EXPERIMENTAL METHODS FOR CLINICAL PRACTICE

Experimental Validation of Efficiency of Probiotics in Combined Therapy of Destructive Pancreatitis

V. S. Tarasenko, A. A. Stadnikov, V. I. Nikitenko, V. A. Kubyshkin,* and A. V. Valyshev

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The mechanism of *E. coli* translocation after its intragastral administration was studied in rats with acute pancreatitis. Bacterial dissemination into visceral organs was shown. Therapy with probiotic sporobacterin was more effective than antibiotic (cefaxon) therapy. Contamination of the viscera with Escherichia was notably decreased.

Key Words: *acute pancreatitis; translocation; probiotics*

Acute pancreatitis (AP) remains an important problem of practical surgery. This disease ranks third among acute abdominal surgical pathology [3,7], and the incidence of its destructive forms increased. The main pathogenetic components of AP were studied in detail and multicomponent etiopathogenetic methods of therapy were developed, but the outcomes of this severe disease are still poor [6]. The outcome of treatment of destructive AP depends on many factors; pyonecrotic complications play a decisive role, leading to lethal outcomes in 40-80% cases. Lethal outcomes of AP are now delayed from the first days of the disease to weeks 2-3, when such complications become the main cause of mortality [2,8,12].

Some authors [9] attribute it to translocation (penetration) of bacteria through the gastrointestinal mucosa into the blood and tissues. Sometimes translocation is regarded as a pathological process caused by stress, immunodeficiency, or intoxication [8,10,11]. On the other hand, translocation of microorganisms from the gastrointestinal tract was revealed in healthy mammals; it is believed to be biologically justified and essential for adequate formation of defense reactions of the organism [4].

Orenburg State Medical Academy; A. V. Vishnevskii Institute of Surgery, Moscow

Endogenous infection, a factor promoting the development of pyoinflammatory complications, received little attention.

MATERIALS AND METHODS

Bacterial translocation was studied on 54 male August rats weighing 170-200 g. Translocation of *E. coli* and *B. subtilis* strain 534 was evaluated in 36 rats with induced AP and 18 intact rats. Bacteria were cultured for 24-48 h on meat-peptone agar with ^{3}H-thymidine and administered in a dose of 5×10^9 bacterial cells (for both strains) in 2.5 ml 0.09% NaCl through a gastric tube 1 h before surgery.

Laparotomy was made under inhalation narcosis; bile with a drop of autological blood was injected into the pancreatic duct and pancreatic tissue was mechanically damaged. All animals developed AP.

The animals were sacrificed under ether narcosis 2, 24, and 48 h after surgery. Histological analysis of the stomach, small and large intestine, liver, pancreas, spleen, mesenteric lymph nodes, and blood was carried out. Histoautographs were prepared as described previously [1]. The number of ^{3}H-thymidine-labeled bacteria was counted in arbitrary visual fields (ocular insert 5×5 mm, ×900). At least 100 visual fields in each histopreparation were analyzed. Radio-

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labels confirmed the presence of bacteria in tissue. Bacteria were cultured by Laulda's method modified by G. V. Rodoman.

Six experimental series were performed: dissemination of *E. coli* and *B. subtilis* was studied in intact animals (series I-II) and rats with experimental AP (series III-IV). In series V and VI dissemination of *E. coli* was studied in rats treated with antibiotics and probiotic (sporobacterin), respectively. A total of 294 analyses were made.

RESULTS

Radiolabeled bacteria were detected in 207 (70.4%) samples.

The data on the presence of *E. coli* and *B. subtilis* in organs and tissues of animals with experimental AP are summed up in Table 1.

Histological analysis showed the development of destructive pancreatitis and exudative (serous and fibrous) peritonitis in experimental animals. Intragastral administration of *E. coli* notably augmented the course of experimental AP. The morphological picture in such cases was characterized by pronounced and more extensive necrotic zones in pancreatic tissue (by 40% and more in comparison with animals administered no bacteria) against the background of intravascular erythrocyte sludge, blood stasis, microthrombosis, and bacterial microembolia.

Dissemination of *E. coli* was analyzed in intact animals. In this group, the number of radiolabels was notably lower than in animals with experimental AP at all terms of the experiment (Table 2).

Studies of histoautographs of animal tissues showed that *E. coli* penetrate through gastrointestinal mucosa into the portal system, small intestinal mesenteric lymph system, and parenchymatous organs (liver, pancreas, and spleen) both in intact animals and in animals with experimental AP.

Migration and pathways of *B. subtilis* interaction with the host histostructures were similar to those of *E. coli*. Unlike *E. coli, B. subtilis* accumulated primarily in the liver, pancreas, red pulp of the spleen, and mesenteric lymph nodes, showing a higher level of dissemination in comparison with the control ($p<0.01$).

We should like to emphasize that *B. subtilis* did not aggravate the course of experimental AP. Structural changes in tissues in this experimental series were mainly similar to those observed in animals with AP receiving no bacterial suspension.

The viability of *B. subtilis* after intragastral administration was verified by isolating its pure culture from tissue homogenate. Live bacteria were detected in 28 of 61 tissue homogenate samples (45.9%).

TABLE 1. Contamination of Organs and Tissues with *E. Coli* and *B. Subtilis* in Animals with Experimental AP ($M\pm m$, n=3)

Time after administration, h	Mucosa			Liver	Pancreas	Spleen (red pulp)	Mesenteric lymph nodes
	gastric	small intestinal	large intestinal				
2	9.82±0.46 1.88±0.06	4.72±0.12 1.07±0.08	1.03±0.06 0.35±0.07	4.96±0.22 1.08±0.07	4.66±0.30 1.66±0.05	3.01±0.08 4.82±0.08	1.83±0.01 5.31±0.31
24	5.77±0.42** 1.09±0.02*	9.72±0.82** 2.23±0.04*	1.16±0.02 2.94±0.21*	9.55±0.08** 3.16±0.21*	11.6±1.04** 5.67±1.03***	5.65±0.12* 6.16±0.94	4.13±0.10* 6.31±1.12
48	2.28±0.62+++ 0.12±0.01+	3.22±0.04++ —	2.14±0.01+ 1.46±0.03++	7.92±0.11+ 1.85±0.44	12.77±0.31 2.07±0.06+++	4.67±0.12++ 3.05±0.14+++	3.34±0.07+++ 2.07±0.17+++

Note. Here and in Table 2: *$p<0.001$, **$p<0.01$, ***$p<0.05$ *vs.* 2 h after administration; +$p<0.001$, ++$p<0.01$, +++$p<0.05$ *vs.* 24 h after administration. Numerator: *E. coli*, denominator: *B. subtilis*.

TABLE 2. Contamination of Organs and Tissues with *E. Coli* and *B. Subtilis* in Intact Animals ($M\pm m$)

Time after administration, h	Mucosa			Liver	Pancreas	Spleen (red pulp)	Mesenteric lymph nodes
	gastric	small intestinal	large intestinal				
2	2.65±0.02 5.01±0.62	1.06±0.02 3.03±0.20	1.18±0.02 1.98±0.05	1.08±0.01 0.85±0.03	0.78±0.02 0.66±0.03	1.77±0.02 1.45±0.04	2.09±0.01 2.04±0.03
24	1.68±0.31 1.82±0.20***	2.56±0.02* 2.16±0.08***	2.08±0.06* 1.81±0.09	1.88±0.03* 1.28±0.04*	1.05±0.01* 1.87±0.06*	2.46±0.33* 2.24±0.22***	2.09±0.07 2.03±0.08
48	— 0.09±0.01++	0.16±0.01+ 0.94±0.03+	— 1.07±0.02++	0.09±0.01+ 1.88±0.03+	0.11±0.02+ 0.98±0.04+	1.27±0.03+ 1.93±0.04+++	1.02±0.03+ 1.75±0.05+++

TABLE 3. Contamination with *E. coli* in Animals with Experimental AP Treated by Cefaxon and Sporobacterin (*M±m*)

Time after administration, h		Mucosa			Liver	Pancreas	Spleen (red pulp)	Mesenteric lymph nodes
		gastric	small intestinal	large intestinal				
2	cefaxon	1.08±0.02*	6.12±1.03	3.66±0.12	1.32±0.11*	1.87±0.08**	3.56±0.06**	1.86±0.03
	sporobacterin	1.98±0.07*+	0.87±0.01*+++	1.46±0.07***+	1.12±0.12*	1.25±0.05*++	1.56±0.01*+	3.06±0.13*+
24	cefaxon	4.55±0.07	3.87±0.14**	2.42±0.31***	6.16±1.08	8.67±1.03	5.09±0.62	3.43±0.06**
	sporobacterin	2.92±0.09*++	3.05±0.11*+++	2.18±0.06*	2.86±0.04*	3.64±0.41*+++	2.55±0.17*++	2.07±0.08*+
48	cefaxon	3.60±0.22	3.17±0.06	5.77±0.51**	7.88±1.12	9.11±1.17	8.81±1.09***	3.15±0.37
	sporobacterin	1.34±0.07+	1.78±0.03*+	2.02±0.09++	1.27±0.04*++	2.68±0.04*++	3.08±0.22*++	3.54±0.14

Note. *p<0.001, **p<0.01, ***p<0.05 *vs.* control in Table 2; +p<0.001, +p<0.01, +++p<0.05 *vs.* cefaxon.

Comparison of *E. coli* and *B. subtilis* dissemination in intact animals and rats with experimental AP showed that the number of radiolabeled *B. subtilis* in gastrointestinal organs, liver, and in the pancreas of intact rats was 1.5-8.9 times higher than of radiolabeled *E. coli* during the entire experiment. In rats with experimental AP the examined organs were contaminated mainly with *E. coli,* except the red pulp of the spleen and lymph nodes, where *B. subtilis* predominated.

The results suggest that bacterial contamination increases with the progress of AP, the most pronounced accumulation of *E. coli* being observed in the involved organ (pancreas), a potential source of generalization of the infection in the abdominal cavity.

The efficiency of antibiotic (cefaxon) and probiotic (sporobacterin) was compared in experimental animals. Probiotic was obtained from *B. subtilis* strain 534. It is characterized by high antibacterial activity: it blocks microorganisms by decelerating migration and decreasing their concentrations in postoperation wounds [5].

Dissemination and location of *E. coli* was similar in animals with experimental AP treated by sporobacterin and cefaxon and did not differ from that described previously (Table 3). Differences consisted in quantitative distribution of the radionuclide. In animals infected with *E. coli* and orally treated with sporobacterin the contamination of organs and tissues decreased by 1.5-4.7 times in comparison with the control and with animals treated by cefaxon. The therapeutic effect of sporobacterin manifested 2 h after intragastral administration and persisted during the entire experiment, being the most pronounced in the liver and pancreas.

Morphological analysis revealed a significant increase in the number of binucleated pancreatocytes in paranecrotic zones of the pancreas. By the and of day 1, the number of binuclears was 8.4±2.1% in intact animals and 5.9±1.4% in rats with AP. After intragastral administration of *E. coli* the number of binucleated cells was lower than in the control 24 h (2.7±0.7%) and 48 h (1.9±0.3%) postinfection. Sporobacterin increased this parameter to 10.3±1.6 and 12.2±0.8% after 24 and 48 h, respectively (p<0.01).

The experiments revealed enhanced proliferative activity of poorly differentiated centracinous cells and the formation of new mono- and bilayer epithelium along the intralobular excretory ducts and islets of poorly differentiated epitheliocytes. All these changes indicates the improvement of the regeneratory potential of intact viable epitheliocytes after administration of sporobacterin.

Hence, animals experiments demonstrated translocation of enteric microflora through the gastrointestinal mucosa in AP. Contamination increased with the progress of pancreatitis. Bacterial agent sporobacterin decreased contamination of the liver and pancreas with *E. coli* more intensively than antibiotic.

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