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Effect of a New Antioxidant Enoxifol on Platelet Aggregation and Blood Rheological Properties in Rats with Experimental Diabetes Mellitus

A. F. Kucheryavenko, A. A. Spasov, and V. A. Anisimova*

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Effect of a new antioxidant enoxifol exhibiting antiplatelet activity *in vitro* and *in vivo* on hemostasis parameters was assessed in laboratory rats with experimental diabetes mellitus. Gliclazide, a hypoglycemic agent with antiplatelet properties, and pentoxifylline, a preparation improving blood rheology, were used as the reference drugs. Enoxifol produced a pronounced inhibitory effect on platelet aggregation in rats with experimental diabetes comparable to the effect of gliclazide and decreased blood viscosity thus demonstrating a significant effect comparable to that of pentoxifylline. In view of the fact that oxidative stress is a pathogenetic components of vascular complications in diabetes, it can be assumed that improvement of hemostasis parameters under the effect of enoxifol is determined by its antiplatelet and antioxidant activities.

Key Words: *enoxifol; platelet aggregation; blood viscosity; antioxidant activity*

The main cause of mortality in patients with diabetes mellitus (DM) is cardiovascular pathology often leading to patients' death [14]. Platelets play the key role in the pathogenesis of these complications. Increased functional activity leads to imbalance in the prostacyclin-thromboxane system, *i.e.* the level of proaggregant and vasoconstrictor TXA_2 increases and the concentration of prostacyclin, vasodilator and antiplatelet agent, decreases [13]. Furthermore, an important role in development of microvascular DM complications is played by increased erythrocyte aggregation and blood viscosity and decreased deformability of cell membrane. These hemorheological disorders are determined by destabilization of the lipid bilayer of the cell membrane [6]. In recent years, the idea on the essential role of LPO in the pathogenesis of elevated thrombogenic potential of the blood was developed. The mechanism of proaggregant action of H_2O_2 is directly related to ROS generation, activation of LPO in platelet membranes, and formation of hydroperoxides of polyunsaturated fatty acids generated, in particular, in case of free radical oxidation of platelet membrane phospholipids under conditions of elevated thrombogenic potential of the blood. All these hemostasis disorders contribute to atherosclerosis progression and increase the risk of thrombosis [12]. Therefore, glycemia correction in DM should be combined with antithrombogenic protection [10,15]. This necessitates the use of antioxidant agents in the pathogenetic therapy of vascular complications of DM.

Benzimidazole derivatives are a promising group of substances for creation of these preparations [1,2]. Previous studies have demonstrated high antioxidant

Department of Pharmacology, Volgograd State Medical University, Volgograd; *Research Institute of Physical and Organic Chemistry, Southern Federal University, Rostov-on-Don, Russia. ***Address for correspondence:*** aidakucheryavenko@yandex.ru. A. F. Kucheryavenko

activity of enoxifol, a representative of this class, and revealed its antithrombotic ability *ex vivo* [8].

The aim of this study was complex evaluation of the effect of enoxifol on platelet aggregation and blood viscosity parameters in experimental alloxan-induced DM.

MATERIALS AND METHODS

The study was carried out on outbred white male rats (n=55) weighing 300-350 g; the animals were kept under vivarium conditions (22-24°C, relative humidity 40-50%) with natural light mode on a standard diet (GOST R 50258-92). Experiments were carried out in accordance with guidance and regulations (GOST Z 51000.3-96 and 51000.4-96; European Convention for the Protection of Vertebrate Animals, Used in Experimental Studies (1997); the Rules of Good Laboratory Practice (GLP) of Russian Federation (Order No. 267 of Ministry of Health of Russian Federation, June 19, 2003). The animals were sacrificed according to the requirements of International Recommendations for Biomedical Research Involving Animals (1997).

Enoxifol substance (Research Institute of Physical and Organic Chemistry, Southern Federal University) was studied. Experimental DM was induced by intraperitoneal injection of alloxan (Merck; 150 mg/kg in 0.01 M acetate buffer) [11]. The studies were performed 2 months later in animals with severe DM manifested in persistent hyperglycemia (glucose >16 mmol/liter). Blood glucose level was measured by glucose oxidase method using Glyukoza FKD set.

The effects of enoxifol on hemostatic parameters were studied at a dose of 29 mg/kg corresponding to ED_{50} on the model of intravascular platelet aggregation in intact animals. The reference drugs gliclazide and pentoxifylline were used in doses of 20 mg/kg (corresponding to ED_{30} of hypoglycemic activity of the drug after single oral administration to rats) and 4 mg/kg, respectively [9]. All compounds were administered to rats with DM daily over 1 week prior to experiments. The effect of enoxifol on platelet aggregation *in vivo* was assessed using a two-channel laser aggregation analyzer (model 220 LA; Biola). Platelet aggregation was determined by the method of G. Born with modifications [3]. The maximum amplitude on the aggregatogram was taken as the degree of platelet aggregation. Blood samples for estimation of platelet aggregation and blood viscosity were taken from the abdominal aorta of rats with DM anesthetized with chloral hydrate (400 mg/kg).

Blood viscosity was determined using rotational viscosimeter AKR-2 with six shear rates (10, 20, 50, 100, 200, and 300 sec^{-1}), simulating different intensity of blood flow in blood vessels [4]. To assess effects of substances on aggregation of erythrocytes they were washed in order to exclude the influence of carbohydrates on blood viscosity. Aggregation was evaluated using erythrocyte aggregation index (EAI), the ratio of blood viscosity shear rate at 10 sec^{-1} to blood viscosity at 300 sec^{-1} [7] measured at standardized hematocrit (40%). Hematocrit was determined by blood centrifugation in capillaries on a Hematocrit Centrifuge GM-70 (Elmi) (8000 rpm, 3 min) and calculated as the ratio of erythrocytic column to plasma column length in the capillary.

Statistical data processing was carried out using Statistica 6.0 and Microsoft Excel 2006 softwares and Mann–Whitney U test.

RESULTS

Alloxan-induced DM was characterized by body weight loss, polyuria, polydipsia, and polyphagia. All these features are characteristic of a severe form of diabetes. In all groups of animals, blood glucose level was assessed immediately before blood sampling.

In rats with severe alloxan DM, the blood glucose concentration 4-fold surpassed the level observed in intact animals. Gliclazide administered daily for 7 days significantly (by 27%) reduced blood glucose concentration (Table 1). Enoxifol had no effect on this parameter. Analysis of blood samples from diabetic rats revealed a 2-fold increase in the platelet aggregation amplitude in comparison with that in intact animals. Enoxifol and gliclazide significantly inhibited functional activity of platelets in rats with DM by 25 and 27%, respectively (Table 2). Thus, *in vivo* antiplatelet effect of the antioxidant was comparable to the effect of gliclazide.

Enhanced platelet aggregation in DM is a result of TXA_2 elevation due to phospholipase activation in platelet membranes and arachidonic acid release [14]. Therefore, significant decrease in platelet aggregation

TABLE 1. Effect of Enoxifol (29 mg/kg) and Gliclazide (20 mg/kg) Administered Orally for 7 Days on Blood Glucose Concentration in Rats with Experimental DM (n=6; $M\pm m$)

Group	Glucose concentration, mmol/liter	
	initially	after 2 month
Control (intact animals)	4.08±0.22	4.22±0.13
Alloxan DM	16.20±0.58	15.6±0.6
Alloxan DM+enoxifol	15.93±0.32	15.47±0.31
Alloxan DM+gliclazide	16.40±0.73	10.99±0.48*

Note. *p<0.05 in comparison with initial values.

TABLE 2. Effect of Enoxifol (29 mg/kg) and Gliclazide (20 mg/kg) Administered Orally for 7 Days on Platelet Aggregation in Rats with Experimental DM (n=6; $M\pm m$)

Group	Amplitude of platelet aggregation, rel. units
Control (intact animals)	21.50±1.28
Alloxan DM	41.50±2.31*
Alloxan DM+enoxifol	23.2±2.0^{+}
Alloxan DM+gliclazide	30.00±0.73^{+}

Note. p<0.05 in comparison with *control, $^{+}$alloxan DM.

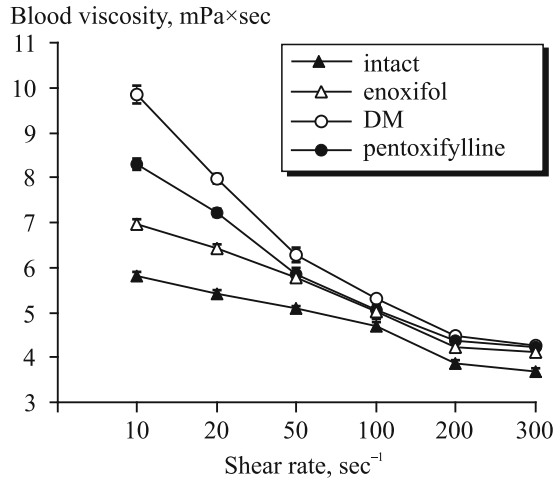

Fig. 1. Effect of enoxifol (29 mg/kg) and pentoxifylline (4 mg/kg) on blood viscosity in rats with alloxan diabetes at shear rates from 300 to 10 sec^{-1}.

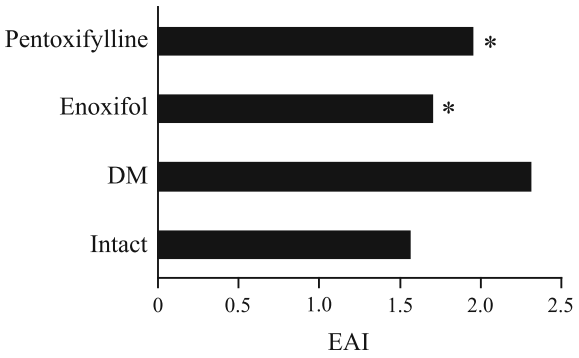

Fig. 2. Effect of course administration of enoxifol (29 mg/kg) and pentoxifylline (4 mg/kg) on EAI in rats with alloxan DM. *p<0.05 in comparison with DM.

in rats with DM treated with enoxifol and gliclazide can be attributed to inhibition of TXA_2 synthesis in platelets by these compounds.

In blood samples of animals with persistent hyperglycemia (≥16 mmol/liter), increased blood viscosity was observed at various shear rates. Enoxifol reduced blood viscosity in rats with experimental DM. The most pronounced effect was observed at low shear rates (Fig. 1). At shear rates of 10 and 20 sec^{-1}, enoxifol significantly reduced blood viscosity by 29.3 and 19.2%, respectively.

Pentoxifylline has a less pronounced effect on blood viscosity at low shear rates in comparison with enoxifol. At shear rates of 10 and 20 sec^{-1}, this reference drug reduced blood viscosity in rats with DM by 16 and 9.2%, respectively (Fig. 1).

EAI in animals with DM was 2.31 (Fig. 2), *i.e.* surpassed the same index in intact animals by 33%. The studied drugs reduced EAI: enoxifol by 26.4% and pentoxifylline by 10.4%.

Blood viscosity at low and higher shear rates is used as numerical characteristics of erythrocyte aggregation and deformability, respectively [5]. The pronounced effect of enoxifol at low shear rates and its minor effect at high shear rates suggest that this preparation modulates erythrocyte aggregation and only slightly affects erythrocyte deformability.

Thus, the new antioxidant enoxifol in case of course administration to rats with experimental DM had an antiplatelet effect and improved hemorheological properties, which can be important for prevention of diabetic complications.

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