Anticoagulant Effects of a Complex of High Molecular Weight Heparin and Arginine

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Heparin forms a complex compound with arginine in a pure system, which was shown by biochemical methods. A method for obtaining the complex *in vitro* has been developed. At arginine/heparin molar ratio of 3:1 the complex exhibited anticoagulant, antiplatelet, and fibrin-depolymerization activities. High fibrin-depolymerization and anticoagulant activities were documented in the blood of animals 10 min after intravenous injection of the arginine-heparin complex, in contrast to the picture after injection of the complex components (heparin and arginine) alone.

Key Words: *arginine-heparin complex; anticoagulant activity; fibrin depolymerization characteristics; platelet aggregation*

It was shown previously that arginine (half-substituted amino acid) promotes a reduction of high cholesterol level, normalization of sugar level in the blood, and stimulates wound healing [1,4,9,14]. Arginine improves the blood rheology and prevents thrombogenesis [11,13]. Arginine inhibition of blood clotting is due to the fact that it is a precursor of NO [8], produced by vascular endothelial cells and involved in the functioning of many body systems, including hemostasis [12]. One of the natural anticoagulants of the hemostasis system, heparin, blocks the clotting activity of thrombin [6] and in parallel with this, exhibits hypolipidemic, hypoglycemic, and antiinflammatory effects. Experimental data, confirmed clinically, indicate amplification of the anticoagulant and antidiabetogenic effects of heparin complexes with some low molecular-weight substances [3,5,10].

The aim of our study was to create an anticoagulant heparin-arginine compound and to study its effects on fibrin polymerization, anticoagulant and antiplatelet activities in circulating blood *in vivo*.

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MATERIALS AND METHODS

High molecular weight heparin (Serva) and L-arginine preparation were used in the study.

Arginine-heparin complex (molar ratio 3:1) was obtained by our method.

Interactions between arginine guanido groups and heparin acid groups were proven by cross electrophoresis. Total and nonenzymatic fibrinolytic (fibrin depolymerization) activities of the compound were evaluated *in vitro* for the concentrations of 10^{-7}-10^{-1} mg/ml. Anticoagulant activity and platelet aggregation were evaluated after addition of the arginine-heparin complex to normal animal plasma.

In vivo experiments were carried out on 54 male rats (7-8 months; 200-220 g). The animals were divided into 4 groups: 1) injection of the complex in a dose of 1 mg/kg; 2) arginine in a dose equivalent to its content in the complex; 3) heparin in a dose equivalent to its content in the complex; and 4) controls, injected with the same volume (0.3 ml) of 0.85% NaCl. The preparations were infused into the *v. jugularis* and blood was collected after 10 min (2 ml; 3.8% sodium citrate served as the preserving agent). The following plasma clotting

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TABLE 1. Anticoagulant Effect of Arginine—Heparin Complex and Its Components in Equivalent Doses 10 Minutes after Intravenous Injection (*M*±*m*)

Experiment conditions	SFA, mm²	NFA, mm²	PA+PAA, mm²	PA, mm²	PAA, mm²
Control (0.85% NaCl)	35.5±1.1	23.6±0.8	14.5±0.4	3.2±0.3	11.3±0.8
AHC, 1 mg/kg	53.0±1.2*	36.1±1.2*	24.8±0.5*	4.0±0.6	20.8±1.6*
Arginine, 0.95% mg/kg	37.5±0.7	23.0±0.9	15.7±0.9	3.9±1.1	11.8±0.7
Heparin, 0.05 mg/kg	38.0±1.5	25.5±1.1	23.1±0.4*	3.8±0.8	19.3±1.2*

Note. PA: plasmin activity; AHC: arginine-heparin complex. Here and in Table 2: *$p<0.01$ *vs.* control.

and anticoagulant system values were evaluated: summary (SFA) and nonenzymatic fibrinolytic activities (NFA) [10], plasminogen tissue activator activity (PAA) [7], and anticoagulant activity (by activated partial thromboplastin time; APTT) [2]. Platelet aggregation in the plasma was evaluated on an aggregometer using ADP (2 μM solution) as aggregation inductor [2]. The results were statistically processed using Student's *t* test.

RESULTS

The arginine—heparin complex exhibited SFA at the expense of NFA with the lysis zones of 25-30 mm² in a pure system (*in vitro*), used in concentrations of 10^{-7}-10^{-1} mg/ml. The components alone (heparin and arginine) in concentrations equivalent to their content in the complex exhibited no fibrinolytic effect or a slight effect (without lysis zones or with zones up to 4 mm²). Anticoagulant activity of the complex in concentrations of 10^{-5}-10^{-1} mg/ml in plasma medium was detected. Heparin (but not arginine) in an equivalent dose exhibited slight (in comparison with the complex) anticoagulant activity. Platelet aggregation in the presence of the complex in concentrations of 10^{-2}-10^{-3} mg/ml reduced by 18-20% in comparison with control samples (platelet-rich plasma after addition of NaCl). Hence, the activity of the complex *in vitro* differed from the activities of its components by a significant effect (according to APTT) and by fibrin depolarization or nonenzymatic fibrinolytic activities.

The SFA and NFA increased significantly (1.5 times) 10 min after intravenous injection of the complex (group 1); summary PAA and plasmin increased significantly, as well as PAA (1.7 times) (Table 1).

Injection of rats with arginine in a dose equivalent to its content in the complex (group 2) caused no changes in SFA and NFA, while the activity of plasminogen slightly (1.2 times) increased in comparison with control rats. Intravenous injection of heparin in a dose equivalent to its content in the complex (group 3) caused no changes in the studied hemostasis parameters, except the summary activity of plasmin and PAA, which increased 1.5 times, and of plasma PAA, which increased 1.7 times in comparison with the values in the control group (Table 1).

Ten minutes after intravenous injection of the complex in a dose of 1 mg/kg and of heparin in an equivalent dose the anticoagulant activity increased (according to APTT test), its level surpassing 5.9 and 2.83 times, respectively, the plasma anticoagulant activity after injection of saline. No sharp changes in the plasma anticoagulant activity were detected after injection of equivalent dose of arginine. The activity of primary hemostasis did not change under these experimental conditions, which was seen from virtually unchanged level of platelet aggregation after injection of arginine-heparin complex and after injections of arginine, heparin, and saline (Table 2).

Hence, comparison of anticoagulant activities after intravenous injections of arginine-heparin complex and its components alone showed a significant effect of the complex on the plasma SFA, its NFA, plasma PAA, APTT, and platelet aggregation.

Arginine-heparin complex exhibited the highest anticoagulant effect *in vitro* and *in vivo*, which indicates its longer existence in circulating blood. Our data recommend the use of the complex (but not its components alone) in prethrombotic conditions. The complex exhibits combined anticoagulant and fibrin depolarization effects *in vivo*, due to

TABLE 2. Changes in Anticoagulant Activity (according to APTT Test) and Platelet Aggregation in the Blood 10 Minutes after Intravenous Injection of Arginine—Heparin Complex and Its Components in Equivalent Doses (*M*±*m*)

Experiment conditions	APTT, sec	Platelet aggregation, %
AHC, 1 mg/kg	590.0±21.6*	105.0±7.9*
Arginine, 0.95 mg/kg	114.0±6.64	112.0±6.4
Heparin, 0.05 mg/kg	283.0±19.8	125.5±15.5
Control (0.85% NaCl)	100.0±5.0	100.0±7.9

which it has good prospects as an effective anticoagulant and fibrinolytic drug.

REFERENCES

1. Z. S. Barkagan and G. I. Kostyuchenko, *Byull. Sib. Otdelen. Rossiisk. Akad. Med. Nauk*, **120**, No. 2, 132-138 (2006).
2. V. V. Dolgov, N. A. Avdeeva, and K. A. Shchetnikovich, *Methods for Hemostasis Studies* [in Russian], Moscow (1996).
3. B. A. Kudryashov, I. P. Ashmarin, L. A. Lyapina, and V. E. Pastorova, *Byull. Eksp. Biol. Med.*, **114**, No. 12, 609-611 (1992).
4. A. V. Simonyan, A. A. Salamatov, Yu. S. Pokrovskaya, and A. A. Avanesyan, *Methodological Recommendations* [in Russian], Volgograd (2007).
5. A. M. Ul'yanov, L. A. Lyapina, V. E. Pastorova, and T. Yu. Smolina, *Izv. Rossiisk. Akad. Nauk*, Ser. Biology, No. 3, 1-4 (2004).
6. R. H. Aster, *J. Thromb. Haemost.*, **4**, No. 4, 757-758 (2006).
7. T. Astrup and S. Mullertz, *Arch. Biochem. Biophys.*, **40**, No. 2, 346-351 (1952).
8. T. M. Brunini, A. C. Mendes-Ribeiro, J. C. Ellory, and G. E. Mann, *Cardiovasc. Res.*, **73**, No. 2, 359-367 (2007).
9. S. Fujita, B. B. Rasmussen, J. G. Cadenas, *et al.*, *Am. J. Physiol. Endocrinol. Metab.*, **291**, No. 9, E745-E754 (2006).
10. B. A. Kudrjashov and L. A. Lyapina, *Thrombosis and Thrombolysis*, Eds. E. I. Chazov and V. N. Smirnov, New York (1986), pp. 33-65.
11. M. Nakayama-Hamada, A. Suzuki, H. Furukawa, *et al.*, *J. Biochem.*, **144**, No. 3, 393-398 (2008).
12. C. Napoli, W. C. Stanley, and L. J. Ignarro, *Cardiovasc. Res.*, **73**, No. 2, 253-256 (2007).
13. T. W. Stief, *Clin. Appl. Thromb. Hemost.*, **13**, No. 2, 146-153 (2007).
14. A. Vasilijevic, B. Buzadzik, A. Korac, *et al.*, *J. Physiol.*, **584**, No. 3, 921-933 (2007).