EFFECT OF TISSUE-TYPE PLASMINOGEN ACTIVATOR FROM CALF KIDNEY CELL CULTURE ON HEMOSTASIS AND FIBRINOLYSIS IN EXPERIMENTAL NEPHRITIS

G. V. Andreenko, L. V. Podorol'skaya, T. N. Serebryakova, A. V. Lyaginskii, B. B. Egorov, and G. A. Afanasenko

UDC 616.611-002-092.9-085.273.53-036.8:[616.151.5+616.153.962.4]-07

KEY WORDS: hemostasis, fibrinolysis, experimental nephritis.

A secondary stage in the pathogenesis of glomerulonephritis, accompanied by a nephrotic syndrome, is fibrin deposition in the basement membranes of the renal glomeruli, making the use of anticoagulant and fibrinolytic agents essential in the treatment of this disease. Urokinase, which activates plasminogen circulating in the blood stream is used most frequently in clinical practice. However, the active plasmin formed may itself be an additional factor causing destruction of the already damaged vascular bed of the renal glomeruli, and thus aggravating the pathology. Cases of temporary worsening of the disease after administration of urokinase in nephritis have been described in the literature [7, 8]. It can be postulated that plasminogen activators of tissue type (TPA), giving rise to local lysis of fibrin without generalized activation of plasminogen, will be more appropriate agents for the removal of fibrin deposits in the capillaries of the kidneys and for the treatment of nephritis.

In the investigation described below the effect of TPA, isolated from culture fluid of calf kidney cells [5], on hemostasis and fibrinolysis in the blood and tissues was studied in experimental Heymann nephritis in animals.

EXPERIMENTAL METHOD

An experimental model of glomerulonephritis was induced in noninbred male albino rats weighing 200-250 g by injection of a 20% emulsion of renal cortex with Freund's complete adjuvant [4]. A preparation of TPA was injected 1.5 months after immunization, when the first signs of nephritis in the animals were appearing, into rats with moderate proteinuria in doses of 100 IU/kg body weight daily for 4 days. The preparation was isolated from the culture fluid of calf kidney cells and had specific activity of 960 IU/mg protein. The animals were killed 4 days after injection of the preparation, a blood sample having previously been taken from the jugular vein. Parameters of hemostasis and fibrinolysis [6] were determined in the blood, and fibrinolytic activity (FA) in liver, kidney, spleen, and heart tissue was tested by Todd's histochemical method in the modification [6]; concentrations of protein and of degradation products of fibrin and fibrinogen (FDP) were determined in the urine [6].

EXPERIMENTAL RESULTS

Table 1 gives data on biochemical changes in the blood plasma and urine of the animals as a result of developing nephritis. The two-sevenfold increase in urinary protein excretion compared with the normal state (an indication of damage to glomeruli and of the development of nephritis) was accompanied by changes in the biochemical parameters accompanying inflammation: the fibrinogen concentration was increased and fibrinolytic activity reduced, as shown by marked lengthening of the euglobulin lysis time and a significant rise of the level of fibrinolysis inhibitors. Destruction of the vascular endothelium of the kidney in glomerulonephritis, exposing collagen, triggers the internal blood clotting mechanism and thrombin formation, and this is accompanied by elevation of the blood level of soluble fibrin monomer complexes (SFMC) by 1.5-6 times.

Laboratory of Enzymic Fibrinolysis, Biological Faculty, M. V. Lomonosov Moscow University. Research Institute of Biomedical Technology, Ministry of Health of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR I. P. Ashmarin.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 106, No. 10, pp. 424-426, October, 1988. Original article submitted October 27, 1987.

 0007-4888/88/0010-1410$12.50

TABLE 1. Biochemical Parameters of Blood and Urine of Animals with Nephritis and of Healthy Rats

Experimental conditions	Fibrinogen concentration, g/liter	SFMC, g/liter	Zones of lysis on fibrin plates, mm			Euglobulin clot lysis time, min	Nonenzymic fibrinolysis, mm^2	Antithrombin III level, %	Level of inhibitors, %	Urine	
			standard plate	heated plate	activator activity					protein concen., g/liter	FDP, g/liter
Exp. (n_p = 32)	6,84±0,34 <0,001	0,24±0,03 <0,001	4±0,4 <0,05	0,3±0,1 >0,5	3,7±0,4 <0,5	539±49 <0,001	68±14,2 <0,05	98±12 >0,5	134±11 <0,05	1,54±0,34 <0,05	0,28±0,07 <0,05
Control (n = 18)	3,56±0,21	0,07±0,016	18±3,2	1,0±0,3	17,0±3,2	221±24	21±10,3	99±10	97±8,1	0,53±0,28	0,104±0,04

Legend. Here and in Table 2, number of animals given in parentheses.

TABLE 2. Biochemical Parameters of Blood and Urine of Animals with Nephritis and Healthy Rats before and after Injection of TPA (100 U/kg)

Experimental conditions	Fibrinogen concentration, g/liter	SFMC, g/liter	Zones of lysis on fibrin plates, mm			Euglobulin clot lysis time, min	Nonenzymic fibrinolysis, mm^2	Antithrombin III level, %	Level of inhibitors, %	Urine	
			standard plate	heated plate	activator activity					protein concentration, g/liter	FDP, g/liter
Experiment											
before inj. (16) p	5,8±0,28 <0,5	0,23±0,04 <0,5	4±1,4 <0,02	2±0,9 >0,5	2±1,4 <0,02	601±43 <0,001	59±12 <0,5	107±8 <0,5	128±12 <0,5	1,46±0,29 <0,5	0,24±0,08 <0,5
after inj. (16)	4,66±0,35	0,13±0,03	29±6,4	4±1,3	25±6,3	321±30,5	26±8	94±12	96±8	1,02±0,31	0,17±0,04
Control											
before inj. (9) p	3,7±0,28 >0,5	0,06±0,03 >0,5	16±4,1 <0,5	2±1,2 >0,5	14±4,0 <0,5	328±41 >0,5	26±6,3 >0,5	87±10 >0,5	92±8,4 >0,5	0,58±0,16 >0,5	0,06±0,02 >0,5
after inj. (9)	3,04±0,25	0,05±0,03	28±6,1	4±1,2	26±6,1	328±33	20±6,2	83±11,2	91±8,5	0,51±0,14	0,04±0,03

Legend. p) Significance of differences between groups before injection and after injection.

Conditions for fibrin formation and deposition in the blood vessels were created in the experimental animals. The criterion of fibrin deposition in the kidney of patients with nephritis is an increase in the FDP concentration in the urine [3]. The FDP level in the urine of animals with nephritis was 1.5-4.5 times higher than in the control. It can be postulated on the basis of these results that the course of nephritis in the experimental animals was complicated by intracapillary fibrin deposition.

Marked exhaustion of FA of the kidney tissue was an important factor facilitating fibrin deposition within the glomeruli.

In the experimental animals the FA level in all tissues was lowered a little, and the greatest fall was observed in tissue of the renal cortex. We know that plasminogen activator (PA) of tissue type in the renal cortex is contained mainly in the vascular endothelium and is readily released in various situations [2]. The marked fall of the PA level in nephritis was most probably the result of continuous stasis of the damaged glomerular capillaries.

Thus, the development of experimental nephritis is accompanied both by hypercoagulation changes and inhibition of fibrinolysis in the systemic blood flow and by inhibition of intramural fibrinolysis in the kidney, leading to fibrin deposition in the glomeruli. Against the background of these changes, TPA was injected into some of the experimental and control animals. Table 2 gives data on changes in hemostasis and FA of the blood and tissues of rats after the fourth injection of TPA. In the control animals the fibrinogen concentration was found to be reduced a little, the SFMC level lowered, and FA increased. These results are in agreement with data in the literature [1] and our earlier observations [5] to show that tissue activator has a very weak effect, below the level of significance, on the hemostasis and fibrinolysis system if the preparation is injected systemically into the blood stream of healthy animals.

In animals with nephritis and with marked hypercoagulation shifts and inhibited fibrinolysis, the tissue activator corrects changes in hemostasis and restores normal FA. The euglobulin clot lysis time was reduced almost by half and plasminogen activator activity was increased 12-fold, the increased nonenzymic fibrinolysis was largely restored to normal, the level of inhibitors was reduced, and the fibrinogen concentration lowered by 23%.

However, the greatest changes took place in the region of maximal damage caused by nephritis, namely in the renal cortex, for that is where the largest quantities both of fibrin and of denatured, degraded proteins, which may also focus TPA, are found [9]. FA in the renal cortex was increased by almost 300%, whereas in the medulla it was increased by only 12%. FA increased a little in other tissues, also showing some changes as a result of the development of experimental nephritis, after injection of the preparation. A significant increase in FA in the liver tissue, where usually none is found in either affected or healthy animals, after injection of TPA is evidence of the important role of the liver in metabolism of the enzyme.

The TPA preparation obtained from the culture fluid of calf kidney, after injection into the blood stream of animals with experimental nephritis, thus restores normal values of parameters of hemostasis and fibrinolysis in the blood. The lowered FA in the renal cortex also is restored to normal.

LITERATURE CITED

1. G. V. Andreenko and L. V. Podorol'skaya, Vopr. Med. Khimii, No. 4, 39 (1984).
2. G. V. Andreenko, Nauch. Dokl. Vyssh. Shkoly, No. 10, 5 (1985).
3. V. A. Varshavskii, L. A. Kupriyanova, R. R. Kaliev, et al., Arkh. Patol., No. 2, 12 (1980).
4. A. M. Vikhert, R. I. Sokolova, and K. I. Bykovskaya, Arkh. Patol., No. 5, 15 (1973).
5. B. B. Egorov, G. V. Andreenko, A. V. Lyaginskii, et al., Farmakol. Toksikol., No. 4, 54 (1983).
6. Methods of Investigation of the Fibrinolytic System of the Blood [in Russian], Moscow (1981).
7. R. H. Goldfarb, G. R. Mürano, and R. Rundage, Semin. Thromb., 12, No. 4, 335 (1980).
8. P. Kincaid-Smith, The Kidney: A Clinico-Pathological Study, Oxford (1975).
9. R. Radcliffe, Biochim. Biophys. Acta, 743, No. 3, 422 (1983).

BLOOD RHEOLOGIC DISTURBANCES IN SHOCK OF VARIED ETIOLOGY

Z. M. Likhovetskaya, T. A. Prigozhina, and N. A. Gorbunova

UDC 616-001.36-07:616.151.4-07

KEY WORDS: blood rheology; hemorrhagic, traumatic, and burn shock.

One of the basic signs in the pathogenesis of shock of whatever etiology is a disturbance of the circulation leading to reduced tissue perfusion and the development of anoxia. Under these circumstances, substantial changes are found in the rheologic properties of the blood [1, 2, 5, 6]. The study of rheologic disturbances of the blood and their dependence on the etiology of shock is important for the elucidation of their role in the mechanism of the circulatory disturbance under extremal conditions.

The aim of this investigation was a comparative study of the rheologic properties of the blood in the stage of relative compensation and decompensation of hemorrhagic, traumatic, and burn shock.

EXPERIMENTAL METHOD

Experiments were carried out on male Wistar rats weighing 300-400 g. Hemorrhagic shock was induced by prolonged posthemorrhagic hypotension by a modified Wiggers' method [6], traumatic shock by Cannon's method [11], and a model of burn shock was created by thermal trauma, using water immersion (the area of the burn was 20-25% of the total body surface area and the duration of exposure was 75-80 sec). The stages of shock were evaluated on the basis of the

Laboratory of Pathological Physiology, Central Research Institute of Hematology and Blood Transfusion, Moscow.

(Presented by Academician of the Academy of Medical Sciences of the USSR O. K. Gavrilov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 106, No. 10, pp. 426-428, October, 1988. Original article submitted September 19, 1987.

0007-4888/88/0010-1412$12.50