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Study of indicators of endothelial dysfunction in rats with experimental peritonitis

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Abstract

The article presents the results of the study of the activity of endothelial and inducible NO-synthase, the level of Willebrand factor and endothelin-1 in rats with experimental peritonitis. The most likely mechanism that is damaged in the endothelium during peritonitis is the activation of the synthesis of inducible NO-synthase by neutrophils/macrophages in response to infection. It is possible that hyperproduction of nitric oxide (NO), on the one hand, is aimed at destroying microflora and oxidizing toxins, and on the other hand, at suppressing the expression of tissue factor and cell adhesion molecules. platelet aggregation and cascade disorders in the hemostasis system. All this indicates that the hyperproduction of NO not only reflects the processes that occur in the focus of damage to the vascular endothelium, but also affects the severity of the inflammatory process and the outcome of the

disease. In animals with experimental peritonitis on the background of OS, an increase in the number of circulating desquamated endothelial cells in the blood, which is a highly specific marker of endothelial dysfunction, was noted. The level of the Willebrand factor also increased, which can serve as a marker of increased risk of thrombus formation and indicate the pathogenetic dependence of the factors that damage the vascular wall endothelium on the concentration of the Willebrand factor, which contributes to the reduction of vascular permeability by adhesion of platelets to the endothelium. Confirmation of the development of endothelial dysfunction in peritonitis is an increase in the concentration of endothelin-1, which is a regulator of the process of vascular neoangiogenesis in response to endothelial damage.

Keywords: peritonitis; endothelial dysfunction; oxidative stress; Willebrand factor; pathogenesis.

Introduction. In recent decades, there has been an increase in surgical inflammatory diseases of the abdominal cavity, and even the frequency of purulent complications. In the structure of purulent complications, peritonitis, destructive lesions of abdominal organs, and, as a rule, advanced forms of these diseases occupy one of the first places - 15-25% of urgent surgical diseases are complicated by peritonitis [6, 14, 15]. Peritonitis is accompanied by a fairly high mortality rate. In the domestic scientific medical literature, there are both optimistic (12-15%) and pessimistic (up to 50%) mortality rates, and in the case of hospital peritonitis (under conditions of pronounced endogenous intoxication and the development of multiple organ failure), this rate can reach 90%. According to foreign authors, peritonitis is the main complication of peritoneal dialysis with mortality: USA – 16%, Hong Kong – less than 18%, which is due to the etiology, the initial state of the patients (somatic, immunological status), the nature of the course of the pathological process, the degree of spread of the peritoneal lesion and, as a result, the severity of clinical manifestations, as well as the use of different effective methods of treating peritonitis [8, 9, 15, 18, 19].

It is generally known that the leading role in the triggering mechanism of the development of peritonitis belongs to the systemic inflammatory reaction, a component of which is phagocytosis, cellular and humoral immunity. The attributive condition of systemic inflammation is the structural and functional rearrangement of endotheliocytes, primarily postcapillary venules, and, indirectly, this disorder of microcirculatory hemodynamics, in which all organs and systems are involved in the pathological process, up to the development of organ dysfunction [16, 17].

The aim of work – study of the activity of NO-synthase, factor Willebrand and endothelin-1 in rats with experimental peritonitis.

Materials and methods. The study was conducted on 24 white nonlinear rats, which were divided into 2 groups: 1st group – intact animals (rats that were on a standard water diet and food); 2nd – rats with control pathology modelling.

According to the "Methodological recommendations for preclinical study of medicinal products", experimental peritonitis was studied on the model proposed by V. A. Lazarenko et al. (2008) [6]. This simulated pathology is close in terms of etiological factors, clinical manifestations and phasic course of the course to the similar process in humans and is acceptable for conducting a dynamic study within 10 days. Experimental rats were injected with 0.5 ml of 10% filtered fecal suspension in the abdominal cavity. The suspension was obtained by mixing isotonic solution and feces from the cecum of 2–3 intact animals, then it was filtered twice through a double layer of gauze. The resulting suspension was administered to the experimental group of animals no later than 20 minutes after preparation. In order to avoid damage to internal organs during the introduction of fecal suspension into the abdominal cavity, the rats were kept vertically, with the caudal end up. Using the method of puncturing the ventral wall in the center of the midline of the abdomen, directing the end of the needle alternately into the right and left hypochondrium, right and left iliac regions, the same amount of fecal suspension was injected [6].

During the working with animals, the International Code of Medical Ethics (Venice, 1983), the "European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes" (Strasbourg, 1986), and the Law of Ukraine "On the Protection of Animals from Cruelty" No. 440 (dated 14.01.2020) were used [3].

NO synthase activity was determined spectrophotometrically by the increase in nitrite content in a reaction mixture containing 50 mM potassium dihydrogen phosphate (pH 7), 1 mM magnesium chloride, 1 mM NADPH, and 2 mM calcium chloride (for measuring eNOS activity) or 4 mM EDTA (for endogenous calcium binding when measuring iNOS activity), for 15 minutes at 37°C [0, 5]. The number of circulating desquamated endothelial cells (DEC) in the blood plasma was determined by a method based on the isolation of endothelial cells together with platelets followed by the precipitation of blood platelets using ADP (Hladovec J. et al., 1978). The content of DEC was calculated in two grids of the Goryaev chamber by the luminescence microscopic method, the result was multiplied by 10^4/л [2]. The level of Willebrand factor (fW) was determined using the Willebrand test reagent kit in citrate plasma, which is based on the ability of fV to cause platelet agglutination in the presence of the

antibiotic ristocetin [0]. The concentration of endothelin-1 in the blood was studied using the immunoenzyme kit "Endothelin 1" of the company "Biomedica gruppe" (Austria) for the quantitative determination of endothelin-1 [0].

Statistical processing of the obtained results was carried out with the help of the "Statistica 10.0" program. The probability of differences between the indicators of the control and experimental groups was determined by Student's and Fisher's tests. The level of reliability was accepted at $p<0.05$ [2].

Results of study and their discussion. In the group of animals with experimental peritonitis, on the first day, a probable decrease in the level of eNOS on 2.3 times ($p<0.05$) and a sharp increase in iNOS on 4.3 times ($p<0.05$) compared to intact animals were established (Fig. 1).

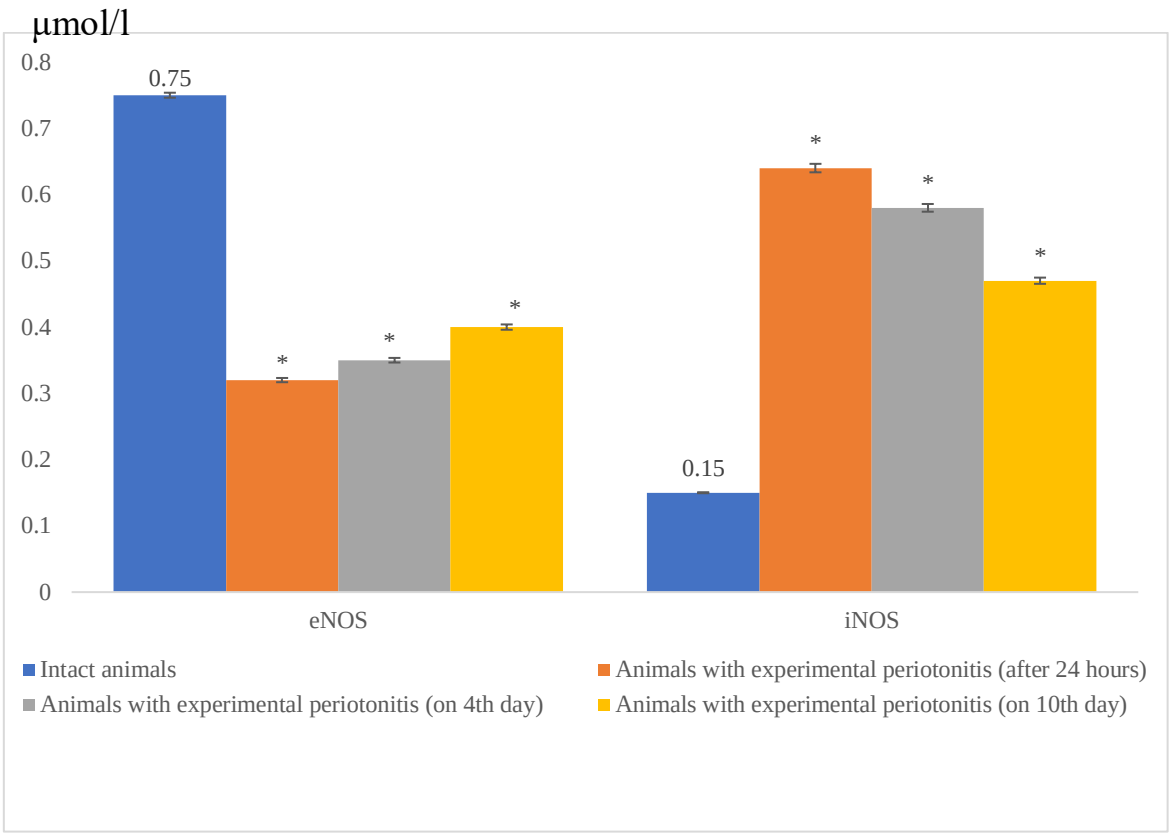

Fig. 1. The level of NO-synthase in the blood of rats with experimental peritonitis at different periods of observation

Notes:

1. * - $p<0,05$ compared to the group of intact animals;

2. n – the number of animals in the group.

On the 4th day of the experiment, the level of eNOS was 0.35±0.02 μmol/l, and the level of iNOS was equal to 0.58±0.04 μmol/l, which probably did not differ from similar indicators obtained on the first day of the study. On the 10th day of the experiment, eNOS increased slightly, but was on 1.9 times lower ($p<0.05$) compared to intact rats; the level of iNOS was slightly lower than the indicators on the first day, but probably higher than the data of intact animals on 3.1 times ($p<0.05$).

The detected significant decrease in eNOS generation is probably due to both its insufficient production and excessive inactivation. The first reason may be a violation of the expression/transcription of eNOS due to the accumulation in the blood of animals of the endogenous inhibitor of NO - ADMA and modified low-density lipoproteins, a decrease in the availability of L-arginine reserves for eNOS due to a decrease in synthesis, the second reason is the development of oxidative stress.

At the same time, the detected hyperproduction of iNOS in plasma is probably a reaction to damage to the endothelium of the vascular wall and increased secretory activity of the endothelium, which contributes to the synthesis of serotonin, ADP, and thrombin by platelets, blocking the oxidation of low-density lipoproteins, and inhibiting the adhesion of monocytes and platelets to the vascular wall. In addition, NO inhibits the expression of pro-inflammatory genes of the vascular wall. All this indicates that NO affects not only the severity of inflammation, but also its course, reflecting the processes that occur in the focus of inflammation and directly in the endothelium of vessels [11]. The leading role in these disorders is attributed to the reaction of the microcirculatory channel, the increased permeability of which leads to exudation, which ensures the transport of histamine, kinins, leukotrienes, thromboxane, prostacyclin and NO to localize the focus of inflammation, which indicates a close interaction of the vascular endothelium and platelets [13]. At the same time, the study of the mechanisms of cellular and endothelial dysfunction as a result of impaired production of NO metabolites, which are one of the universal regulators of cellular and tissue metabolism, attracts special attention.

In animals with experimental peritonitis against the background of oxidative stress, an increase in the amount of DEC in the blood was noted (up to $10,2±0,8*10^4$/l against $3,2±0,3*10^4$/l in intact animals), which is a highly specific marker of ED. On the 4th day, the level of desquamated endothelial cells was $9,8±0,6*10^4$/l, and on the 10th day - $7,5±0,5*10^4$/l.

When simulating peritonitis on the first day, the level of fW increased on 1.7 times ($p<0.05$); on the 4th day – on 1.5 times ($p<0.05$); on the 10th day – on 1.3 times ($p<0.05$)

compared to intact animals. A significant difference ($p<0.05$) was established between the indicators obtained on the first and 10th day (Table 1).

Table 1

Indicators of the functional state of the endothelium in rats with experimental peritonitis ($X\pm S_x$)

Indicators	Intact animals (n=6)	Acute peritonitis (n=18)		
		after 24 hours	4th day	10th day
DEC, $*10^4$/l	3,2±0,3	10,2±0,8*	9,8±0,6*	7,5±0,5*
FW, %	82,8±3,6	140,6±5,4*	124,7±4,7*/**	105,1±4,2*/**
Endothelin-1, fmol/ml	3,5±1,1	9,6±1,7*	8,4±1,5*	6,8±1,6*/**

Notes:

1. * - $p<0,05$ compared to the group of intact animals;
2. ** - $p<0,05$ compared to the result after 24 hours;
3. n – the number of animals in the group.

fW is assigned the role of a rheological glue, a kind of bridge, connecting the receptors of the platelet membrane with the subendothelial structures of the damaged vessel wall. Thus, an increase in the functional activity of fW in the experimental group of animals relative to intact rats can serve as a marker of ED and an increased risk of thrombosis and testify to the pathogenetic dependence of vascular wall endothelium damage factors on the concentration of fW, which contributes to the reduction of vascular permeability by adhesion of platelets to the endothelium.

It was established that during experimental peritonitis already on the first day, the level of endothelin-1 sharply increases (9.6±1.7 fmol/ml against 3.5±1.1 fmol/l of the intact group) on 2.7 times ($p<0$,05). On the 4th day, this indicator slightly decreases compared to the first day, but it exceeds the data of intact rats on 2.4 times ($p<0.05$). On the 10th day of observation, the level of endothelin-1 was 6.8±1.6 fmol/l, which is on 1.4 times ($p<0.05$) lower than the similar results obtained on the first day of the experiment.

An increase in the concentration of endothelin-1 in rats with experimental peritonitis confirms the presence of ED and indicates that this indicator is a regulator of the process of vascular neoangiogenesis in response to endothelial damage. After all, endothelin-1 is formed under the influence of many factors (adrenaline, thrombin, angiotensin, vasopressin) and is produced inside the vascular wall, where specific high-affinity receptors are localized. That is,

we can conclude that endothelin-1 plays a significant role in the modulation of vascular resistance [10, 11, 12].

Against the background of endogenous intoxication and free radical oxidation processes in peritonitis, damage to the cell membrane, vascular endothelium or violation of its secretory function occurs. The implementation of protective mechanisms in case of damage to the endothelium of vessels is accompanied by an increase in the adhesive activity of platelets. In turn, NO regulates leukocyte adhesion and platelet aggregation to the vascular endothelium. Endothelial cells directly due to NO secretion increase the intracellular level of cyclic guanosine monophosphate in platelets, which helps inhibit their adhesion and aggregation. Moreover, this process is carried out according to the principle of negative feedback, since platelets also have the ability to synthesize NO and can activate aggregation [4].

Thus, the obtained results indicate that in the early stages of the development of peritonitis in the animal body against the background of endogenous intoxication, metabolic and functional disorders occur, the cause of which is ED with a violation of the synthesis of such biologically active substances as endothelin-1 and fW. Identified violations of the production of the studied ED markers in experimental peritonitis indicate the possibility of developing a new direction in improving the diagnosis and correction of this disease.

Conclusions:

1. The most likely mechanism that damages the endothelium during peritonitis is the activation of the synthesis of inducible NO-synthase by neutrophils/macrophages in response to infection.

2. In animals with experimental peritonitis against the background of oxidative stress, an increase in the amount of CED in the blood, which is a highly specific marker of ED, was noted. The level of fW also increased, which can serve as a marker of increased risk of thrombus formation and testify to the pathogenetic dependence of the factors of damage to the vascular wall endothelium on the concentration of fW, which contributes to the reduction of vascular permeability by adhesion of platelets to the endothelium. Confirmation of the development of ED in peritonitis is an increase in the concentration of endothelin-1, which is a regulator of the process of vascular neoangiogenesis in response to damage to the endothelium.

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