FUNCTIONAL VASCULAR LESIONS AND INTRAVASCULAR PLATELET AGGREGATION IN BURNS

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Disturbance of the function of the hemostasis and intravascular blood clotting are components of the pathogenesis of burns [1, 3, 4]. The prostacycline-generating system of the blood vessel wall participates in the mechanism of maintenance of hemostatic homeostasis, for by continuously synthesizing prostacycline and secreting it into the blood stream it prevents the onset of spontaneous intravascular aggregation of platelets and their adhesion to the intact endothelium, prevents thrombocytic thrombogenesis, and dilates the vessels of the microcirculation [7]. Depression of prostacycline synthesis leads to disturbance of hemostatic homeostasis and to the formation of intravascular platelet aggregates. However, the state of the prostacycline generating system of the vascular wall in burns and the role of its functional disturbances in the pathogenesis of this pathological entity have not yet been studied.

In the investigation described below the effect of burn trauma on antiaggregation antithrombogenic properties of the vascular wall, mainly dependent on functional activity of the prostacycline generating system [6, 7], and on functional activity of platelets, was studied.

EXPERIMENTAL METHOD

Experiments were carried out on 138 male Wistar rats weighing 180-220 g. A flash burn of the IIIB degree covering 15% of the body surface was inflicted. Under pentobarbital anesthesia (1 ml of 1% pentobarbital solution/200 g body weight) the abdominal aorta was excised, rinsed in Tris-HCl buffer solution (0.05 M, pH 7.5), and the prostacycline activity of the aortic wall was determined by the method in [1]. To obtain platelet-rich plasma, blood was taken from the abdominal aorta of normal control animals, stabilized with 3.14% sodium citrate solution (9:1), and subjected to differential centrifugation in order to obtain platelet-deprived and platelet-enriched plasma. The disodium salt of ADP (from Reanal, Hungary) in a final concentration of 10^{-5} M was used as inducer of platelet aggregation. The state of platelet function in the animals with burns was assessed from changes in synthesis of malonic dialdehyde (MDA) and the presence of spontaneous intravascular platelet aggregation [11]. MDA, an indicator of TXA_2 synthesis in platelets, was determined by the method in [10], using thrombin (DATA-FL Thrombin Reagent) in a final concentration of 2.5 units/ml to induce aggregation.

EXPERIMENTAL RESULTS

The antiaggregation activity of the aortic wall from animals with burns showed considerable changes (Table 1). During the first hours after burning it fell on average by 43%, on the first and third days it returned to the control level, but fell again toward the 7th day on average by 87%. On the 15th day after burn trauma the antiaggregation activity of the aortic wall increased, and in the late period of observation (30th day) it fell again.

The functional activity of the platelets, tested by determining MDA release, in thrombin-induced aggregation was found to be increased throughout the period of observation. For instance, the MDA concentration in the platelets (10^{11}/liter) at the end (5th minute) of thrombin-induced aggregation, 6 h after burning, was increased on average by 163%, after 24 h it was increased by 84%, at the height of the pathological changes (7th day) it was increased by 179%, and it remained high until the end of observation (increased by 113%, $P < 0.01$).

In burns spontaneous intravascular platelet aggregation developed (Table 1, Fig. 1).

It will be clear from Fig. 1 that negative correlation was observed between the function-

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TABLE 1. Disturbance of Blood Vessel–Platelet Component of Hemostasis System in Burns

Time after burning	Antiaggregation activity of vessel wall, percent	MDA accumulation during induced platelet aggregation relative to initial level taken as 100%	Index of spontaneous intravascular platelet aggregation
Control	100±4,4	142±9,8	1,01±0,08
Hours			
1	57±7,3*	158±9,6	2,35±0,13*
6	—	305±3,4*	3,1±0,28*
Days			
1	109±3,6	226±4,5*	2,53±0,13*
3	115±2,7	200±2,1*	1,48±0,11*
7	12±3,8*	321±4,1*	3,63±0,2*
15	115±0,8	189±7,6*	1,9±1,2
30	62±3,6*	255±7,0*	5,47±0,47*

*$P < 0.05$.

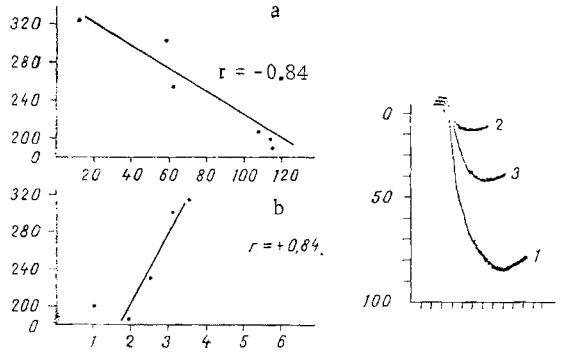

Fig. 1. Character of relationship between platelet function (MDA level and index of spontaneous intravascular aggregation) and antiaggregation activity of vascular wall in burns. Abscissa: a) antiaggregation activity of vascular wall (in percent); b) index of spontaneous intravascular platelet aggregation; ordinate, ratio of MDA accumulation during induced platelet aggregation and initial level (in percent).

Fig. 2. Effect of endotoxin on antiaggregation activity of vascular wall. 1) ADP-induced platelet aggregation in normal animals; 2) under the influence of vascular wall from normal animals; 3) under the influence of vascular wall treated with endotoxin solution for 3 min. Abscissa, time (in min); ordinate, platelet aggregation (in percent).

al disturbance of the prostacycline generating system of the vascular wall and the thromboxane generating system of the platelets.

The mechanism of disturbance of the homeostatic balance between the thromboxane generating system of the platelets and the prostacycline generating system of the vascular wall is a multicomponent mechanism. Depression of the antiaggregation properties of the aortic wall and enhancement of MDA synthesis in the platelets during the first few hours after burning are linked with the animal's stress response to the action of the extremal factor, which is accompanied by release of catecholamines, cortisone, and other biologically active substances. The inhibitory effect of adrenalin and cortisone on prostacycline synthesis by endothelial cells was demonstrated previously [1, 5].

Since in burns a septicotoxemia arises and an endotoxin is produced, a series of experiments was carried out to study the effect of endotoxin on the antiaggregation antithrombogenic properties of the vascular wall. In experiments *in vitro*, during incubation of platelet-rich plasma from normal animals with the wall of the abdominal aorta from normal animals, ADP-induced platelet aggregation was inhibited on average by 14 times, but after treatment of the aortic wall with endotoxin (50 μmoles/ml; Endotoxin Reference, from Sigma, USA) for 3 min its antiaggregation properties were reduced; platelet aggregation was inhibited under these circumstances only by half ($P < 0.01$; Fig. 2).

Maximal disturbances of functional activity of the vascular wall and platelets, observed on the 7th day of observation (the septicotoxemic period of burns), were evidently connected with the endotoxemia, with the marked inflammatory changes, and also with the appearance of burn toxin in the blood. According to data in the literature [8], in burns cell membrane phospholipid degradation products, which are inhibitors of the enzyme prostacycline synthetase, responsible for prostacycline synthesis and the antiaggregation properties of the vascular wall [9], enter the blood stream in burns.

The mechanism of onset of spontaneous intravascular platelet aggregation in burns is due, on the one hand, to functional disturbances of the vascular wall, affecting the prostacycline generating system, as the result of which prostacycline synthesis is disturbed and its constant inhibitory influence on circulating platelets is abolished, and on the other hand, it is due to the appearance of platelet aggregation inducers (adrenalin, thrombin, endotoxin, serotonin) in the blood stream in quantities sufficient to induce activation of the platelet thromboxane generating system.

Disturbance of hemostatic homeostasis, giving rise to spontaneous intravascular platelet aggregation, is a component of the pathogenesis of burns and it leads to a microcirculatory block in the capillary system of the parenchymatous organs, with the development of acute respiratory and renal failure, and also to cerebral edema [4].

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