in the early period after trauma may be useful for restoring metabolism in the myocardium and liver, if not injured during trauma, but may nevertheless induce additional disturbances in the lung tissue itself.

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ACTIVATION OF THE BLOOD KALLIKREIN – KININ SYSTEM DURING DISSEMINATED INTRAVASCULAR CLOTTING IN RATS. ROLE OF THE PULMONARY COMPONENT AND ATTEMPTED CORRECTION WITH ASPIRIN

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Disseminated intravascular clotting (DIC) and associated disturbances of the rheologic properties of the blood and of the microcirculation are widespread complications of severe trauma [7]. Disturbances of regulation of the clotting and fibrinolytic systems of the blood with multiple microthrombus formation arising in this form of pathology lead to disturbance of functions of the brain, kidneys, lungs, and other systems of the body and may frequently cause the formation of "shock organs." In the multicomponent mechanism of the initial phase of the pathogenesis of DIC disturbances of regulation of the liquid state of the blood must be distinguished: marked activation of the clotting system, a fall in platelet concentration, massive formation of degradation products of fibrinogen, release of vasoactive substances into the blood stream leading to changes in capillary permeability and the microcirculation [1, 8]. To formulate the purpose of the investigation three interconnected lines can be distinguished: 1) The kallikrein–kinin system (KKS) is one of the most important systems in regula-

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TABLE 1. Dynamics of PT, PTT, TT, and F/FDP Parameters in Venous Blood Plasma of Rats during Intravenous Infusion of Physiological Saline, and also of Physiological Saline and Thrombin (M ± m)

Experimental conditions	Duration of infusion, min	Parameter					n
		PT, sec	PTT, sec	TT, sec	F/FDP ethanol test	F/FDP protamine sulfate test	
Intact animals (control)	—	14,1±0,3	17,4±2,3	36,6±2,5	( )	( )	18
Infusion of physiological saline	40	14,5±0,8	14,2±0,8	45,9±2,9*	( )	( )	14
	120	14,4±0,8	14,1±0,7	40,2±1,8	( )	( )	14
	180	14,0±0,4	12,1±1,4	51,0±3,5*	( )	( )	14
Infusion of physiological saline and thrombin	40	26,0±1,2*	50,0±3,3*	60,7±1,5*	( )	( )	14
	120	Complete incoagulability of blood			( )	( ) ( )	24
	180	The same			( )	( )	16

Legend. *)P < 0.05 compared with control; n) number of animals.

tion of microcirculatory homeostasis and it reacts directly on disturbance of equilibrium between clotting and fibrinolysis. The KKS has been shown, in particular, to participate in the pathogenesis of severe skeletal trauma [6], and also in shock and acute leukemia complicated by the development of DIC [3, 11]; 2) active participation of platelets in the DIC syndrome raises the question of a link between the latter and the prostaglandin (PG) system and also with attempts to correct the process by inhibitors of prostaglandins; 3) the lungs are among the organs most likely to be affected by DIC. Disturbance of the metabolic function of the lungs under these circumstances, considering their role in biotransformation of several groups of physiologically active substances, may be a significant factor in the pathogenesis of DIC.

The aim of this investigation was to study parameters of the KKS in arterial and venous blood of rats at intervals in the course of DIC caused by prolonged infusion of thrombin. In the second part of the investigation these parameters were determined after preliminary administration of small doses of aspirin (acetylsalicyclic acid), an inhibitor of PG synthesis.

EXPERIMENTAL METHOD

Experiments were carried out on 332 male Wistar rats weighing 180-360 g. DIC was produced in the animals of one group by preliminary subcutaneous injection of the fibrinolysis inhibitor p-aminomethylbenzoic acid (100 mg/kg), followed after 30 min by prolonged infusion of thrombin solution (550 units/kg/h). The infusion was given through a catheter introduced into the right ventricle through the jugular vein. The rate of infusion was 0.55 ml/100 g body weight/h. The duration of continuous infusion in separate series of the investigation was 40, 120, and 180 min. The animals of the other group received an injection through the same catheter of a standard 0.3% pharmaceutical solution of aspirin (final concentration 20 mg/kg body weight). Control rats were given an injection of physiological saline (the duration of injection was the same as in the experimental groups). Blood for investigation was taken from both ventricles after thoracotomy, with artificial respiration. All manipulations were performed under pentobarbital anesthesia (5 mg/100 g body weight, intramuscularly). Spontaneous arginine-esterase activity of the plasma (SAA), the prekallikrein concentration (PKK), and total plasma activity of kallikrein inhibitors (KI) were determined in test samples of blood. To diagnose DIC the thrombin time (TT) and prothrombin time (PT) [10], partial thomboplastin time (PTT) [13], and fibrin/fibrinogen degradation products (F/FDP) [9, 14] were determined to diagnose DIC. The p-aminomethylbenzoic acid used in the work was obtained from Germed, East Germany, the N-α-tosyl-L-arginine methyl ester hydrochloride was from Reanal, Hungary, Ca-thromboplastin, thrombin reagent, and the kaolin suspension were from Boehringer-Mannheim (West Germany), thrombin was produced by the Kaunas Bacteriological Preparation Factory, and the chromotropic acid was from the P. L. Voikov Factory. The results were subjected to statistical analysis.

EXPERIMENTAL RESULTS

Intravenous infusion of thrombin led to the development of a marked DIC syndrome in the animals' blood (Table 1): 40 min after the beginning of infusion PT was 179%, PTT 352%, and TT 132% compared with the control. A characteristic reaction of the blood system to injection of thrombin is the appearance of F/FDP, as shown by the positive protamine sulfate and ethanol tests at this time. An increase in the duration of thrombin infusion led to total incoagulability of the blood, and F/FDP could no longer be determined under these circum-

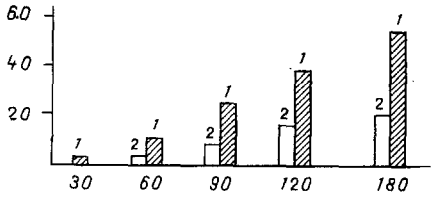

Fig. 1. Mortality among rats after intravenous injection of thrombin solution (1) and also of solution of aspirin and thrombin (2). Abscissa, time of death (in min); ordinate, mortality among animals (in %).

stances. With an increase in the duration of rhombin infusion (development of DIC) mortality among the animals also increased (Fig. 1), up to 55% of the total number of rats of this series dying.

In all the animals an increase in weight of the lungs, edema of the lungs, and signs of hematuria were observed.

Determination of KKS parameters in venous and arterial blood showed (Table 2) that prolonged infusion of physiological saline had virtually no effect on the values of SAA, PKK, and KI. A completely different picture was observed against the background of developing DIC. Evidence of activation of KKS in the venous blood was observed 30 min after the beginning of thrombin infusion: a marked increase in SAA (+88%), a significant decrease in PKK (−19.2%), and a tendency for KI to fall. These changes became even more marked after 120 and 180 min, when the PKK level was reduced by 42 and 53% respectively, and KI activity fell by 29 and 46.5%. Such a type of change in activity of KKS is characteristic of pathological processes with an acute course [4]. It must be pointed out, however, that the changes in activity of KKS in venous blood described above do not correlate exactly with its changes in arterial blood. After infusion of thrombin for 40 min, only SAA was significantly increased in arterial blood samples and values of PKK and KI were indistinguishable from the control. After 120 min the arteriovenous difference for PKK and KI became greater. This means that activation of KKS, linked with the development of DIC, is accompanied during the first 2 h by an outflow of PKK and KI from the lungs into the arterial blood. A similar phenomenon was described by the writers previously: During activation of KKS, as a result of immobilization stress in the acute phase outflow of the components of this system into arterial blood was observed [5]. However, during infusion of thrombin for 3 h no arteriovenous difference for PKK and KI could be observed any longer. In other words, progressive development of DIC leads to exhaustion of the compensatory reserves of the KKS. This type of response – a sharp fall in the values of PKK and KI – reflects activation of KKS under limited control and disturbance of the biochemical equilibrium of the components of this system of regulation of microcirculatory homeostasis [4].

In the group of animals receiving aspirin, activation of KKS due to DIC took place only in the final stage of thrombin infusion. The difference in the values of PKK and KI in venous and arterial blood under these circumstances must be emphasized. The impression was obtained that injection of aspirin led to a considerable time shift in activation of KKS due to the development of DIC. Correspondingly, the decrease in concentration of kallikrein precursor and KI in the venous blood after 180 min was accompanied by a compensatory increase in these parameters in arterial blood passing through the lungs. Mortality among the animals receiving aspirin also was less than half that in those not so treated, and in the last stage of thrombin infusion it was only 22%.

Infusion of thrombin usually led to activation of the platelet stage, as shown by multiple aggregation of platelets, a reduction in their total number in the blood stream, and development of a liberation reaction [8]. It was thus a question of disturbance of both coagulation and platelet hemostasis during DIC. These two mechanisms, coupled with function of the KKS, are quite sufficient for its mass activation. The appearance of vasoactive products (kinins) in the blood may be the cause of disturbance of permeability and generalized disturbance of the hemodynamics, leading to the formation of "shock organs." A pathogenetic role of the KKS also is possible in the DIC syndrome.

Facts indicating the importance of the pulmonary component of regulation of the activity of KKS are accumulating: In acute pathological situations an arteriovenous difference for PKK and KI has been found in blood taken "before the lungs" and "after the lungs." This fact is evidence that reserves of the KKS, utilizable in the first phase of development of the pathological process, are present in the pulmonary endothelium (or other struc-

TABLE 2. Parameters of KKS of Arterial (A) and Venous (B) Blood of Rats during Intravenous Infusion of Physiological Saline, Thrombin, and Aspirin and Thrombin (M ± m)

Series of experiments	Duration of infusion, min	Blood	Parameter			n
			SAA	PKK	KI	
I. Initial state (control)	—	V	14,7±2,3	91,9±4,4	0,91±0,08	6
		A	11,4±1,6	97,5±4,9	0,95±0,08	
II. Infusion of physiological saline (control)	40	V	13,3±1,5	86,1±2,4	0,94±0,04	12
		A	11,9±1,4	86,4±3,1	0,92±0,03	
	120	V	10,5±1,2	79,7±3,0[a]	0,97±0,07	13
		A	10,8±1,1	80,3±4,4[a]	1,02±0,07	
	180	V	18,5±1,4	80,1±3,4[a]	0,95±0,04	12
		A	16,6±1,0	88,9±2,7	0,93±0,03	
III. Infusion of thrombin	40	V	25,0±1,8[a]	69,5±3,1[a]	0,83±0,04[b]	12
		A	22,1±1,6[a]	89,7±2,4[b]	1,0±0,04[b]	
	120	V	16,7±1,7[a]	46,2±3,2[a]	0,69±0,08[a]	12
		A	17,3±1,5[a]	75,9±3,0[b]	1,03±0,03[b]	
	180	V	21,2±2,8	37,5±3,8[a]	0,52±0,07[a]	15
		A	17,8±2,2	42,0±5,2[a]	0,53±0,08[a]	
IV. Infusion of aspirin and thrombin	40	V	18,4±2,1[c]	90,3±3,0[c]	1,08±0,05[a,c]	6
		A	17,5±2,5	93,1±2,4	1,08±0,03[a]	
	120	V	17,5±1,2[a]	68,0±1,4[a, c]	1,12±0,05[c]	6
		A	13,8±1,2	76,6±4,9	1,18±0,05[c]	
	180	V	15,9±1,7	27,9±4,1[a]	0,44±0,04[a]	6
		A	13,5±1,3	51,9±2,4[a, b]	0,80±0,07[b, c]	

Legend. a) $P < 0.05$ compared with series I and II; b) $P < 0.05$ for arteriovenous difference; c) $P < 0.05$ compared with series III.

tures of the pulmonary microcirculatory system). Realization of these products of KKS is independent of a direct disturbance of the pulmonary microcirculation: The outflow of PKK and KI into the arterial system also has been observed in acute craniocerebral trauma [2] and in immobilization stress [5].

The facts described above are evidence that a single preliminary injection of a small dose of aspirin can reduce mortality among animals and delay activation of the KKS. Considering the probable participation of a platelet mechanism in the pathogenesis of DIC, a link between it and activity of the prostaglandin system was deemed likely. The use of inhibitors of PG synthesis in conjunction with administration of PGE prevents the development of thrombocytopenia due to operative trauma [15].

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