Experimental Study of the Use of Perftoran for Preventing the Formation of Postoperative Adhesions in Peritonitis

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The effect of Perftoran (perfluorocarbon compound) on cells of the peritoneal exudate and small intestinal serosa was studied in Wistar rats with experimental peritonitis and mechanical injury. The peritoneal cavity was treated with Perftoran for preventing postoperative adhesions and the mechanisms of reparative regeneration of the mesothelium after this treatment were studied by immunohistochemical methods and scanning electron microscopy. The increase in macrophage population during the early postoperative period and its decrease at later terms prevented fibroblast activation and promoted epithelialization as early as by day 5.

Key Words: *postoperative adhesions; Perftoran; peritonitis*

The incidence of adhesion disease increased in recent years. Postoperative adhesive processes in the peritoneal cavity develop in 64-87% surgical patients [6]. Despite modern achievements and methods for preventing and treatment of adhesion formation, there is still no reliable method preventing the development of adhesive process in the peritoneal cavity [3,6].

Predominant formation of adhesive complications in patients with inflammatory diseases of the peritoneal cavity [10,11], especially in peritonitis [7,9] attests to important role of inflammation in the pathogenesis of adhesion formation. The main cause of peritoneal adhesions in peritonitis is inadequate reaction of phagocytic cells during the early postoperative period, which leads to long persistence of fibrin precipitate, bacterial products, tissue detritus, and formation of chronic inflammation [10].

We studied proliferative activity of macrophages, lymphocytes, mast cells, mesothelium, and fibroblasts in experimental animals with inflammation in the peritoneal cavity.

MATERIALS AND METHODS

Peritonitis was induced in Wistar rats by the method developed by V. M. Buyanov. The mesothelium of the small intestinal loops was mechanically damaged at 10 sites (0.25 cm^2 each site). In order to prevent the formation of postoperative adhesions, 2 ml Perftoran emulsion was injected intraperitoneally after pus evacuation. For visual evaluation of changes in the wound surface, peritoneal exudate and intestinal wall samples were collected on days 3, 5, 7, and 14 and stained with monoclonal antibodies (PCNA).

RESULTS

Activation of macrophage proliferation under conditions of experimental peritonitis was observed on days 3-5 in experimental rats. By days 7-14 the mitotic activity of these cells decreased. This was paralleled by a drastic increase in the lymphocyte count, mainly at the expense of the T-cell population (NK cells labeled with monoclonal antibodies). By day 14 their count decreased, but still 2-fold surpassed the normal. The number and proliferative activity of mast cells also tended to increase, but these changes were less pronounced (in percent). By days 7 and 14 we observed exhaustion of mitotic division in cell populations (Table 1).

Experimental peritonitis was associated with activation of macrophages and lymphocytes and rapid exhaustion of inflammation mediators, which eventually disordered the immune status and regulation of the regeneration processes.

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TABLE 1. Proliferative Activity of Peritoneal Exudate Cells and Mesothelium in Animals with Peritonitis (*M*±*m*)

Group, day of experiment		Macrophages	Lymphocytes	Mast cells	Mesotheliocytes	Fibroblasts
Intact animals		1.49±0.03	4.18±0.11	1.17±0.01	0.58±0.01	—
Experimental animals	3	6.71±0.88	11.41±1.14	4.91±0.57	2.81±0.14	9.67±1.01
	5	5.93±0.73	14.11±1.23	3.17±0.23	6.15±0.91	17.13±2.11
	7	3.85±0.41	12.53±1.03	2.43±0.11	10.31±1.07	11.20±1.17
	14	2.24±0.18	8.07±0.81	0.74±0.08	2.14±0.21	4.17±0.78

Note. Here and in Table 2: the data are significant ($p<0.01$).

Cell sticking during their stochastic migration can lead to their accumulation. In this case any cell reaching the center of inflammation ("adhesive spot") will be restrained there.

Using the index of labeled epitheliocyte nuclei we found that activity of the mesothelium was maximum on day 7, while granulation tissue was covered by epitheliocytes by day 14.

The maximum proliferation of fibroblasts was observed on day 5 of the experiment, while by day 14 their proliferative activity decreased. This led to the formation of the so-called adhesive spots during the first week and to the formation of adhesions. The basic substance of adhesions is interstitial proteins (product of fibroblasts). We studied their component hyaluronic acid (fibroblast product) by cytophotospectrometry. The content of nucleic acids in the fibroblast exocytoplasm on day 5 more than 2-fold surpassed that in intact animals.

Well discernible fibrin threads were seen macroscopically between intestinal loops on days 3 and 5 of the experiment. Fibrin threads detected on days 7-14 were presented by well-formed collagen fibers, somewhere covered with mesotheliocytes.

Injection of Perftoran into the peritoneal cavity stimulated migration of phagocytic cells and their antibacterial phagocytic activity [2], that is, increased the resorptive cell resistance, an important mechanism of the barrier functions of the body. Macrophages play the key role in inflammatory reactions and elimination of microorganisms, as well as in the formation of immune response (activation of T and B lymphocytes).

Cell adhesions were detected at 56% of damaged areas. The effect of Perftoran possessing pronounced membrane-stabilizing activity is presumably due to the following mechanism: macrophages capture Perftoran particles, which leads to quantitative increase of their population early after the operation and its decrease during the later postoperative period.

Macrophage count in experimental animals was higher in the control group, but their proliferative activity rapidly decreased. Proliferation of mesothelium decreased on days 7 and 14 due to contact inhibition (Table 2).

The number of fibroblasts in experimental group was notably lower than in intact animals; the number of "adhesive spots" was also lower, and granulation tissue presented mainly as a monolayer. According to cytospectrophotometry data, the content of nucleic acids in experimental animals was lower in comparison with the control.

Adhesion of damaged sites in the intestinal wall was observed in 21% cases (total surface of mechanical injuries was taken as 100%). Fibrous structures formed by day 14 were more fine in experimental animals in comparison with intact rats.

Slight exudation and few fibrin threads were seen in the peritoneal cavity on day 3 after Perftoran injection. The peritoneum was slightly thickened, smooth; there were solitary fine adhesions. In many cases there were no adhesions at all.

TABLE 2. Proliferative Activity of Peritoneal Exudate and Mesothelium in Animals with Peritonitis Treated with Perftoran (*M*±*m*)

Cells	Day of experiment			
	3	5	7	14
Macrophages	8.17±0.21	4.12±0.18	1.03±0.09	0.97±0.71
Lymphocytes	14.13±1.41	16.21±1.63	10.47±1.09	5.01±0.71
Mast cells	6.14±1.03	7.27±0.97	3.21±0.11	1.47±0.09
Mesotheliocytes	8.91±1.07	16.11±1.41	4.50±0.51	1.19±0.07
Fibroblasts	7.57±0.87	5.87±0.41	3.41±0.17	1.15±0.11

Thus, treatment of the peritoneal cavity with Perftoran emulsion leads to migration of phagocytic cells to the focus of inflammation, which improves the resorptive cellular resistance, an important mechanism of barrier functions of the body. The increase in macrophage population during the early postoperative period and its decrease during later period prevented activation of fibroblasts and promoted epithelialization (at the expense of membrane-stabilizing factors) as early as by day 5. Adhesive processes decreased 2-fold in comparison with animals, in whom the peritoneal cavity was treated traditionally.

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