Antithrombotic Activity of Heparin-ATP Complex

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The complex of high-molecular-weight heparin and ATP prevented thrombus formation in the blood flow. Repeated intramuscular injection of the complex increased total fibrinolytic activity of the blood, nonenzymatic fibrinolysis, and plasma anticoagulant activity and significantly decreased platelet aggregation.

Key Words: *heparin; adenosine triphosphate; fibrinolysis; platelet aggregation; antithrombotic effect*

Intramuscular injection of complexes of high-molecular-weight heparin with natural low-molecular-weight components (amino acids, amines, and amides) increase plasma anticoagulant activity [11,12].

The possibility of using heparin as a natural anticoagulant of direct and rapid action was extensively studied [5,10,13], but hemorheological properties of heparin complexes with low-molecular-weight ligands (*e.g.*, pharmacological preparations) are poorly known. It was shown that complexes of heparin with aspirin [11] and some oligopeptides [2,6,7] exhibit anticoagulant and fibrin-depolymerizing activity and produce an antithrombotic effect in the blood of animals. Adenosine triphosphoric acid, a natural low-molecular-weight ligand, plays a role in various metabolic processes (*e.g.*, aminosugar metabolism) and serves a natural constituent of tissues in humans and animals.

ATP improves cerebral and coronary circulation and is used in combination therapy for muscular dystrophy, atrophy, cardiovascular failure, heart rhythm disorders, and peripheral vasospasm [4].

Here we studied the antithrombotic effect induced by repeated intramuscular injection of heparin-ATP complex to prethrombotic animals. We also evaluated whether this compound can modulate functional activity of the anticoagulant system in the organism.

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MATERIALS AND METHODS

Experiments were conducted with high-molecular-weight heparin (Serva) and sodium adenosine triphosphate (L'vovdialek).

We prepared a complex of heparin and ATP (H-ATP) at a 1:20 w/w ratio [3]. The formation of H-ATP was verified by crossed electrophoresis [9].

The study was performed on male outbred albino rats weighing 300-350 g. The animals received daily intramuscular injections of H-ATP (1 mg/kg), ATP (0.95 mg/kg), and heparin (0.05 mg/kg) for 4 days at 24-h intervals. An equivalent volume of 0.85% NaCl was injected intramuscularly to control rats (0.5 ml per 350 g body weight).

For evaluation of biochemical parameters of hemostasis, the blood (2 ml) from the jugular vein was taken 1 h after the last injection. Sodium citrate was used as the anticoagulant (9:1 blood/anticoagulant ratio).

The following parameters of the coagulant and anticoagulant system of blood plasma were estimated: total and nonenzymatic fibrinolytic activity [11], tissue plasminogen activator activity (PAA) [8], and activated partial thromboplastin time (APTT) [1]. Platelet aggregation in platelet-rich plasma was studied using 2 μM ADP as the aggregant. The intensity of aggregation was estimated from the maximum optical density of a sample and expressed in percents. Platelet aggregation in control samples was taken as 100% [1].

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Experimental thrombus formation was induced in the segment of the jugular vein isolated with metal clamps. The study was performed by the standard method [14] in our modification (thrombin administration into the isolated vein segment). One hour after thrombus formation, they were dissected, dried at 37°C for 3 h, and weighted.

The results were analyzed by Student's *t* test.

RESULTS

In vivo experiments revealed a significant increase in total and nonenzymatic fibrinolytic activity (by 1.7-1.8 times), PAA (by 3.2 times), and anticoagulant activity (by 1.24 times according to APTT test) and decrease in platelet aggregation (by 32.8%) 1 h after the last intramuscular injection of the H-ATP complex (compared to control animals receiving 0.85% physiological saline, Table 1). These changes persisted for 4 days. Administration of ATP in a dose equivalent to its content in the complex was followed by a significant increase in PAA and total and nonenzymatic fibrinolytic activity and decrease in platelet aggregation. Administration of heparin induced a significant increase in anticoagulant activity compared to the control.

The weight of freshly isolated experimental thrombi from the jugular vein of animals subjected to repeated treatment with the H-ATP complex was 0.1 mg (14-fold lower than in control animals, Fig. 1).

The weight of thrombi was 0.38 and 0.3 mg after pretreatment with constituents heparin and ATP, respectively (3.4 and 4.5 times lower than in the control, respectively). The antithrombotic effect of the test complex exceeded that of its constituents by more than 3 times.

Our results indicate that repeated intramuscular injection of the H-ATP complex produced antithrombotic, anticoagulant, and fibrin-depolymerizing effects in rat blood plasma. This complex increased PAA. The weight of experimental thrombi in H-ATP-receiving animals was insignificant compared to the control. The antithrombotic effect of H-ATP was more significant compared to that of high-molecular-weight heparin or ATP. We conclude that H-ATP complex can be used in clinical practice for preventing thrombus formation.

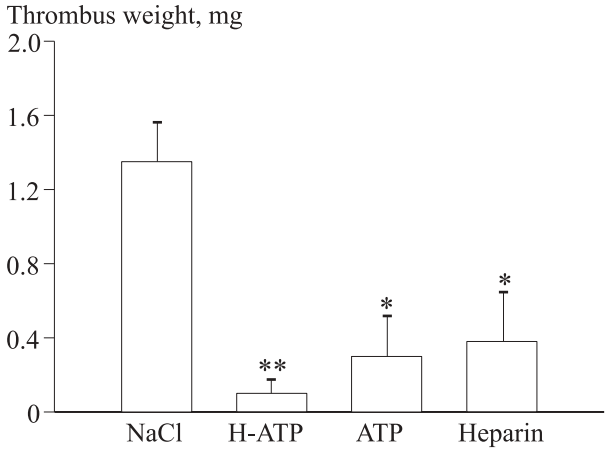

Fig. 1. Antithrombotic effect observed 1 h after 4-day intramuscular injection of the H-ATP complex and its constituents. *$p<0.05$ and **$p<0.01$ compared to the control.

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TABLE 1. Parameters of Hemostasis in Rat Blood 1 h after 4-Day Intramuscular Injection of the H-ATP Complex (1 mg/kg) or Its Constituents (*M*±*m*)

Parameters of hemostasis	NaCl (control)	H-ATP complex	ATP	Heparin
APTT, sec	24.50±1.02	30.40±1.77*	35.7±4.3**	31.9±1.36**
Total fibrinolytic activity, mm^2	31.5±0.8	54.5±1.3**	41.2±1.1**	37.0±1.3*
Nonenzymatic fibrinolytic activity, mm^2	20.0±0.9	37.5±1.5**	31.5±0.8**	26.0±0.9*
PAA, mm^2	11.2±0.8	36.2±4.4**	25.5±1.0**	14.5±0.9
Platelet aggregation index	6.10±0.35 (100%)	4.10±0.17** (67.2%)	4.30±0.73* (70.5%)	4.95±0.73 (81.1%)

Note. Platelet aggregation is shown in brackets (%). *$p<0.05$ and **$p<0.01$ compared to the control.

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