Effects of α_1-Acid Glycoprotein on Hemostasis in Experimental Septic Peritonitis

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Pathogenesis of hemostasis disorders in septic peritonitis and the possibility of their correction with acute phase protein (α_1-acid glycoprotein; two doses of 150 mg/kg) were experimentally studied on outbred albino rats. Platelets count in the peripheral blood and their adhesion to endothelium did not change during peritonitis, while aggregation activity increased due to increased rate and shorter time of aggregation, which was associated with the development of hypercoagulation involving the intrinsic and common coagulation pathways and reduction of antithrombin activity. α_1-Acid glycoprotein increased platelet count above the normal level, normalized aggregation rate, some blood clotting parameters, and antithrombin activity. Hence, α_1-acid glycoprotein is a polyfunctional protein modulating all pathogenetic components in the development of blood clotting disorders during septic peritonitis.

Key Words: *α_1-acid glycoprotein; hemostasis; peritonitis*

Septic peritonitis (SP) is a severe complication of surgical diseases of the abdominal organs often associated with the development of the multiple organ failure syndrome. High incidence of disseminated intravascular coagulation syndrome suggests that hemostasis disorders play a role in the pathogenesis of SP and prompts regarding the therapeutic measures aimed at their elimination as an obligatory component of pathogenetic therapy of this condition.

We studied hemostasis disorders in experimental SP and the role of α_1-acid glycoprotein (AGP) as a possible therapeutic agent. AGP is a highly sialated plasma glycoprotein with a molecular weight about 44,100 Da. Its concentration increases 2-5-fold in the course of acute phase reaction 24 h after extreme exposure [3]. This protein is characterized by several hemostasiological effects: inhibits ADP-induced platelet aggregation and plasma coagulation cascade and modifies activity of the fibrinolysis system [2].

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MATERIALS AND METHODS

The study was carried out on 104 outbred albino male rats (200-220 g) divided at random into 3 groups. Group 1 consisted of 55 intact rats. In groups 2 (n=26) and 3 (n=23), SP was induced by intraperitoneal injection of 10% suspension of feces in buffered phosphate saline with 0.1% autoblood (1 ml/100 g). The development of SP was verified by leukocytosis with left shift towards stab neutrophils, elevation of rectal temperature, and presence of purulent exudation in the abdominal cavity. Group 3 rats received 150 mg/kg AGP (Orozin, Chelyabinsk Regional Blood Transfusion Station) simultaneously with the suspension and 48 h after SP induction, with consideration for the AGP half-life period (19.3 h).

The animals were sacrificed 72 h after SP induction. The blood was collected by puncture of the heart and stabilized with sodium citrate (9:1 for studies of coagulation hemostasis and 5:1 for evaluation of platelet aggregation). Peripheral blood counts of platelets and leukocytes were evaluated

by the melangeur method. Platelet adhesion to the endothelium was evaluated by an original method: autologous blood was perfused through a fragment of the aorta under a pressure of 100 mm Hg and cell counts before and after passage through the vessel were evaluated, after which the percent of adherent cells was estimated. Platelet-rich (PRP) and platelet-poor plasma (PPP) were prepared by centrifugation at 150*g* and 1200*g*, respectively. Platelet aggregation activity was evaluated using ADP as the aggregation inductor. Coagulation hemostasis parameters were evaluated: time of recalcification in PRP and PPP, activated recalcification time in PRP and PPP, activated partial thromboplastin time, prothrombin time, and thrombin time. The level of factor P_3 and antithrombin activity were measured [1].

Reagent kits (Tekhnologiya-Standart) and reagents (Zero-Med) were used.

The results were statistically processed using Statistica 6.0 software with Student's *t* and Mann—Whitney's *U* tests.

RESULTS

The development of SP was associated with a statistically significant increment in the total leukocyte count (from $4.59\pm1.39\times10^9$/liter to $8.98\pm3.06\times10^9$/liter; $p<0.001$) and elevation of fibrinogen level (Table 1). These shifts reflect acute phase reaction in SP, which was confirmed by increased plasma ceruloplasmin activity (from 44.6±5.1 to 56.2±4.7 arb. units; $p<0.005$).

Platelet count virtually did not change during SP development. Presumably, platelet consumption was effectively compensated by their mobilization from splenic and bone marrow pools. Adhesion of formed elements of the blood is the first stage of

TABLE 1. Effect of AGP on Coagulation Hemostasis in SP (*M*±*m*)

Parameter	Group 1 (*n*=17)	Group 2 (*n*=8)	Group 3 (*n*=8)
Thrombin time, sec	19.1±3.7	11.8±0.8*	14.2±0.8*+
Recalcification time, sec			
in PRP	82.7±16.2	67.4±21.3	89.4±12.5
in PPP	155±21	102.0±34.1*	139.0±19.3+
Activated recalcification time, sec			
in PRP	46.6±11.4	31.8±7.5*	36.2±8.6
in PPP	107.0±22.8	64.2±15.8*	94.2±9.8+
Activated partial thromboplastin time, sec	23.1±2.5	17.8±3.3*	21.6±5.9
Prothrombin time, sec	13.9±2.8	12.2±1.3	11.4±0.6**
Factor P_3, sec	59.4±19.7	34.4±11.7*	58.0±3.5+
Antithrombin activity, sec	194.0±28.6	149.0±33.3*	207±38+
Fibrinogen, g/liter	4.6±0.8	5.7±0.8*	4.4±0.5+

Note. Here and in Table 2: $p<0.05$ compared to group 1: *according to Student's *t* test, **according to Mann—Whitney *U* test; +$p<0.05$ compared to group 2.

TABLE 2. Effect of AGP on Platelet Aggregation Activity in SP (*M*±*m*)

Parameter	Group 1 (*n*=18)	Group 2 (*n*=8)	Group 3 (*n*=8)
Maximum amplitude, %	39.4±4.1	42.8±2.9	35.6±9.9
Aggregation time, min	6.5±1.0	5.0±0.8*	5.1±1.1*
Aggregation rate, %/min	6.2±1.1	7.9±2.3*	7.0±1.3
Wave 1 amplitude, %	33.8±8.1	35.3±3.6	30.4±9.2
Duration of wave 1, min	3.4±0.6	2.7±0.3*	3.1±0.8
Rate of wave 1, %/min	9.7±1.7	13.3±1.1*	10.0±2.6+
Wave 2 amplitude, %	7.6±2.2	7.8±2.2	5.0±1.9**
Duration of wave 2, min	3.1±0.7	2.38±0.90	2.0±0.5*
Rate of wave 2, %/min	2.5±0.6	3.4±0.7*	2.5±0.5+

the hemostasis process, and therefore platelet adhesion to the vascular wall endothelium was evaluated under conditions maximally approximating the physiological. Platelet adhesion activity virtually did not change during SP development: 31.8±7.5% platelets adhered to the endothelium *vs.* 28.5±7.7% cells in intact animals. Platelet aggregation in SP changed at the expense of changed rate of the process in general and of both aggregation waves without changes in their amplitudes (Table 2). Due to this, the total duration of aggregation and duration of wave 1 decreased significantly. Hence, the development of SP stimulated platelet response to the aggregant and platelet secretion process. The coagulation hemostasis parameters in SP were characterized by the development of hypercoagulation with participation of factors of the common (thrombin time) and intrinsic coagulation pathways (recalcification time, activated partial thromboplastin time), and reduction of antithrombin activity (Table 2). Prothrombin time characterizing the status of the extrinsic coagulation pathway, virtually did not change, while the level of platelet coagulation activity (platelet factor P_3) decreased significantly, which was presumably linked with its previous release from platelets and consumption during SP development. The development of hypercoagulation caused by activation of the intrinsic coagulation pathway in SP, was described [4].

Hence, the development of SP leads to complex hemostasis disturbances including increased aggregation activity of platelets and hypercoagulation due to activation of the intrinsic and common coagulation pathways, and reduced activity of the anticoagulant system, which can be regarded as signs of disseminated intravascular coagulation syndrome.

The use of AGP normalized leukocyte count and fibrinogen level, which could result from inhibition of acute phase reactant synthesis in hepatocytes by the negative feedback mechanism. Platelet count increased significantly during AGP treatment from $852\pm62\times10^{12}$/liter to $976\pm148\times10^{12}$/liter ($p<0.05$; Wald—Wolfowitz test). This was presumably due to inhibition of platelet consumption during continuing stimulation of their mobilization from depots. Adhesion activity of platelets virtually did not change: 29.2±3.3% *vs.* 31.9±7.5% in SP. The effect of AGP on platelet aggregation activity was characterized by normalization of the rates of aggregation waves 1 and 2 and hence, inhibition of accelerated receptor interaction and secretion of endogenous proaggregants by the platelets (Table 1). AGP normalized standard and activated recalcification time in PPP and activities of antithrombin and factor P_3. Thrombin time was prolonged, though did not reach the normal value.

AGP produced a positive effect on all pathogenetic components involved in the development of hemostasiological disorders in experimental SP. Hence, it can be regarded as a perspective therapeutic agent for correction of hemostasis disorders in critical states.

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