Effect of Antithrombin III on Local Hemostasis in the Kidneys during Experimental Nephritis

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Administration of antithrombin III-enriched plasma to rabbits with acute Masugi nephritis inhibited prothrombinase formation and increased the release of component C_3 from the kidneys. This treatment had a cytoprotective effect and was probably followed by dissociation of antigen—antibody complexes.

Key Words: *hemostasis; Masugi nephritis; antithrombin III*

Kidney diseases, *e. g.* glomerulonephritis, are accompanied by an increase in blood heparin level and decrease in antithrombin III concentration (AT-III, plasma heparin cofactor) [3]. However, these patients are prescribed to receive heparin [1,2].

It remains unclear whether treatment with exogenous heparin is effective in patients with high concentration of endogenous heparin and low content of AT-III in the blood. On the other hand little is known on the effects of AT-III on local hemostasis in the kidneys. It cannot be excluded that prescribed drugs produce different effects on local and total hemostasis.

MATERIALS AND METHODS

Experiments were performed on 70 rabbits weighing 2.0-3.7 kg. Acute Masugi nephritis in animals and glomerulonephritis in humans have the same clinical and morphological signs. The development of Masugi nephritis was verified histologically by thickening of Bowman's capsules, plethora of the glomeruli, proliferation of endothelial and mesangial cells, alternative and exudative changes, edema of the intercanalicular stroma, and degeneration of proximal tubules. These signs corresponded to changes in laboratory indexes: proteinuria (≤7), presence of hyaline and granular cylinders, and 2.5-fold increase in blood urea concentration. The system of hemostasis was studied in animals with similar changes in the blood, urine, and morphology indexes, which allows differentiation of kidney diseases (Fig. 1).

Therapy with AT-III-enriched plasma (1.2 ml/kg intravenously) was started from the 10th day of renal inflammation. Laparotomy was performed under ethaminal anesthesia (40 mg/kg intraperitoneally). The blood was sampled with silicon syringes.

The blood from the renal artery and renal vein was stabilized with heparin in low dose (5000 U, 0.01 ml per 4-5 ml blood). It should be emphasized that the patients usually receive heparin in high therapeutic doses (20,000-60,000 U) 2 times a day. We obtained 4-5 ml blood from each vessel (less than 3% of total blood volume in rabbits). Blood plasma from the renal artery and renal vein of 20 healthy animals served as the control.

We studied parameters of the blood coagulation and anticoagulation systems [4].

The results were analyzed by methods of variational statistics.

RESULTS

In rabbits with Masugi nephritis coagulation activity of the blood significantly increased after minimum and maximum contact with the kidneys (Table 1). The silicon time for plasma samples from the renal artery and vein increased by 11.2 and 30%, respectively. The

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kaolin time in the renal artery and vein increased by 10.2 and 23.5%, respectively. The index of contact activation range (ICAR) for blood samples remained unchanged in the renal artery, but slightly decreased in the vein. These results suggest that coagulant activity increases in renal blood from rabbits with Masugi nephritis. These changes are mediated by phospholipid activation and to a lesser extent by the contact mechanism. During measurement of the partial thromboplastin time, clotting time for plasma samples from the renal vein in rabbits with Masugi nephritis was shorter than in healthy animals. Kallikrein activity increased, while the content of complement component C_3 decreased in the blood from renal veins of rabbits with Masugi nephritis. Fibrinogen concentration in the venous blood was lower than in the arterial blood. This can be explained by its partial degradation with the formation of fibrinogen degradation products (FDP). The concentration of FDP in the blood from renal vein and artery increased by 27.5 and 11 times, respectively. Heparin time in arterial and venous plasma increased by 21.5 and 27.2%, respectively.

AT-III concentration in the blood from renal vein significantly increased. These results illustrate abnormalities in AT-III synthesis in the kidneys or its consumption for inactivation of blood coagulant factors. We also observed inhibition of enzymatic fibrinolysis and activation of nonenzymatic fibrinolysis in the blood of rabbits with Masugi nephritis. The complex of plasminogen+plasmin—heparin in the blood from renal vein had maximum activity. Lytic activity of epinephrine—heparin and fibrinogen—heparin complexes in the arterial blood was higher than in the venous blood (Table 1).

Despite sharply increased heparin concentration in the blood of patients with glomerulonephritis, therapy of this disorder includes treatment with exogenous heparin [2]. Native plasma from healthy rabbits was maintained at room temperature for several hours. AT-III concentration in these plasma samples was high. The plasma was stabilized with heparin (Heparin-Richter, 5000 U/ml, 0.01 ml per 5 ml blood). Administration of the plasma to rabbits with Masugi nephritis significantly lengthened silicon and kaolin times (Table 1). Kephalin time in the blood from the renal vein increased by 49.9% compared to that observed before treatment. The heparin time underwent insignificant changes, which was probably related to the increase in blood AT-III concentration. AT-III concentration increased most significantly in the arterial blood.

Treatment with AT-III-enriched plasma increased kallikrein activity in the venous and arterial blood by 36.9 and 26%, respectively, which plays a role in the inhibition of prothrombin formation. Therapy with AT-III-enriched plasma increased the content of complement component C_3 in the blood from the renal vein. It prevents fixation of complement component C_3 on the membranes of kidney cells and their destruction.

Administration of AT-III-enriched plasma slightly decreased fibrinogen concentration, but increased the amount of FDP in the blood from the renal vein.

AT-III-enriched plasma induced lysis of blood clot and euglobulin fraction in effluent blood from the kidneys (Table 2). We revealed a considerable increase in nonenzymatic fibrinolytic activity. Lytic activity of the plasminogen+plasmin—heparin complex most significantly increased in the arterial blood. Activity of fibrinogen—heparin and epinephrine—heparin complexes was higher in the venous blood. AT-III converts heparin into immediately reacting anticoagulant, which probably determines the development of these changes.

Hence, infusion of AT-III-enriched plasma during the therapy of animals with Masugi nephritis is more

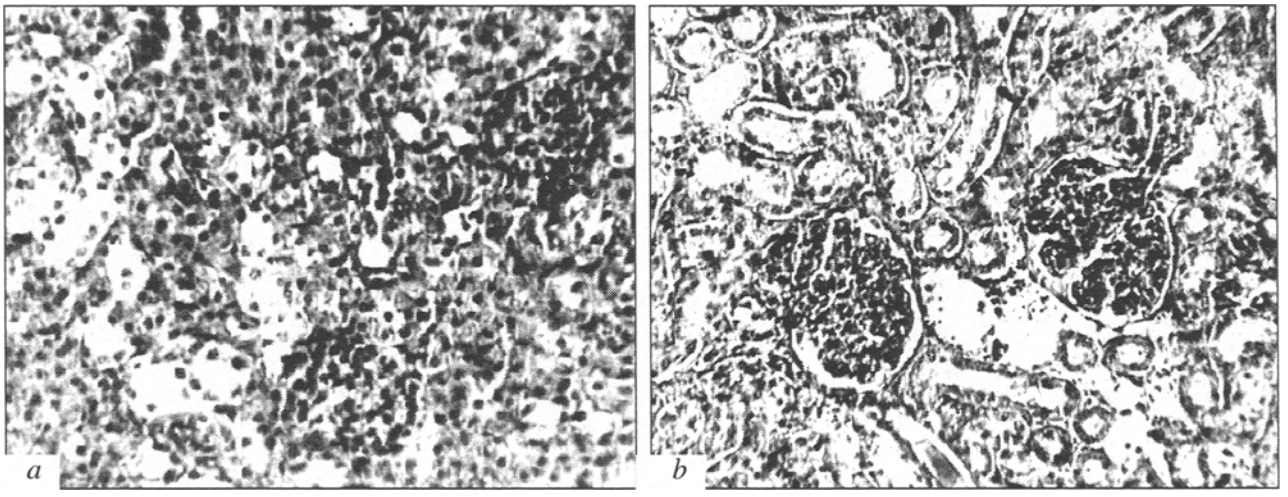

Fig. 1. Kidney tissue in healthy rabbit (*a*) and animal with acute Masugi nephritis (*b*). Staining with hematoxylin and eosin, ×25.

TABLE 1. Effect of Antithrombin III-Enriched Plasma on Procoagulant and Anticoagulant Activity of the Blood from Animals with Acute Nephritis ($M \pm m$)

Index	Healthy animals		Masugi nephritis			
			before therapy		after therapy	
	renal artery	renal vein	renal artery	renal vein	renal artery	renal vein
Plasma silicon time, sec	221.0±15.7	218.5±15.5	183.0±15.1	153.0±11.0	235.4±3.1^{+}	203.±3.3^{++}
Plasma kaolin time, sec	74.6±3.9	68.2±3.8	65.8±4.0	52.2±3.0	82.7±1.5^{+}	76.7±1.8^{+}
ICAR, %	64.1±2.4	66.4±2.7	62.0±2.0	62.7±2.6	57.9±6.5	62.2±0.6
Plasma kephalin time, sec	58.3±3.5	60.6±3.5	48.7±1.9	38.7±1.9	62.0±1.2	58.0±1.0^{+}
Plasma prothrombin time, sec	30.6±1.5	30.2±1.8	25.6±1.3	19.2±1.4	40.8±2.3^{+}	33.1±1.8^{+}
Kallikrein, U/ml	0	0.40±0.05*	22.7±1.1	31.7±1.2	16.8±0.6^{+}	20.8±0.8^{+}
Complement component C_3, %	11.6±0.3	13.4±0.7***	8.5±0.7	3.3±0.6	13.2±0.4^{+}	9.2±0.4^{+}
Fibrinogen, mg%	275.2±6.9	266.6±7.1	392.8±9.1	359.2±13.8	380.3±7.5	317.3±7.5^{++}
FDP, mg%	0.10±0.02	0.60±0.05*	6.6±0.6	16.5±0.7	8.8±1.6	23.8±1.9^{++}
Plasma thrombin time, sec	31.7±1.0	36.4±1.0**	38.5±1.7	46.3±1.6	54.0±6.2^{++++}	79.7±1.6^{+}
Plasma heparin time, sec	6.1±0.3	12.7±0.6*	14.7±0.8	20.5±1.0	17.2±1.5^{+++}	24.5±0.8^{+++}
AT-III, sec	87.4±2.1	101.6±2.6*	70.9±3.2	55.3±2.9	93.4±1.0^{+}	80.4±1.0^{++++}

Note. Here and in Table 2: *p<0.001, **p<0.01, and ***p<0.05 compared to the renal artery; ^{+}p<0.001, ^{++}p<0.002, ^{+++}p<0.01, and ^{++++}p<0.02 compared to indexes before therapy.

TABLE 2. Effect of Antithrombin III-Enriched Plasma on Fibrinolytic Activity of the Blood from Animals with Acute Nephritis ($M \pm m$)

Index	Healthy animals		Masugi nephritis			
			before therapy		after therapy	
	renal artery	renal vein	renal artery	renal vein	renal artery	renal vein
Fibrinolysis of euglobulins, min	167.9±4.8	122.4±3.8*	171.7±4.1	161.6±4.2	117.3±8.9^{+}	128.3±3.2^{+}
Fibrinolysis of whole blood, %	13.5±0.4	19.2±0.6*	8.7±0.3	6.8±0.4	5.7±0.8^{+++}	9.2±0.5
Fibrinolytic activity, mm^2						
total	109.0±2.1	120.2±2.6**	127.4±2.9	139.4±2.9	187.6±4.0^{+}	172.6±4.0^{+}
total nonenzymatic	94.8±1.8	101.1±2.4***	107.0±2.3	126.9±2.2	161.5±3.2^{+}	148.5±2.6^{+}
fibrinogen—heparin complex	45.1±1.0	51.6±1.1*	47.9±1.8	61.9±2.2	60.1±7.1	79.2±1.2^{+}
epinephrine—heparin complex	26.6±0.8	36.9±1.2*	52.9±1.7	45.2±1.4	64.8±1.0^{+}	50.8±2.0
plasminogen+plasmin—heparin complex	0	0.40-0.05*	2.4±0.8	10.2±1.0	14.5±0.9^{+}	23.5±0.8^{+}

effective than administration of heparin alone [3]. AT-III more significantly inhibited prothrombinase formation and stimulated enzymatic and nonenzymatic fibrinolysis in rabbits with Masugi nephritis. The heparin—AT-III complex is probably involved in the inhibition of cytolysis in the kidneys. Kidney diseases are accompanied by complement activation by the classic and alternative pathways. Heparin inhibits the classic pathway, but often activates the alternative pathway. These findings explain little inhibitory effect of endogenous heparin on fixation of complement in the kidneys. Heparin does not prevent destruction of cell membranes in the kidneys. However, AT-III and heparin in low doses modulate this process and stimulate the release of complement component C_3 from the kidneys. These data suggest that administration of heparin in excessive amounts is not pathogenetically substantiated. It cannot be excluded that AT-III causes dissociation of the antigen—antibody complex, which is followed by the release and excretion of complement from the kidneys. This fact is of particular importance from immunological point of view.

Our results suggest that the therapy of patients with nephritis should include AT-III or AT-III-enriched plasma. The use of these drugs is more pathogenetically substantiated compared to treatment with heparin alone.

REFERENCES

1. S. Kh. Al'-Shukri, V. N. Tkachuk, and E. G. Goloshchapov, *Urol. Nefrol.*, No. 3, 13-18 (1999).
2. M. Ya. Ratner and N. D. Fedorova, *Ibid.*, No. 2, 32 (1996).
3. N. V. Sokratov, *Changes in the Stationary State of Hemostasis during Kidney Diseases* [in Russian], Orenburg (2001).
4. M. A. Bazarova, A. I. Vorob'ev, Z. S. Barkagan, *et al.*, *Manual on Clinical Laboratory Diagnostics* [in Russian], Kiev (1991).