Phospholipase A_2 Activity and Lipid Peroxidation during Endotoxemia under Conditions of Experimental Peritonitis

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The severity of endotoxemia in peritonitis depends on morphofunctional state of the intestine determined by the intensity of free-radical lipid peroxidation and phospholipase A_2 activity, which are the highest on day 1 postoperation.

Key Words: *endotoxemia; intestine; microcirculation; lipid peroxidation; phospholipase A_2*

Endogenous intoxication accompanies various diseases including peritonitis. In spite of high efficiency of drug therapy and elaboration of new surgical procedures, peritonitis is still the main cause for postoperation mortality [1,2,6]. Severe intoxication is the chief cause of death from acute peritonitis [3,6,7]. Dysfunction of the gastrointestinal tract contributes to the development of endotoxemia during peritonitis [5]. However, the relationship between endotoxemia and changes in the morphofunctional state of the intestine caused by peritonitis and free-radical lipid peroxidation (LPO) and phospholipase A_2 (PLA_2) activity remains unclear [4,8].

MATERIALS AND METHODS

Experiments were performed on 24 adult dogs. The animals were narcotized with 0.04 g/kg sodium thiopental and intraperitoneally injected with 20% fecal suspension (0.5 ml/kg). Laparotomy, revision of the abdominal cavity, intestinal biopsy, and blood sampling from subcutaneous and mesenteric veins were performed on the next day under anesthesia. Repeated laparotomy, intestinal biopsy, and blood sampling were conducted on days 1, 3, and 5 postoperation. During the postoperative period, the animals received antibacterial and infusion therapy.

The redox potential (RP) was measured using an EV-74 universal ionometer. Changes in the blood-tissue permeability were evaluated by capillary filtration and protein loss. The total and effective concentrations of albumin in the plasma were measured fluorometrically using an AKL-01 analyzer (Zond). The content of medium-weight molecules (MWM) was estimated spectrophotometrically on a SF-46 spectrophotometer at 280 nm. PLA_2 activity was measured in 10 mM Tris-HCl buffer (pH 8.0) containing 150 mM Triton X-100, 10 mM $CaCl_2$, and 1.2 mM yolk phosphatidylcholine as the substrate. The catalytic activity was estimated potentiometrically by the formation of free fatty acids. The contents of conjugated dienes and trienes in lipids were determined spectrophotometrically at 233 and 275 nm, respectively. The concentration of malonic dialdehyde (MDA) was estimated by the reaction with thiobarbituric acid (TBA). The preparations stained with hematoxylin and eosin were examined microscopically.

RESULTS

Intraperitoneal injection of 20% fecal suspension caused diffuse purulent peritonitis with severe acute intesti-

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nal inflammation persisting to day 5 postoperation. Circulatory disturbances were manifested by edema, venous plethora, hemorrhages, and polymorphonuclear leukocyte infiltration.

Microcirculatory studies showed that the vein-to-vein gradient increased on day 1 of peritonitis, which was confirmed by intense protein loss (256.7%, $p<0.001$) and pronounced capillary infiltration (169%, $p<0.001$). Electrogenesis in intestinal tissues was impaired: RP decreased by 39.4% ($p<0.001$). One day postoperation these disturbances became more severe: capillary infiltration and protein loss increased to 196.1% ($p<0.001$) and 275.8% ($p<0.001$), respectively, and RP decreased by 44.5% ($p<0.001$) compared with the initial levels. On day 5 postoperation, the vein-to-vein gradient and RP returned to normal, and the volume of capillary infiltration surpassed the initial level by 48.9% ($p<0.001$).

The effective concentration of albumin decreased by 35.3 ($p<0.01$), 60.2 ($p<0.001$), and 48.1-38.3% ($p<0.001$) on day 1 of peritonitis, after cleaning of the abdominal cavity, and on days 3-5 postoperation, respectively (Table 1). In the blood taken from mesenteric veins, this parameter decreased by 52.8 ($p<0.001$), 68.5, 59.8, and 54.3% ($p<0.001$) on day 1 of peritonitis and 1, 3, and 5 days postoperation, respectively.

In the blood taken from subcutaneous veins, the reserve albumin binding capacity decreased by 33.8% during peritonitis ($p<0.001$) and was below the initial level by 59.9, 44.8, and 34.7% ($p<0.01$) on days 1, 3, and 5 postoperation, respectively. In the blood taken from mesenteric veins, this parameter decreased by 50.5% during peritonitis ($p<0.001$) and was below the initial level by 69.5, 57.4, and 52.9% ($p<0.001$) on days 1, 3, and 5 postoperation, respectively.

The toxicity index of the plasma taken from subcutaneous veins increased by 156 ($p<0.001$) and 494.6% ($p<0.001$) on day 1 of peritonitis and 1 day after cleaning of the abdominal cavity, respectively. On days 3 and 5 postoperation this parameter decreased but surpassed the initial level by 246.5 and 173% ($p<0.01$), respectively. The toxicity index increased to a greater degree in the blood taken from mesenteric veins.

The content of MWM in the blood from subcutaneous veins surpassed the initial level by 2 ($p<0.001$) and 2.3 times ($p<0.001$) on day 1 of peritonitis and 1 day after operation, respectively. On days 3 and 5, this parameter was above the initial level by 95 ($p<0.001$) and 42.5% ($p<0.01$), respectively. In the blood from mesenteric veins, this parameter increased to a greater degree (Table 1).

PLA_2 activity in the intestine increased by 46.4 ($p<0.001$), 113.3 ($p<0.001$), 41.7, and 57.8% ($p<0.01$) during peritonitis and on days 1, 3, and 5 postoperation, respectively. PLA_2 activity in the blood from

TABLE 1. Biochemical Parameters of the Blood Taken from Subcutaneous (I) and Mesenteric (II) Veins during Experimental Peritonitis ($M\pm m$)

Parameter	Initial level	Peritonitis	Postoperation period, days		
			1	3	5
PLA_2, μM/sec/g protein					
I	0.042±0.011	0.51±0.034*	0.45±0.093*	0.30±0.042*	0.091±0.032
II	0.043±0.012	0.70±0.054*+	0.73±0.047*+	0.56±0.055*+	0.20±0.021*+
MDA, nM/g protein					
I	1.81±0.24	3.97±0.11*	4.90±0.33**	4.22±0.48**	2.65±0.50***
II	1.91±0.13	5.38±0.32*+	6.20±0.37*+	5.18±0.37*	3.26±0.15*
Effective albumin concentration, g/liter					
I	26.6±1.72	17.2±1.29**	10.60±1.15*	13.8±1.19*	16.40±0.76*
II	25.4±1.44	12.0±0.79*+	8.00±0.61*	10.20±0.65*+	11.6±0.57*+
Albumin reserve binding capacity, arb. units					
I	0.67±0.04	0.45±0.02*	0.27±0.03*	0.37±0.02*	0.44±0.04**
II	0.66±0.04	0.33±0.03*+	0.20±0.02*	0.28±0.02*+	0.31±0.01*+
Toxicity index, arb. units					
I	0.48±0.08	1.23±0.08*	2.87±0.56*	1.67±0.13*	1.32±0.21**
II	0.51±0.09	2.17±0.30*+	3.42±0.52*	2.53±0.22*+	2.17±0.11*+
MWM, arb. units					
I	0.327±0.016	0.658±0.046*	0.754±0.042*	0.640±0.031*	0.466±0.031**
II	0.338±0.01	0.809±0.029*+	0.919±0.028*+	0.847±0.021*+	0.593±0.027*+

Note. *$p<0.001$, **$p<0.01$, and ***$p<0.05$ compared with the initial level; +$p<0.05$ compared with the blood from subcutaneous veins.

subcutaneous and mesenteric veins increased by 12 and 15 ($p<0.001$), 11 and 16 ($p<0.001$), and 7 and 11 times ($p<0.001$), respectively, during peritonitis and on days 1 and 3 postoperation.

The contents of conjugated dienes and trienes and MDA concentration increased by 48.2, 38.4 ($p<0.05$), and 101.6% ($p<0.001$), respectively, during peritonitis and surpassed the initial levels by more than 40% ($p<0.05$) throughout the observation period.

The dynamics of LPO processes in the plasma was similar. One day after peritonitis modeling, the contents of conjugated dienes and trienes and MDA concentration in the blood from subcutaneous veins increased by 65.9 ($p<0.001$), 51.3 ($p<0.05$), and 118.9% ($p<0.001$), respectively; in the blood from mesenteric veins, these parameters increased by 112, 150, and 181% ($p<0.001$), respectively. Further increase in the content of these substances was observed during the early postoperation period (days 1 and 3), but the concentration of TBA-reactive substances decreased on day 5 postoperation.

Thus, peritonitis markedly impairs homeokinesis, especially over the first 3 days postoperation. Intestinal disturbances play an important role in the pathogenesis of endotoxemia, which was confirmed by considerable shifts of the intestinal parameters. Our experiments demonstrated microcirculatory disorders, impaired electrogenesis, and delayed normalization of homeostasis in the intestine. The activation of LPO and increase in PLA_2 are the major pathogenic mechanisms of such alterations: changes in these parameters correlated with the index of endotoxemia (r=0.58-0.65).

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