Antithrombotic Activity of Russian Antithrombin III Preparation on the Model of Induced Venous Thrombosis

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 142, No. 7, pp. 75-77, July, 2006
Original article submitted November 24, 2005

Antithrombotic activity of Russian preparation Antithrombin III was studied on rat model of induced venous thrombosis. Optimal doses of antithrombin and heparin preventing thrombus growth were determined.

Key Words: *antithrombin III; heparin; antithrombotic activity*

Antithrombin III (AT-III) is a physiological anticoagulant playing an important role in the regulation of hemostasis. It inhibits serine proteases activated at various stages of blood coagulation [2,3,6]. Congenital or acquired AT-III deficiency leads to thromboses. Thrombin, blood coagulation factors (Xa, IXa, XIa, and XIIa), and plasmin serve as the targets for AT-III [5]. Preparations based on AT-III concentrate derived from donor plasma are used in the therapy of various diseases associated with congenital or acquired AT-III deficiency and thrombosis [4].

Here we studied antithrombotic activity of a Russian drug product Antithrombin III in rats with experimental venous thrombosis.

MATERIALS AND METHODS

AT-III concentrate was obtained from virus-inactivated raw materials by affinity chromatography on Heparin-Sepharose Fast Flow sorbent (Amersham Biosciences). This method allowed us to obtain AT-III concentrate with specific activity ≥6 U/mg protein. The chromatographic yield was ≥500 U AT-III per l plasma.

Preclinical studies of lyophilized AT-III preparation were performed on the model of induced venous thrombosis in male Wistar rats (250-350 g). The animals received intraperitoneal injection of nembutal in a dose of 60 mg/kg (1 ml per 200 g body weight). A skin flap was prepared on the neck and cranial part of the ventral chest area. The left and right jugular veins were separated (0.7-1.0 cm). A cannula for repeated administration of substances was inserted into the right jugular vein. Atropine sulfate in a dose of 5 mg/kg was used to prevent a protective response of the anticoagulant system. Venous thrombosis of the left jugular vein was induced as described elsewhere [9]. The anticoagulant system in rat blood was activated with human serum 15 min after administration of anticoagulants. A 0.5-0.7-cm segment of the vein was ligated. Experimental animals were consecutively treated with AT-III (55, 75, and 100 U/kg) and heparin (60, 55, and 50 U/kg; 5000 U/ml; Belmedpreparat). The rats of control groups 1 and 2 received 0.9% NaCl and 100 U/kg AT-III, respectively.

Antithrombotic activity was scored by thrombus shape [9], wet thrombus weight (analytical balance), and protein concentration (Lowry method) [1] in thrombus homogenate (in 0.2 ml phy-

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TABLE 1. Effect of Combined Intravenous Treatment with AT-III and Heparin on the Course of Experimental Venous Thrombosis in Rats 15 min after Administration of Anticoagulants (*M*±*m*)

Dose of substance		Thrombus size, points	Thrombus weight, mg	Protein concentration in thrombus homogenate, μg/ml	Plasma antithrombin activity, U/ml
heparin, U/kg	AT-III, U/kg				
0	0	4±0	6.1±0.6	93±8	0.18±0.03
	100	4±0	5.8±0.7	89±9	0.20±0.04
50	0	3.1±0.4	2.9±0.4	71±5	0.25±0.07
	100	0±0*+	0±0*+	19±2*+	1.20±0.03*+
55	0	2.8±0.5	2.3±0.3	64±7	0.29±0.09
	75	0±0*+	0±0*+	25±4*+	0.84±0.12*+
60	0	2.2±0.4	1.2±0.4	58±5	0.41±0.05*+
	55	0±0*+	0±0*+	21±3*+	0.70±0.05*+

Note. Each dose was administered to 10-20 animals. $p<0.05$: *compared to individual treatment with AT-III in a dose of 0 U/kg; +compared to administration of 0 U/kg heparin and 100 U/kg AT-III.

siological saline, Potter homogenizer). Protein concentration was evaluated by the calibration curve constructed using fibrinogen (Behring) in concentrations of 10-400 μg/ml.

Antithrombin activity of blood plasma was determined by the ability of heparins to activate AT-III and inhibit amidolytic activity of thrombin [8]. Antithrombin (250 μl, Behring; 1 U/ml 0.05 M Tris-HCl buffer with 0.0075 M Na_2-EDTA and 0.175 M NaCl, pH 7.4) was incubated with platelet-depleted plasma (100 μl) at 37°C for 3 min, then bovine thrombin (Behring, 2 NIH U/ml Tris-EDTA buffer) was added. Thrombin-specific chromogenic substrate S-2238 (Behring, 200 μl solution, 2 mM Tris-EDTA buffer) was added after 30 sec. Optical density of the solution was recorded on an Akvilon spectrophotometer at 405 nm over 1 min. The calibration curve for plasma antithrombin activity was constructed using international standard unfractionated heparin (WHO) obtained from the National Institute for Biological Standards and Control (NIBSC, United Kingdom).

The differences were evaluated by Student's *t* test. Correlation study was performed.

RESULTS

AT-III concentrate is administered in combination with heparin for the treatment of some thrombotic disorders, because unfractionated heparin catalyzes inhibitory activity of serpin relative to serine proteinases in blood plasma.

Table 1 shows antithrombotic activity of intravenous AT-III and unfractionated heparin.

Thrombus size (in points), thrombus weight, and protein concentration in the thrombus homogenate linearly correlated with antithrombin activity of rat plasma. The linear correlation coefficients were -0.92, -0.80, and -0.91, respectively ($p<0.05$). A strong correlation was found between the increase in plasma antithrombin activity and decrease in thrombus size.

Combined intravenous injection of heparin and AT-III sometimes resulted in 100% antithrombotic

TABLE 2. Duration of Antithrombotic Effect after Combined Intravenous Treatment with 100 U/kg AT-III and 50 U/kg Heparin (*M*±*m*)

Parameter	Time after anticoagulant treatment, min					
	0	15	30	45	60	90
Thrombus size, points	4±0	0±0*	1.3±0.4*	2.1±0.3*	3.1±0.3*	3.9±0.1
Thrombus weight, mg	6.3±0.5	0±0*	0.6±0.3*	0.7±0.4*	2.2±0.5*	6.4±0.6
Protein concentration, μg/ml	92±7	19±2*	44±6*	60±9*	73±7	99±9
Plasma antithrombin activity, U/ml	0.18±0.03	1.20±0.03*	0.87±0.07*	0.62±0.10*	0.48±0.06*	0.33±0.02*

Note. Each dose was administered to 10-20 animals. *$p<0.05$ compared to the parameter observed before anticoagulant treatment.

activity (50 U/kg heparin and 100 U/kg AT-III; 55 U/kg heparin and 75 U/kg AT-III; 60 U/kg heparin and 55 U/kg AT-III; Table 1). The anticoagulant effect was most pronounced after injection of 50 U/kg heparin and 100 U/kg AT-III. Antithrombotic effect of 55 and 60 U/kg heparin alone was 35-60%. Therefore, treatment with 50 U/kg heparin and 100 U/kg AT-III is optimal for 100% antithrombotic activity.

Optimal dosages of AT-III and heparin were effective over 60 min (Table 2). Antithrombin activity of the plasma remained high even 90 min after treatment. Published data show that human plasma with antithrombin activity of 0.35-0.70 U/ml is therapeutically effective [7].

We conclude that optimal dosages of Russian drug products AT-III and heparin completely prevent thrombus growth in rats with experimental venous thrombosis.

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