METHODS

Coagulation and Fibrinolysis in Rats after Surgery with Monopolar Electrical Scalpel

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Blood coagulation, fibrinolysis in plasma and peritoneal fluid, and activity of tissue plasminogen activator in the peritoneum and uterine horns were studied in albino rats after surgery on the uterine horns with a monopolar electrical scalpel. This instrument induced severe inflammatory reaction and disturbances in the fibrinolysis and coagulation systems.

Key Words: *plasminogen activator; plasmin; fibrinogen*

Thrombosis and adhesions are most common complications of gynecological surgery with ordinary scalpel [3,4]. New surgical techniques and instruments such as laser, ultrasound, and electrical scalpels were recently elaborated and applied in reconstruction and plastic surgery. Postoperation adhesions are largely determined by activation of the coagulation and inhibition of the anticoagulation systems [9,10]. This necessitates elaboration of new optimal surgical techniques ensuring minimal influence on hemostasis and fibrinolysis. Our aim was to study changes in the hemostasis and fibrinolysis systems induced by different surgical interventions.

We studied coagulation and fibrinolysis in the plasma and peritoneal fluid (PF), and activity of tissue plasminogen activator in rat organs after surgery with a monopolar electrical scalpels (EkhVCh-500-4 and OLYMPUS).

MATERIALS AND METHODS

Experiments were performed on 45 female randombred albino rats weighing 200 g anesthetized with Hexenal (100 mg/kg, intramuscularly). A 1.5-2.0 cm section along the abdominal middle line and uterine horns was made with a monopolar electrical scalpel. The uterus was then sutured with prolene.

On days 1, 7, 15, 21, and 45 after surgery the blood was drawn from the jugular vein into tubes with 3.8% sodium citrate (9:1). The animals were sacrificed, tissue samples were taken from the uterus and peritoneum, and fibrinolytic activity was evaluated by the Todd histochemical method modified by L. V. Lyutova.

Plasma was isolated by centrifugation at 2000 rpm for 10 minutes. The concentration of fibrinogen, recalcification time, parameters of the thromboelastogram (TEG) recorded on a Hellige thromboelastograph, activities of plasmin and tissue plasminogen activator (TPA), and euglobulin clot lysis time (ECLT) were measured to characterize the state of hemostasis and fibrinolysis. The state of fibrinolysis inhibition system was assessed by antiactivation activity [1].

To estimate hemostasis and fibrinolysis in PF, it was mixed with plasma from healthy animals (1:1), and the above listed indices were measured. Cell populations in PF were studied using a Leika light microscope.

Parameters of hemostasis and fibrinolysis in blood plasma and PF, and activity of TPA in samples from

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the uterine horns and abdomen in intact rats served as the control.

The data were processed statistically using Student's *t* test.

RESULTS

On day 1 after surgery, ECLT in operated animals increased 3-fold compared to the initial level; while on day 15 it increased 13-fold (Fig. 1). TPA activity increased significantly (2-fold) on days 7 and 15. Plasmin activity was 2-fold below the normal from day 1 to 15, and the titer of plasminogen activator inhibitor was near the baseline throughout the experimental period (Fig. 2, *a*). By the end of the experiment (day 45), fibrinolysis was completely restored. The concentration of fibrinogen in operated rats 3-fold surpassed the initial level from day 1 through 21 (Table 1) and returned to normal by day 45. Fibrinogen is an acute phase protein and a primary inflammation marker. The recalcification time on days 1, 7, and 45 and index *k* of TEG indicated on hypocoagulation, which could be a defense reaction of healthy organism to increased risk of thrombosis (enhanced thromboplastin release from injured tissues during surgery, appearance of fibrin strips (adhesion precursors), and hypofibrinolysis).

Starting from day 1 after surgery, addition of PF to donor plasma prolonged ECLT (Fig. 1), 7-fold reduced plasmin activity, and 1.5-fold increased activity of antiactivator. Activity of TPA 10-fold surpassed the initial values (Fig. 2, *b*). After 15 days, the inhibition of fibrinolysis assessed by ECLT did not progress, TPA increased 8-fold compared to the control, while activity of plasminogen activator inhibitor sharply decreased. These shifts persisted until day 45, although plasmin activity was 1.3-fold below the control (Fig. 2, *b*). Fibrinogen in PF was found only on days 1 and 7 postoperation (205.0±32.6 and 208.0±16.4 mg%, respectively), which coincided with the formation of

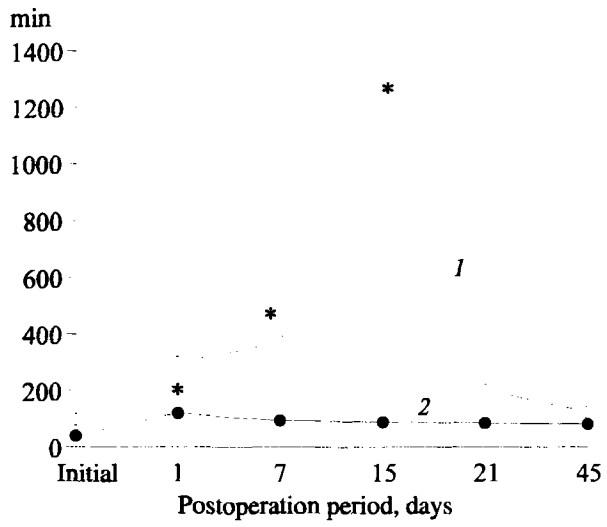

Fig. 1. Euglobulic clot lysis time in rat plasma (*1*) and peritoneal fluid (*2*) after surgery on uterine horns with a monopolar electric scalpel.

primary adhesions in the abdominal cavity. In intact rats no fibrinogen was found in PF. Addition of 0.025% $CaCl_2$ to the mixture of PF and donor plasma did not induce clotting. PF from operated animals prolonged recalcification time from 380.0±212.8 sec on day 1 to 1943.0±810.8 sec on day 15. On day 21, this index decreased to 176.7±5.8 sec and by the end of the experiment no coagulation was observed. Parameters *r* and *ma* of TEG were 6.9±2.4 mm and 30.0±4.4 mm, respectively, throughout the entire experiment. Parameter *k* peaked on day 15 (20.7±11.5 *vs.* 6.7±2.6 mm on day 1). Thus, the changes observed in PF were similar to those in blood plasma, but were more pronounced, probably because generalized response was a culmination of local inflammation first developed in the peritoneal cavity. Macrophages, lymphocytes, eosinophils, and mesothelial cells in PF rapidly responded to mesothelium damage.

On day 1, activity of TPA in the uterine horns increased 2-fold compared to the baseline value. It re-

TABLE 1. Hemostatic Indices in Rat Plasma after Surgery on Uterine Horns with a Monopolar Electric Scalpel (*M*±*m*)

Indices	Baseline	Postoperation period, days				
		1	7	15	21	45
Fibrinogen, mg%	245.0±21.2	728.0±120.3**	768.0±144.3**	494.3±19.7**	789.3±269.3*	446.0±59.2***
Recalcification time, sec	60.0±1.2	85.0±23.1	82.5±15.0	62.5±8.6	52.5±6.4	80.8±10.8
TEG parameters, mm						
r	3.0±0.3	3.5±0.5	1.75±0.50***	3±1	2.3±0.5	3±1
k	1±0	1.6±0.5	1±0	1.8±0.9	1.7±0.5	1.8±0.8
ma	26.0±1.8**	48.6±5.1***	43.5±6.3***	41.0±7.8	48.3±7.2	34.6±2.8***

Note. Here and in Table 2: *$p<0.001$, **$p<0.01$, and ***$p<0.05$ compared to the baseline.

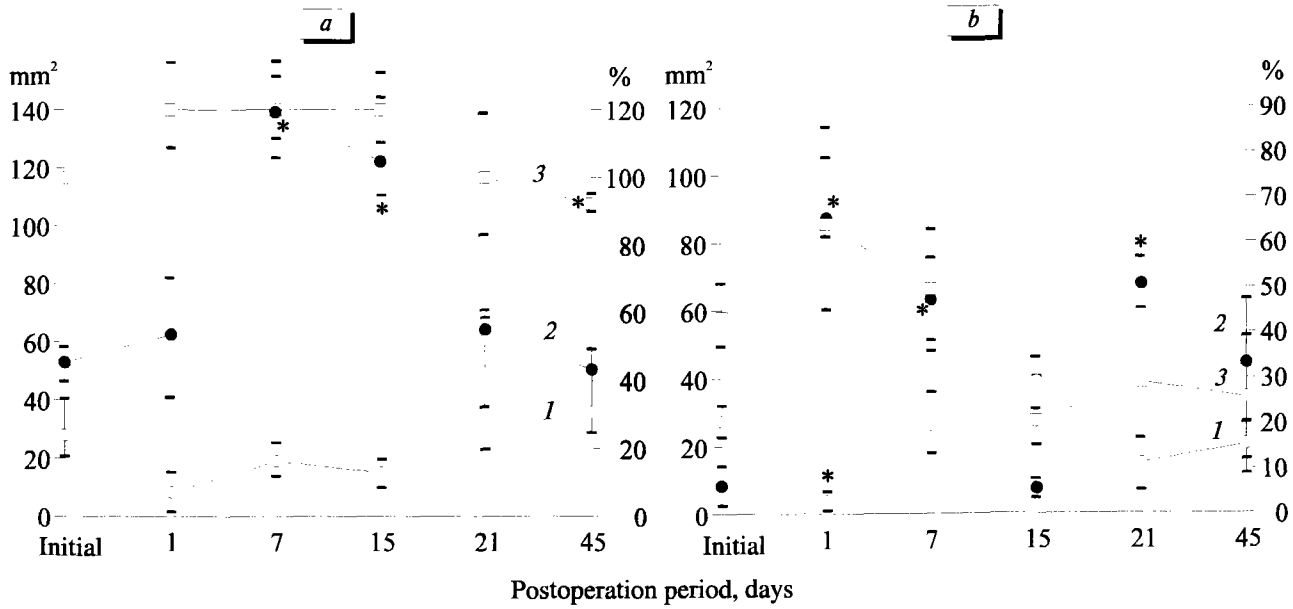

Fig. 2. Levels of plasmin (mm², *1*), plasminogen activator (mm², *2*) and plasminogen activator inhibitor (%, *3*) in rat plasma (*a*) and peritoneal fluid (*b*) after surgery on uterine horns with a monopolar electric scalpel.

mained high until day 15 and returned to normal on days 21-45 postoperation. TPA activity in abdominal tissue increased starting from postoperation day 7 and peaked on day 21. On day 45 it 3.5-fold surpassed the initial value (Fig. 3). TPA activity in the control group was 5.6±1.1 mm/h.

Enhancement TPA activity in tissues and PF seems to disagree with inhibition of fibrinolysis. High TPA activity can result from enzyme release from damaged cells, defense reaction of the organism to fibrin and thrombin appeared in the abdominal cavity, and activation of protein C systems [2,7]. Moreover, some chemical mediators (cAMP and fibroblast growth factor-β), as well as thrombin [8] in the interstitial space during inflammation stimulate TPA synthesis by vascular endothelium. It was shown that activated macrophages also secrete TPA in addition to various mediators (growth factors, prostaglandins) [12]. Inability of this enzyme to initiate plasmin synthesis was probably due to activation of the inhibitory system. It is known that inflammatory transmitters interleukin-1β and tumor necrosis factor-α elevate the content of plasminogen activator inhibitor-1 (PAI-1) [12]. We previously demonstrated that repeated injections of interleukin-1β prolong ECLT and enhanced activities of antiplasmins and plasminogen activator inhibitors in animals [5]. In our experiments (days 1-15) we observed no activation of blood plasminogen activator inhibitors. However, this does not exclude high level of PAI-1 antigen reported by other investigators [10]. The decrease in the content of free plasminogen activator inhibitor in the plasma after incubation with PF taken in the late postoperation period can result from inhibitor binding with TPA excess.

Analysis of cell populations in PF revealed intensive inflammatory reaction in the abdominal cavity caused by surgery on the uterine horns performed using the described techniques. This was justified by visual observation, increased fraction (by 25%) of polymorphonuclear leukocytes carrying powerful bactericidal enzymes compared with the baseline subpopulation (25-30% in PF from intact rats), and decreased lymphocyte counts (by on average 15% compared to baseline) on days 1 through 45 postoperation. The

TABLE 2. Cell Populations in PF (%) after Surgery on Rat Uterine Horns with a Monopolar Electric Scalpel (*M±m*)

Cells	Baseline	Postoperation period, days				
		1	7	15	21	45
Polymorphonuclear leukocytes	30.2±0.5	57.7±5.7**	30.9±1.9**	42.8±1.2*	41.7±1.4*	40.8±2.5
Lymphocytes	44.3±0.2	23.7±5.7**	30.3±8.8	31.5±0.3*	27.9±0.5*	37.4±0.6*
Macrophages	26.0±0.4	18.7±1.7**	28.1±1.7	24.5±0.4***	29.8±1.8***	21.8±1.7***

number of macrophages changed insignificantly throughout the experiment. Macrophages are the main type of resident phagocytes in peritoneal tissues and fluid [6], and this is why we revealed no changes in their activity in PF samples (Table 2).

Inhibition of fibrinolysis, pronounced inflammatory reaction, and high levels of blood fibrinogen provide conditions for adhesion formation irrespective of the enhanced activity of TPA. Normally, fibrous adhesions near the peritoneal wound appear on days 1-7 after surgery. If fibrin matrix is not resolved, it provides basis for adhesions under conditions of fibrinolytic system dysfunction, which was seen in our study. The density of adhesions was 3.19 according to Mynbaev's 5-point scale.

Our results suggest that the use of monopolar electric scalpel induce inflammation and provide conditions for adhesion formation. Thus, additional antiinflammatory therapy and stimulation of fibrinolysis are required.

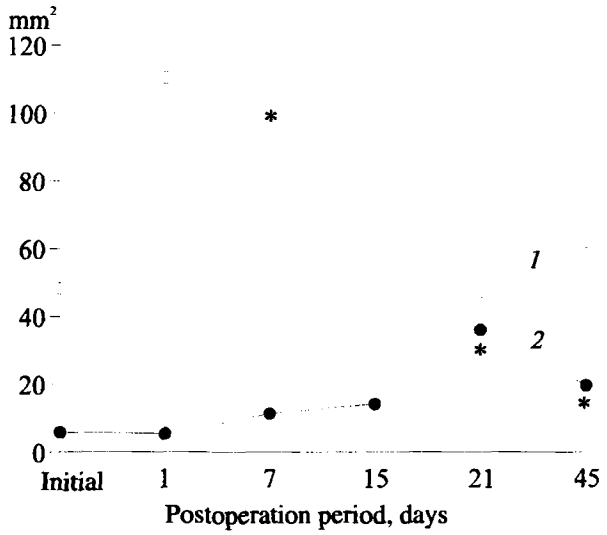

Fig. 3. Activity of plasminogen activator in uterine horns (*1*) and abdomen (*2*) after surgery on uterine horns with a monopolar electric scalpel. *$p<0.01$ compared to the baseline.

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