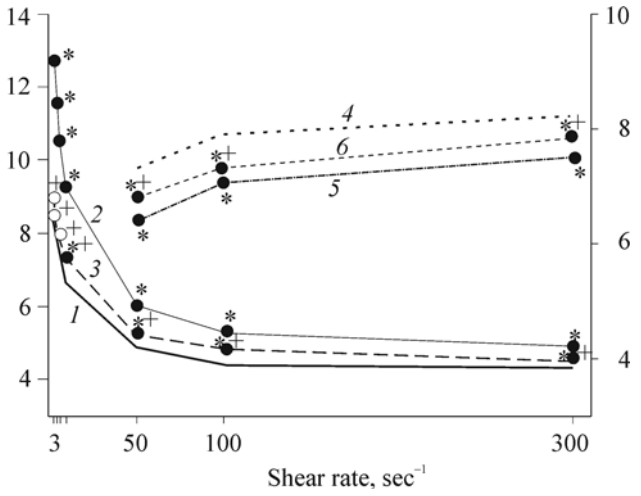

Fig. 1. Effects of a course of intragastric MD on blood viscosity (*1-3*) and oxygen availability for tissues (*4-6*) in rats with CI. *1*, *4*) SO; *2*, *5*) control; *3*, *6*) MD treatment. Ordinates: left: blood viscosity, mPa×sec; right: oxygen availability for tissues, rel. units. $p<0.05$ compared to: *SO, +control.

RESULTS

Blood viscosity increased in the controls for the entire range of shear rates on day 5 after CI modeling. At low shear rates (3-10 sec^{-1}), blood viscosity increased by 39-51%, at high ones (50-300 sec^{-1}) by 14-22% in comparison with SO animals (Fig. 1). It seems that the increase in blood viscosity (by 1.2 times) contributes significantly to deterioration of plasma viscosity, which, in turn, was caused by high (by 1.9 times higher than normally) fibrinogen content in the plasma. In addition to these shifts in macrorheology, the increase of blood viscosity in rats with CI was caused by microrheological changes: increased erythrocyte aggregation and reduction of their deformability. The increase in erythrocyte aggregation activity manifested by 44% shortening of the aggregation half-time in comparison with SO animals, which could be partially due to increase of plasma fibrinogen level ($r=0.92$; $p<0.05$; Table 1). Morphological changes in erythrocytes forming under conditions of oxidant stress lead to more intense spontaneous aggregation of erythrocytes and to reduction of their deformability [7]. In addition, we found that erythrocyte deformability deteriorated in control rats with CI: the deformability index at all shear rates was 23-26% lower than in SO rats (Table 1).

These shifts in the studied parameters in the controls indicated disorders of blood rheology in rats with CI and the formation of blood hyperviscosity syndrome. This is in line with the data indicating the presence of this syndrome in patients with acute cerebrovascular disorders and in animals with experimental CI [5,13].

TABLE 1. Effects of a Course of Intragastric MD on Blood Characteristics of Rats with CI (*M±m*)

Parameter		SO	Control	MD
Plasma viscosity, mPa×sec		1.44±0.02	1.66±0.04*	1.48±0.03+
Fibrinogen content, mg/ml		2.85±0.15	5.40±0.43*	3.78±0.26*+
Hematocrit, %		48±1	49±1	49±1
Erythrocyte aggregation half-time, sec		20.5±2.6	11.4±1.2*	16.5±1.8+
Erythrocyte deformability index, rel. units	at 90 sec^{-1}	0.132±0.004	0.098±0.002*	0.115±0.004*+
	at 180 sec^{-1}	0.240±0.009	0.185±0.007*	0.226±0.009+
	at 360 sec^{-1}	0.328±0.010	0.248±0.004*	0.285±0.008*+
	at 890 sec^{-1}	0.398±0.009	0.296±0.004*	0.339±0.007*+

Note. $p<0.05$ compared to: *SO, +control.

Deterioration of deformability and increase of erythrocyte capacity to spontaneous aggregation have a negative impact on blood viscosity and on the main function of erythrocytes, oxygen supply to tissues [12]. The parameter characterizing oxygen availability for tissues was 10-12% reduced in CI rats in comparison with SO ones (Fig. 1).

Treatment with MD during the development of CI improved blood rheology. Blood viscosity was 8-30% lower ($p<0.05$) in comparison with the control for all shear rates at the same hematocrit values in experimental and control groups (Fig. 1). Reduction of plasma viscosity in this group in comparison with the control correlated with reduction of plasma fibrinogen content ($r=0.86$; $p<0.05$). Treatment with the studied compound promoted a clear-cut improvement of microrheology values, which was shown by reduction of erythrocyte aggregation capacity and improvement of these cells' deformability. Erythrocyte deformability index in this group increased by 14-22% in comparison with the control (Table 1). A less intense aggregation of erythrocytes, manifesting by prolongation of its half-time, correlated with reduced plasma fibrinogen level in animals treated with MD ($r=0.94$; $p<0.05$). Improvement of blood rheology in experimental rats indicated a 4-8% improvement of oxygen availability for tissues in comparison with animals in the control group (Fig. 1).

Hence, the course of MD treatment (100 mg/kg) effectively corrected blood rheology in rats with CI by modulating blood macrorheology (reduction of plasma viscosity and fibrinogen level) and microrheology (improvement of erythrocyte deformability and reduction of their aggregation).

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