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HYPOXIA-INDEPENDENT MECHANISM OF ORGAN INJURY IN HYPERTHROMBOPLASTINEMIA

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Injury to organs during intravascular hemolysis, trauma, and certain obstetric complications is linked with disturbances of regulation of the aggregate state of the blood (RASB), caused by the entry of thromboplastin into the bloodstream [5]. It is considered that hyperthromboplastinemia causes activation of thrombin and the formation of hypercoagulation, followed by response activation of plasmin and the development of hypocoagulation. Disturbances of the microcirculation under these circumstances lead to hypoxic damage of organs [2, 13, 14].

EXPERIMENTAL METHOD

The consequences of hyperthromboplastinemia were studied in three series of experiments on 20 dogs and 85 albino rats after infusion of a suspension or extract of allogeneic (AGB) or xenogeneic (XGB) brain in a dose of 25 ml/kg body weight. In the experiments of series I (four dogs, 25 rats) a suspension of fragments of XGB cell membranes was injected intravenously. An extract of XGB was injected into the animals in the experiments of series II (25 rats, eight dogs — three males and five females). In the experiments of series III (25 rats, eight dogs — three males and five females) extract of AGB was used. In the control, 10 rats received an injection of isotonic NaCl solution, pH 7.4, in a dose of 25 ml/kg body weight.

To obtain 25 ml of a suspension of XGB, 1 g of thromboplastin was mixed with 55 ml of physiological saline, homogenized, and the sample was centrifuged for 3 min at 17 sec^{-1}. To obtain 25 ml of extract 10 g of native material of AGB was mixed with 45 ml of physiological saline and homogenized; the homogenate or suspension of XGB was then centrifuged twice at 58 sec^{-1} for 20 min each time. The supernatant with activity of 16-24 sec^{-1} [1] was used for infusion. The level of free kinins in arterial and venous blood was determined in the male dogs before and at the 2nd minute of infusion, and 5 min and 1 h after infusion, by a biological method [4] after stabilization [9], and the blood pressure was measured. The ureters of the female dogs were exteriorized by the Pavlov—Tsitovich method and partial renal function was studied [7] before and 2, 5, and 10 days after infusion. Parameters of the coagulogram of the dogs [1] were determined before and at the 1st-2nd minute of infusion, and again 5, 60, and 120 min and 24 h after infusion. Under ether anesthesia the rats were decapitated 3-4 and 10 min and 1 and 10 days after infusion. Under these circumstances the liver, kidneys, and heart were removed and treated for histological investigations by staining with hematoxylin and eosin [11] and by Selye's method [12]. The ultrastructure of these organs 3-4 min after infusion was studied by the acid phosphatase (AP) reaction and with ordinary contrast staining [6].

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TABLE 1. Blood Pressure in Femoral Artery (in kPa) and Free Kinin Level (in pg/ml) of Arterial and Venous Blood Plasma from Dogs after Infusion of XGB Extract and AGB Extract (M ± m)

Test	Initial data	Second minute of infusion	After infusion	
			5 min	60 min
Infusion of XGB extract				
Blood pressure	19,9±1,1	8,6±0,4* (11,3)	10,9±1,6* (9,0)	15,9±0,6* (4,1)
Arterial blood kinins	25,8±0,8	1000±42* (—974,2)	—	—
Venous blood kinins	66,3±2,7	1000±42* (—933,7)	—	—
Infusion of AGB extract				
Blood pressure	19,9±1,1	8,0±0,7* (11,9)	10,7±0,8* (9,2)	12,6±0,9* (7,3)
Arterial blood kinins	25,8±0,8	1200±56* (—1174,2)	—	—
Venous blood kinins	66,3±2,7	1200±56* (—1133,7)	—	—

Legend. Here and in Table 2, number in parentheses denotes difference between values and initial data; *P < 0.05.

TABLE 2. Time Course of Partial Renal Functions of Dogs after Infusion of AGB (M ± m)

Test	Initial data	Days after infusion		
		2-	5-	10-
Glomerular filtration, ml/min	51,0±1,50	8,0±0,75* (43)	14,0±0,88* (37)	150,0±1,50* (36)
Urea clearance, ml/min	30,0±0,25	11,0±1,25* (21)	13,0±0,75* (17)	14,0±1,00* (16)
Effective renal plasma flow, ml/min	300,0±9,90	88,0±4,00* (232)	138,0±3,88* (162)	138,0±3,75* (162)
Filtration fraction	1,7±0,06	0,9±0,09* (0,8)	1,0±0,20* (0,7)	1,1±0,20* (0,6)
Maximal tubular secretion, mmoles/min	56,0±5,50	17,0±1,25 (39)	24,0±1,63 (32)	27,0±2,10 (29)

Infusion of a suspension of XGB caused rapid death of the animals from intravascular blood clotting.

At the 1st-2nd minute of infusion of XGB extract the blood lost its ability to coagulate. Its platelet count fell by half and the fibrinogen B and kinin levels rose. The blood pressure fell (Table 1). For a period of 2 h it was impossible to determine the parameters of the coagulogram. After 24 h the clotting power of the blood was restored and the animals' condition was the same as initially. A reversible decrease of the partial renal functions was noted.

The ultrastructure of the cells was changed in the organs studied. Edema, enlargement of the mitochondria, and an increase in the number of lysosomes were observed. AP activity was detected only in lysosomes.

Dilatation of the lumen of the capsules of the glomeruli and tubules, which contains masses of albumin, and also vacuolation of the hepatocytes and fucsinophilia of the myocardium were observed histologically 3-4 and 10 min after infusion. After 24 h dilatation of the lumen of the tubules, vacuolation of the hepatocytes, and fucsinophilia of the myocardium were still present. On the 10th day no changes were found. Infusion of physiological saline caused no changes.

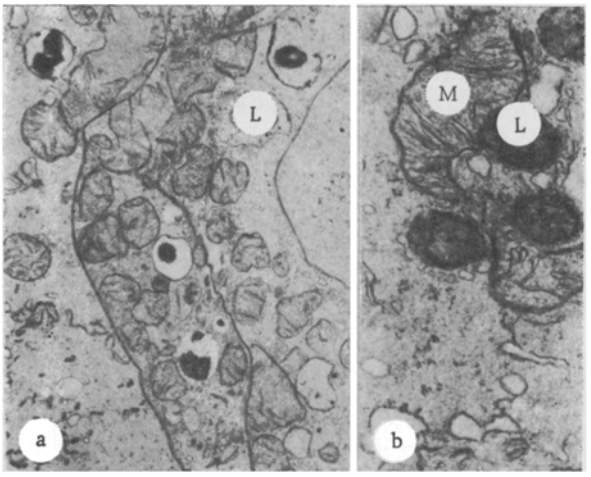

Fig. 1. Kidney (a) and liver (b) of a rat 3-4 min after infusion of AGB extract. Destruction of membranes of lysosomes (L), edema, injury to mitochondria (M). Usual treatment, impregnation with osmium [6]. Magnification: a) 5000, b) 17,000.

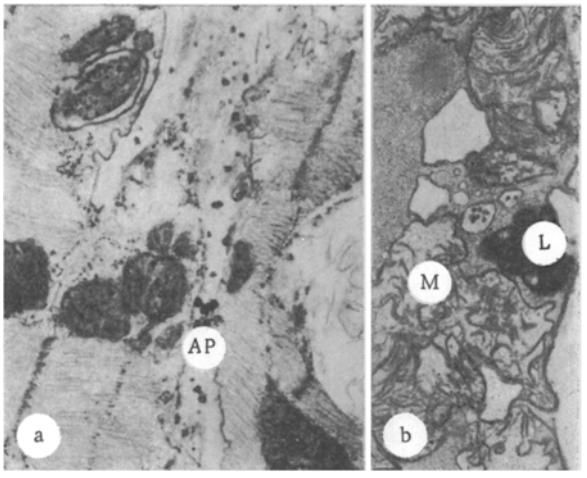

Fig. 2. Myocardium of a rat 3-4 min after infusion of AGB extract. a) Method of Ericson and Trump [6]. AP activity monitored as electron-dense granules in cytoplasm, mitochondria, bundles of myofibrils, surrounded by zones of lysis: b) ordinary treatment, impregnation with osmium [6]. Lysosomal membranes fragmented, mitochondria adjacent to them, bundles of degenerated myofibrils, with homogeneous droplets. Magnification: a) 5000, b) 6500.

After 1-2 min of infusion of AGB extract the blood also lost its ability to coagulate, no platelets could be detected in it, but the levels of fibrinogen B and kinins were raised. The blood pressure was lowered (Table 1). For 2 h the parameters of the coagulogram could not be determined. After 24 h the clotting power of the blood was restored. The animals were not restored to health on the 10th day. The parameters of renal function are given in Table 2.

The ultrastructure of the cells of the organs studied was damaged. The lysosomal membranes were fragmented (Fig. 1) and AP activity could be detected in bundles of myofibrils, in mitochondria, and in the interstices (Fig. 2). Zones of lysis and homogeneous droplets could be seen around the sites of AP activity and the damaged lysosomes. Many cells were destroyed. The intercellular spaces in the epithelium of the renal tubules were widened. Platelets with destroyed cytolemma were found in the lumen of the capillaries.

Dilatation of the lumen of the capsules of the glomeruli and tubules, which contained masses of albumin and single nuclei, was found 3-4 and 10 min after infusion. Widening of the

circumsinusoidal spaces and loss of the complex structure or fusion of the hepatocytes were found in the liver. The cytoplasm of the hepatocytes was vacuolated and basophilic. Many myocardiocytes were fragmented and basophilic and had lost their cross-striation. After 24 h the lumen of the tubules contained free nuclei. The number of mitoses in the liver was increased to 400-450 per 10,000 cells and vacuolation of the hepatocytes was observed. Fragmented myocardiocytes were found. The foci of injury were replaced by connective tissue on the 10th day.

During infusion of the XGB suspension was connected with isolation of cell membrane fragments with fibrin and platelets [8]. Primary hypocoagulation, hypotension, and elevation of the fibrinogen B and kinin levels during infusion of XGB and AGB extracts indicates simultaneous activation of thrombin, plasmin, and kinins and simultaneous fibrin formation and fibrinolysis. Severe damage to the organs took place after infusion of AGB, whereas infusion of the XGB extract caused reversible changes. The essence of this phenomenon is unknown.

Hypoxia damages organs after an exposure of 1 h [3, 10, 15]. A damaging action of the lysosomal enzymes was found 3-4 min after infusion of AGB, and it cannot therefore be associated with hypoxia. Thrombin and plasmin damaged the glycocalyx, as was shown by the widened intercellular spaces of the epithelium of the renal tubules and the breakdown of hepatocyte complexes or their fusion. Damage to the glycocalyx reduced the positive potential of the cytolemma and the pH of the cytoplasm, which led to basophilia of the cells. In an acid medium phospholipases destroyed the membranes and liberated the lysosomal enzymes. The mechanism of these phenomena requires detailed elucidation.

Consequently, the protective response of RASB is predetermined by the species of the animal and the degree of dispersion of the thromboplastin entering the bloodstream, and in extremal situations it may give rise to intravascular clotting, primary hypocoagulation, and hypoxia-independent activation of lysosomal enzymes, which cause damage to the organs.

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