ORIGINAL ARTICLE

CONTENTS

Morphological evaluation of the effectiveness of probiotic disinfectants in the treatment of experimental widespread peritonitis

Olga O. Bilyayeva[1], Ivan V. Karol[2], Olena O. Dyadyk[1]

[1] SHUPYK NATIONAL HEALTHCARE UNIVERSITY OF UKRAINE, KYIV, UKRAINE

[2] ODESA REGIONAL CLINICAL HOSPITAL, ODESA, UKRAINE

ABSTRACT

Aim: to conduct a morphological evaluation of the effectiveness of probiotic disinfectants for abdominal cavity sanitation in experimental widespread peritonitis.

Materials and Methods: The experimental study was conducted on 45 white male Wistar rats aged 4-5 months, which were modelled with peritonitis. The rats were divided into 3 groups of 15 animals each. The treatment of animals in the main group (group I) consisted of washing the abdominal cavity with a 5% solution of probiotic disinfectant, applying a probiotic disinfectant spray and a gel with probiotics to the surgical wound. For the treatment of rats in the comparison group (group II), a sorbent solution was used. The treatment of rats in the placebo control group (group III) consisted of abdominal lavage with 0.9% NaCl. Histological, histochemical and immunohistochemical studies were performed.

Results: In the main group, 10 (66.7 %) rats survived, in which peritonitis was eliminated, as evidenced by clinical and morphological results. In the comparison group, 8 (53.3 %) rats survived, in which peritonitis was eliminated. All animals in the placebo control group died of severe peritonitis. The best repair and renewal of mesothelial cells with a pronounced intensity of parietal peritoneal expression was in group I. In group II on the 7th day there were mesothelial cells with moderate expression, in group III - there was a weak intensity of expression in single cells.

Conclusions: According to our data, probiotic disinfectants are effective for abdominal cavity sanitation in peritonitis, which was proved by experimental study.

KEY WORDS: peritonitis, experiment, endogenous intoxication, multiple organ failure, probiotics

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INTRODUCTION

Peritonitis is an acute inflammation of the peritoneum accompanied by high intoxication, one of the most severe complications of diseases and injuries of the abdominal cavity with a high mortality rate. The relevance of the problem of peritonitis is due to its prevalence, high mortality, complexity and multiple disorders of body homeostasis in peritonitis [1].

Secondary peritonitis and intra-abdominal sepsis are a global health problem. The life-threatening systemic damage that results from intra-abdominal sepsis has been thoroughly studied for many years and, despite this, remains poorly understood. While local surgical management of abdominal perforations is well established, there is little systemic therapy to control the subsequent systemic inflammatory response. At the same time, improvements in intensive care have led to improved outcomes for secondary peritonitis. Understanding the impact of secondary peritonitis on the human microbiome is an emerging field, and this provides potential opportunities for improving treatment tactics [2].

The Lancet Commission on Global Surgery concluded that surgery is 'an indivisible, essential part of health care and that surgical and anaesthetic treatment should be an integral component of the national health system in countries at all levels of development'. Therefore, the treatment of secondary peritonitis, which is used in all parts of the world, is particularly noteworthy [3].

Antibiotic resistance of microorganisms causing peritonitis and abdominal sepsis is a major problem today. Antimicrobial resistance and persistence are associated with an increased risk of ineffective treatment and recurrent infections. Thus, they are important factors in the increase in morbidity and mortality, which leads to higher healthcare costs. Measures are being taken worldwide to reduce the development of resistance and the spread of resistant microorganisms [4].

A new direction for overcoming antibiotic resistance in the treatment of patients with peritonitis is the use of

probiotic disinfectants. Bacillus subtilis is a model microorganism used to study cell motility, biofilm formation, protein secretion, cell division, and biosynthesis of secondary metabolites [5]. B. subtilis is a commercially important probiotic known to produce secondary metabolites with antibacterial, antifungal, antiviral, and anti-inflammatory effects [6]. There are many studies on the impact and effectiveness of probiotic disinfectants. For example, some authors note their effectiveness not only in controlling microbial contamination of surfaces but also in reducing the resistance of microorganisms to drugs [7]. The collected data showed that B. subtilis is effective as a probiotic for targeting and destroying human enteropathogens and has a high safety profile without significant health hazards. The findings highlight the promising prospects of B. subtilis as a potent candidate probiotic for probiotic disinfection and clinical applications [8].

As can be seen from the above, this study is relevant and necessary to improve the outcomes of treatment of patients with disseminated peritonitis.

AIM

The aim of the study was to conduct a morphological evaluation of the effectiveness of probiotic disinfectants for abdominal cavity sanitation in experimental widespread peritonitis.

MATERIALS AND METHODS

The experimental study was conducted at the Shupyk National University of Health of Ukraine on 45 white male Wistar rats aged 4-5 months. The animals were kept in the vivarium in accordance with generally accepted standards. All painful manipulations were performed under general anaesthesia. The experimental study was conducted in accordance with generally accepted international and national ethical laws and with the permission of the Ethics Committee of the Shupyk National University of Health of Ukraine (Protocol No. 8 of 7.11.2022). Peritonitis was modelled by injecting a 10% solution of faecal suspension into the abdominal cavity at the rate of 0.5 ml per 100 g of animal weight [9]. Animals were withdrawn from the experiment by an overdose of anaesthetic drugs.

Rats were divided into 3 groups of 15 animals each. The development of peritonitis occurred 1-2 days after the modelling, which was determined clinically and confirmed intraoperatively. Treatment of animals in the main group (group I) consisted of washing the abdominal cavity with a 5% solution of probiotic disinfectant 'SVITECO PHS', followed by application of probiotic disinfectant spray 'AREDERMA' to the abdominal organs and application of gel with probiotics 'SVITECO PPG' to the surgical wound after its suturing. In the treatment of rats in the comparison group (group II), a solution of the sorbent 'Enterosgel' was used for abdominal cavity sanitation. The treatment of rats in the placebo control group (group III) consisted of abdominal lavage with 0.9% NaCl solution. All animals received analgesic and antibacterial therapy. Programmed abdominal cavity rehabilitation was performed at intervals of 24-48 hours.

We conducted a comprehensive pathological examination of the excised areas of the parietal peritoneum. The pathological examination was performed at the Department of Morphology, Clinical Pathology and Forensic Medicine of the P.L. Shupyk National Medical University of Ukraine (Head of the Department: O.O. Dyadyk). Samples of parietal peritoneum 5-7 mm^2 in size, which were obtained intraoperatively, were studied. Changes in the parietal peritoneum on days 1, 3, 5 and 7 after the start of treatment were studied. Histological, histochemical and immunohistochemical studies were performed. Fragments of the parietal peritoneum were fixed in a 10% solution of neutral buffered formalin (pH 7.4) for 24-48 hours. After fixation, the obtained material was routinely wired in an Excelsior AS (Thermo Fisher Scientific, UK), then embedded in paraffin using a HistoStar (Thermo Fisher Scientific, UK). Serial histological sections of 2-3 μm thickness were made from the obtained paraffin blocks on a rotary microtome HM 325 (ThermoShandon, UK), which were then stained with hematoxylin and eosin, and picrofuchsin according to Van Gieson [10].

To determine the morphological features and the term of mesothelial repair, we performed immunohistochemical examination (IHC) with a rabbit monoclonal antibody (RA) to calretinin/Calretinin (Clone SR 13) (Master Diagnostica, Spain). For IHCS, sections were placed on Super Frost Plus adhesive slides (Menzel, Germany). The study was performed using the Master Polymer Plus Detection System (Peroxidase), (Incl.DAB Chromogen) (Master Diagnostica, Spain). The assessment and intensity of the marker expression was carried out according to the absence or presence of brown cells of varying degrees of intensity according to the visual analogue scale from 0 - 'absent' to +++ - 'expressed' [11].

Microscopic examination and photoarchiving were performed using light-optical microscopes 'ZEISS' (Germany) with the data processing system 'Axio Imager. A2' at magnifications of 5x, 10x, 20x, 40x lenses, 1.5 binocular objective and 10 eyepieces with ERc 5s camera, Carl Zeiss PrimoStar with Axiocam105 colour camera.

Statistical processing of the study results was performed using Statistical software EZR v. 1.64 (graphical user interface for R statistical software version 4.3.1, R Foundation for Statistical Computing, Vienna, Austria).

Table 1. Survival of rats in an experimental study

Research group	Survived rats		Died rats	
	Abs.	%	Abs.	%
Group I (n = 15)	10	66,7	5	33,3
Group II (n = 15)	8	53,3	7	46,7
Group III (n = 15)	0	0	15	100

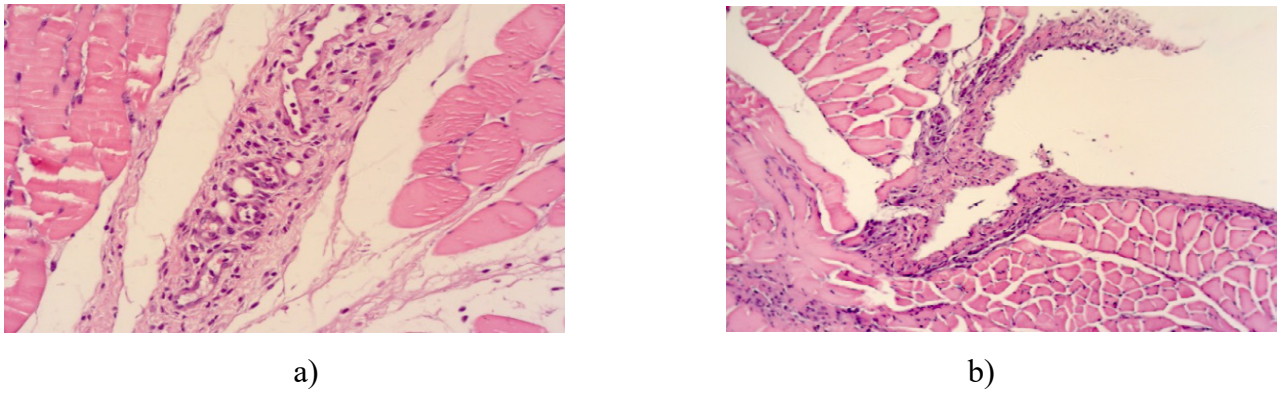


Fig. 1. a) Group I, day 1. Expressed leukocyte infiltration of the peritoneum, vascular haemorrhage. Hematoxylin and eosin staining, magnification x100; b) Group I, day 3. Segmental lymphocytic infiltration of the peritoneum with leukocytes, proliferation of young connective tissue. Hematoxylin and eosin staining, magnification x100

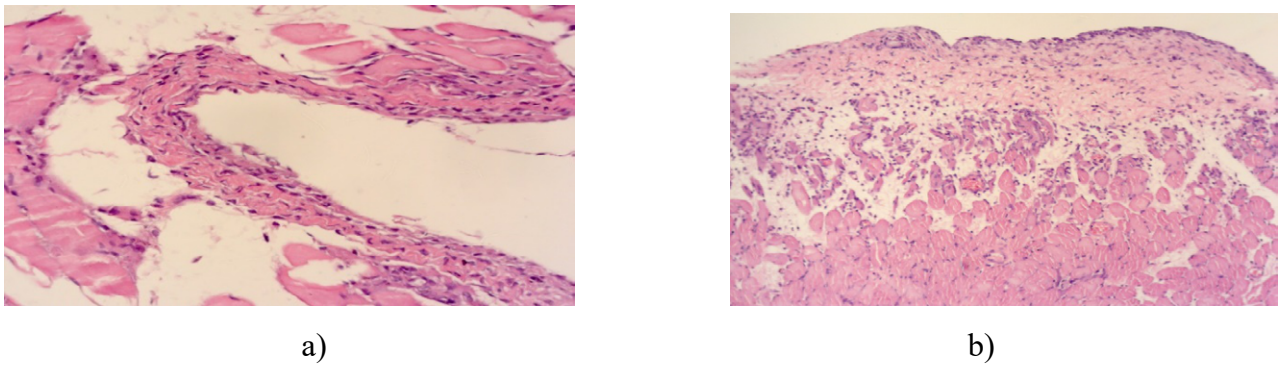


Fig. 2. a) Group I, day 5. Slight cellular infiltration of peritoneal regeneration sites, edema in the regeneration zone. Hematoxylin and eosin staining, x200 magnification.
b) Group I, day 7. Edema, slight lymphohistiocytic cell infiltration, mesothelial regeneration. Hematoxylin and eosin staining, magnification x100

RESULTS

After conducting the experimental study, we obtained clinical data on the survival rate of rats in each group, which are shown in Table 1.

Table 1 shows that 10 (66.7 %) rats survived in the main group, 8 (53.3 %) rats survived in the comparison group, while in the placebo control group all animals died from severe peritonitis.

In group I, on day 1, there was a marked leukocyte infiltration with an admixture of lymphocytes, edema, and full blood flow of the microcirculatory vessels (Fig. 1a). On day 3, a change in the phenotype of cells was observed in the inflammation zone, the vast majority being represented by lymphocytes, histiocytes with a small admixture of neutrophilic leukocytes (Fig. 1b). The initial processes of peritoneal tissue regeneration were established - the growth of young connective tissue.

On the 5th day, there was a slight infiltration of the peritoneum with a small number of lymphocytes, histiocytes, macrophages and monocytes in small numbers (Fig. 2a). Along with this, there were areas of slight disorganisation and edema in the area of connective tissue regeneration. On the 7th day of the experiment, slight lympho-histiocytic cell infiltration, moderate edema, and signs of mesothelial cell regeneration were observed in the peritoneal areas (Fig. 2b).

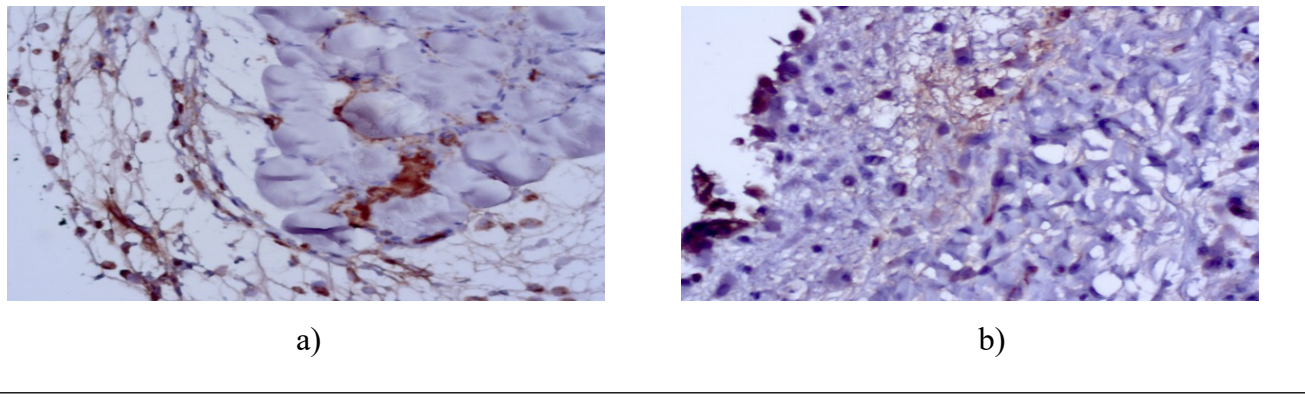


Fig. 3. a) Group I, day 1. Positive expression of mesothelial cells in the parietal peritoneum, positive mast cells. Immunohistochemistry with MCA to Calretinin, magnification x200.
b) Group I, day 7. Expressed positive expression of mesothelial cells in the parietal peritoneum, focally positive mast cells in the underlying tissues. Immunohistochemistry with MCA to Calretinin, magnification x400

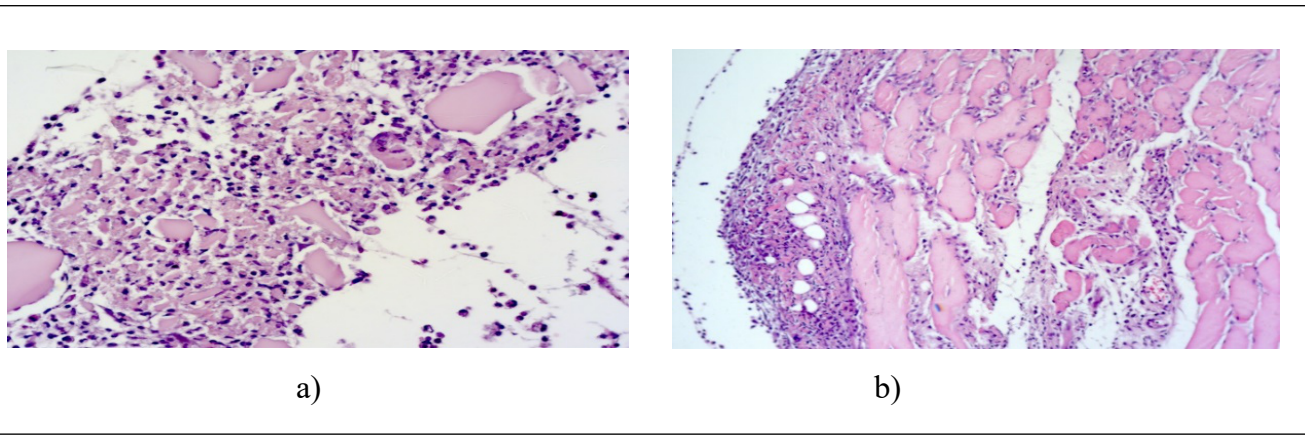


Fig. 4. a) Group II, day 1. Expressed diffuse leukocyte infiltration of the parietal peritoneum. Hematoxylin and eosin staining, magnification x100.
b) Group II, day 3. Extension of cellular infiltration to soft tissues, edema, vascular haemorrhage. Hematoxylin and eosin staining, magnification x100

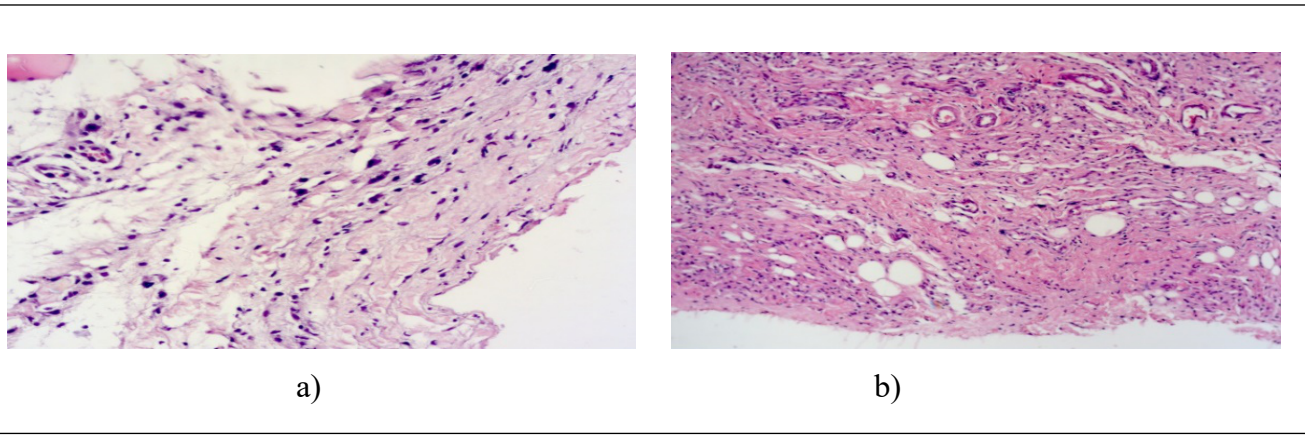


Fig. 5. a) Group II, day 5. Moderately expressed lympho-histiocytic cell infiltration in the regeneration zone of the parietal peritoneum, edema, full blood flow of microcirculatory vessels in the regeneration zone. Hematoxylin and eosin staining, magnification x200.
b) Group II, day 7. Edema, moderate lymphohistiocytic cell infiltration, areas of regeneration of the parietal peritoneum mesothelium, full blood flow of the microcirculatory vessels. Hematoxylin and eosin staining, magnification x100

In group I of rats with MCA to calretinin/Calretinin at day 1, positive, mostly moderately expressed expression was observed in some parietal mesothelial cells and mast cells in the area of cellular infiltration (Fig. 3a). On day 7, the expression of parietal mesothelial cells was observed with a pronounced intensity in the area of parietal peritoneal repair, positive expression of mast cells in the cellular infiltrate, as shown in Fig. 3b.

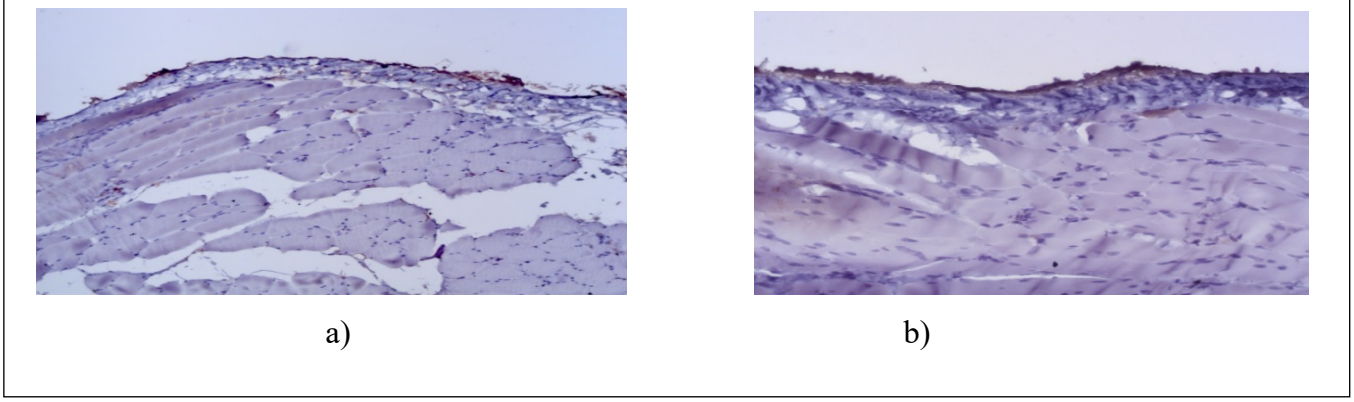


Fig. 6. a) Group II, day 1. Positive segmental expression of some mesothelial cells in the parietal peritoneum, focally positive mast cells. IHCI with MCA to Calretinin, magnification x100
b) Group II, day 7. Positive diffuse expression of mesothelial cells in the parietal peritoneum, single positive mast cells. Immunohistochemistry with MCA to Calretinin, magnification x100

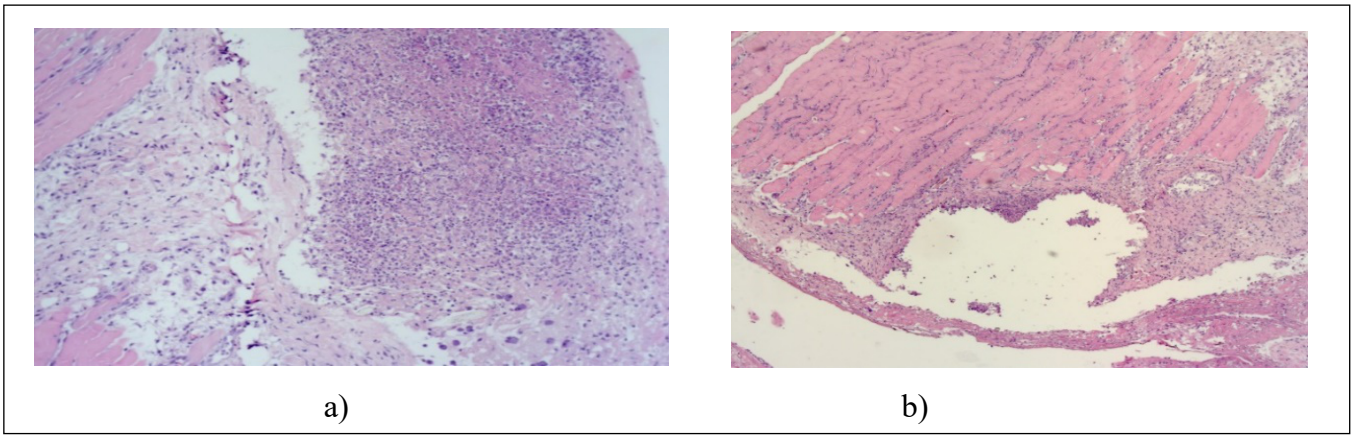


Fig. 7. a) Group III, day 1. Areas of the parietal peritoneum with severe leukocyte cell infiltration with abscess formation, widespread cell infiltration into the underlying soft tissues, focal melting of them. Hematoxylin and eosin staining, magnification x100.
b) Group III, day 3. Leukocyte cell infiltration with abscess formation, spread of cell infiltration to soft tissues, focal myomalacia in the area of parietal peritoneum. Hematoxylin and eosin staining, magnification x100

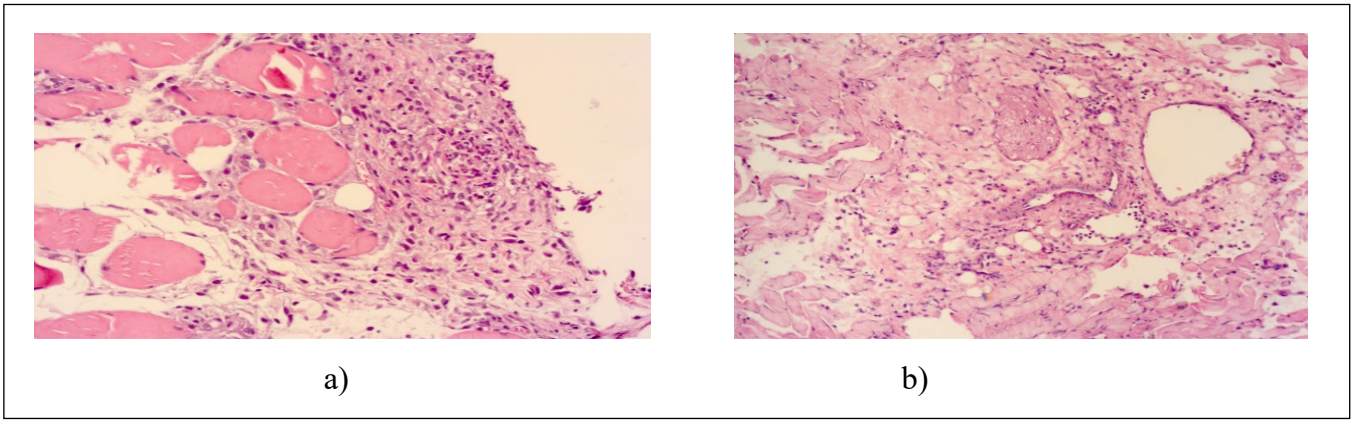
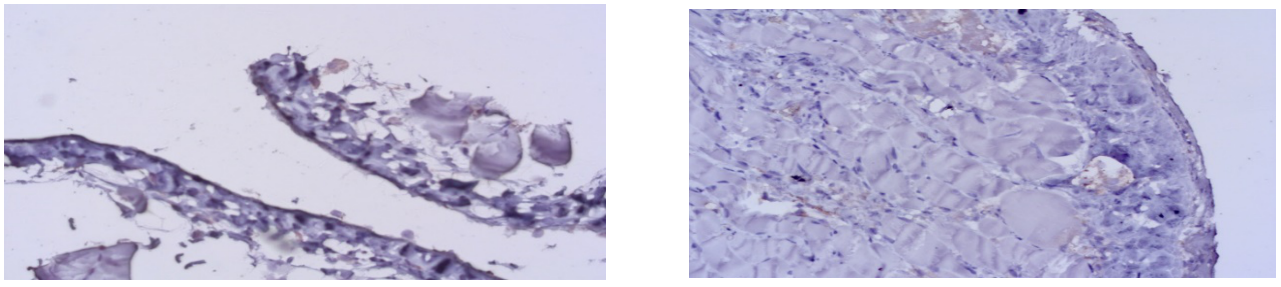


Fig. 8. a) Group III, day 5. Areas of the parietal peritoneum with leuko-lymphocytic cell infiltration with spread of cell infiltration to soft tissues, signs of vasculitis. Hematoxylin and eosin staining, magnification x100.
b) Group III, day 7. Connective tissue proliferation of varying degrees of maturity, leuko-lymphocytic cell infiltration with spread of cell infiltration to soft tissues, signs of vasculitis. Hematoxylin and eosin staining, magnification x100

In the second group of rats, morphological manifestations were similar to the first group on day 1 of the experiment. There was a pronounced cellular infiltration of the lesions mainly from leukocyte cells, edema, soft tissue destruction, and vascular hemorrhage of the microcirculatory bed (Fig. 4a). On day 3 and day 5 of the



a) b)

Fig. 9. a) Group III, day 1. Positive expression of single mesothelial cells in areas of the parietal peritoneum. Immunohistochemistry with MCA to Calretinin, magnification x200.
b) Group III, day 7. Single mesothelial cells of low intensity expression in areas of parietal peritoneum, single positive mast cells. Immunohistochemistry with MCA to Calretinin, magnification x200

study, cellular infiltration of the parietal peritoneum and the spread of inflammatory cellular infiltration to soft tissues were preserved. The composition of the cellular infiltrate gradually changed, along with leukocytes, mast cells, lymphocytes, histiocytes, macrophages, monocytes were present (Fig. 4b).

In the areas of parietal peritoneum regeneration, connective tissue disorganization and edema were noted (Fig. 5a). On day 7, uneven moderately pronounced lymphohistiocytic cell infiltration, edema, connective tissue proliferation of varying degrees of maturity, areas of mesothelial regeneration, and areas of microcirculatory vessel formation remained in the parietal peritoneum (Fig. 5b).

In group II of rats with MCA to calretinin/Calretinin at day 1, focal positive expression of some mesothelial cells in the parietal peritoneum was noted, as well as focal positive mast cells in cell infiltrates (Fig. 6a). On day 7, there was a moderately pronounced predominantly diffuse expression of mesothelial cells in the areas of parietal peritoneum repair, positive expression of a small number of positive mast cells in the underlying tissues (Fig. 6b).

In group III of rats, at all periods of pathological examination, predominantly pronounced leukocyte cell infiltration was noted, especially on days 1 and 3 (Fig. 7a and Fig. 7b), with limited leukocyte cell infiltration in some areas of limited abscess formation.

On day 5, there was a pronounced leukocyte-lymphocyte cell infiltration that spread to the soft tissues, severe damage, edema, soft tissue melting zones, vascular hemorrhage of the microcirculatory bed, and signs of vasculitis (Fig. 8a). On the 7th day, in the areas of inflammation, there was an increase in connective tissue of varying degrees of maturity, edema, dysmucoidosis, focal leukocyte cell infiltrates, unevenly distributed dilated vessels, and inflammatory infiltrates in the wall of some of them (Fig. 8b).

In group II of rats with MCA to calretinin/Calretinin at day 1, there was a weakly positive expression of single mesothelial cells of the parietal peritoneum (Fig. 9a). On day 7, there was a weakly positive focal expression of a small number of mesothelial cells in the areas of parietal peritoneal repair, and positive expression of a small number of positive mast cells in the underlying tissues (Fig. 9b).

DISCUSSION

Thus, after analysing the results of the study, we found that the best repair and renewal of mesothelial cells with a pronounced intensity of parietal peritoneal expression was in group I. In group II, mesothelial cells with moderately expressed expression were present on day 7, and in group III, there was a weak intensity of expression in single cells.

Our study shows a new direction in the development of a comprehensive treatment for widespread peritonitis. In the available literature, we did not find any reports on the effectiveness of abdominal cavity sanitation with probiotic disinfectants. In recent years, there have been many studies on the effect of probiotic preparations in the treatment of gastrointestinal diseases [12-14].

There are studies on the use of probiotic disinfectants in the treatment of purulent wounds. Probiotics have the ability to accelerate wound healing due to their excellent antipathogenic, antibiofilm and immunomodulatory effects. Wound dressings with probiotics are now the main candidates for the development of therapeutic approaches to wound healing, to fight infections and promote the healing process [15].

Some authors have shown that probiotic therapy can prevent the development of spontaneous bacterial

peritonitis in liver cirrhosis [16]. There are reports of studying the effect of enteral probiotics on the body in peritonitis. It was found that probiotic strains showed tolerance to antibiotics, but their use did not show significant clinical efficacy, although it had a positive effect on intestinal dysbiosis [17].

Therefore, we believe that this study is relevant and can make a significant contribution to improving the treatment outcomes of patients with peritonitis.

CONCLUSIONS

1. According to our data, probiotic disinfectants are effective for abdominal cavity sanitation in peritonitis, which was proved by an experimental study. Thus, in group I, 66.7 % of animals survived, in group II - 53.3 %, while in group III all animals died, which indicates the ineffectiveness of abdominal cavity sanitation with 0.9 % NaCl solution in case of widespread peritonitis.
2. The pathological and immunohistochemical examination using monoclonal antibodies to calretinin showed that the best regeneration of the parietal peritoneum occurred in group I. At the same time, there was mesothelial regeneration in group II, but the intensity of mesothelial cell expression was moderate, indicating a slowdown in cell differentiation in this group. In group III, regeneration was weakly expressed in single cells.

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CONFLICT OF INTEREST
The Authors declare no conflict of interest

CORRESPONDING AUTHOR
Ivan V. Karol
Odesa Regional Clinical Hospital
26/32 Academika Zabolotnoho St., 65025 Odesa, Ukraine
e-mail: drkarol@ukr.net

ORCID AND CONTRIBUTIONSHIP
Olga O. Bilyayeva: 0000-0003-2862-0423 A F
Ivan V. Karol: 0000-0003-3684-0127 C D
Olena O. Dyadyk: 0000-0002-9912-4286 B E

A – Work concept and design, B – Data collection and analysis, C – Responsibility for statistical analysis, D – Writing the article, E – Critical review, F – Final approval of the article

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