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L.I. Sydorchuk

Higher State Educational Establishment of Ukraine "Bukovinian State Medical University", Chernivtsi

ACUTE EXPERIMENTAL PERITONITIS: MICROECOLOGICAL INDEXES, SPECIES COMPOSITION AND POPULATION LEVEL OF LARGE INTESTINE MICROBIOTA OF EXPERIMENTAL ANIMALS AFTER 6 HOURS OF INITIATION

Key words: *microbiota of large intestine, contamination, experiment, acute peritonitis.*

Abstract. *The aim of research. This experimental issue deals with microbiological investigation of quantitative and qualitative composition of large intestinal cavity microflora in albino rats with acute experimental peritonitis (AEP) within 6 hours of its initiation.*
Methods. *Case-control experimental study conducted on white 25 albino rats with weight from 200 to 220 g: 10 intact animals (control group) and 15 rats in basic group with acute experimental peritonitis (AEP). The content of large intestine served as research material for microbiological investigation with obtaining pure cultures of microorganisms. Population level of microflora of large intestine content displayed in logarithm of colony-forming units (lg CFU/g). In the current study, few important indexes had used for evaluation of character of microbiota violations - constancy index, Margalef index, Berger-Parker index, Simpson index.*
Results. *It had observed the reduction of dominant Bifidobacteria on 33,5%, Lactobacilli on 36,72%, Peptostreptococci in 2 times and Enterococci in 2,5 times in the large intestine cavity of experimental animals with AEP within 6 hours. However, quantitative dominance in microbiocenosis of large intestine cavity increases for opportunistic Bacteroides in 38,55%, Peptococci in 2,97 times, E. coli in 9,17% and Proteus in 59,37%.*
At the same time there significantly increased role of opportunistic Enterobacteriaceae in the formation of microbiota of large intestine cavity in animals with AEP after 6 hours: Bacteroides - by 42,86%, Peptococci - in 3 times, Escherichia coli - by 10,53%. Opportunistic pathogenic Enterobacteriacea (genus Klebsiella, Edwardsiella), which contaminate and colonize the large intestine cavity, reach a moderate population level and microecological indexes.
Conclusions. *The study of qualitative and quantitative composition, micro-ecological indexes and coefficients that demonstrated interactions and co-existence of dominant, residual and random gut microflora of albino rats with AEP established minor violations of species composition within 6 hours after modelling: deficiency of Bifidobacteria, Lactobacilli, Peptostreptococci on the background of the increased number of opportunistic pathogenic bacteria. The development of acute experimental peritonitis in 6 hours accompanied by the formation of dysbiosis in the cavity of large intestine of first (30,0%), second (60,0%) and fourth (10,0%) degrees.*

Introduction

Peritonitis is the primordial problem of emergent surgery and medicine; more than hundred years ago the famous surgeon Wagner said: "my generation was growing up in a fear of God and peritonitis". Meanwhile this citation sounds like precaution because really the problem of peritonitis is far from final resolve [10].

In spite of the implementation into medical practice of modern technologies, the improvement of surgical technics, rational application of antibiotics and wide spectrum antiseptics the mortality of patients with peritonitis is still high [8]. This affirmed about necessity of new conception according to pathogenesis, role of pathogenic and conventionally pathogenic microorganisms, diagnostics, treatment and forecast of clinical course of acute peritonitis [11]. Especially important meaning has

autochthonous obligatory and facultative anaerobic and aerobic gut microbioma [4, 5]. It is necessary to study a status of dominant, additional and residual microflora, because it can influence on a therapeutic choice in multimodality treatment of patients with peritonitis. The important meaning have results of investigation of intestinal microbioma on the first stages of acute peritonitis development [6]. Author has studied qualitative and quantitative composition of intestinal cavity microflora in albino rats with experimental acute peritonitis (AEP) after 6 hours of its developing, because of such investigation never conducted before.

The aim of study is to establish qualitative and quantitative composition of intestinal cavitary microflora in albino rats with experimental acute peritonitis (AEP) after 6 hours of its modelling.

Material and methods

Experiments have been conducted on white outbreed albino rats with weights from 200 to 220 g. Animals were divided into two groups (basic group includes 10 albino rats with stimulated AEP; control group includes 10 intact animals), all underwent quarantine for 10-14 days in vivarium. Before the starting of investigation all rats were examined for presence of any pathology and reaction on visual and acoustic irritants was studied. Water has been given in unlimited quantity. The animals were fed once a day in the morning, with calorific value from 5.6 to 6.2 kJ per kg daily.

All intervention and slaughter animals were conducted in compliance with the European Convention for the Protection of Vertebrate Animals and "General Principles of animal experiments" approved by Fourth National Congress on Bioethics (Kyiv, 2010). The Commission on Biomedical Ethics of Bukovinian State Medical University (BSMU) didn't found any breaches of ethical standards during experimental study.

In sterile conditions an abdomen cavity was opened, a pieces (1,5-2 sm) large intestine had cut and its content was stamped into sterile wax paper; put it into the sterile porcelain mortar; with addition of isotonic solution in the tenfold volume, carefully grinded to getting of homogenous mass in dilution 1:10 (10-1). From the homogenate of colon wall the row of tenfold serial dilution on the base of isotonic solution from 10-2 to 10-7 were prepared. Every time it was used a new sterile pipette. From each tube row by sterile micropipette were taken 0.1 ml of solution and applied to the corresponding solid nutrient medium optimal for each kind of microbe.

Cultures of facultative anaerobic and aerobic bacteria were cultured in incubator (37° C) for 24-48 hours. Cultures of obligate anaerobic bacteria were cultivated in stationary anaerostat "CO2-Incubator T-125" (Sweden) for 5-7 days (to appearance of growth), sometimes up to 14 days. Then received single-type colonies were studied for each genus of the microbes. Pure culture were identified by genus (species) by morphological, tinctorial, cultural and biochemical properties.

Because the number of bacteria and yeast-fungi of the genus Candida per unit volume reaches millions and billions, for easier data presentation a quantity where calculated in logarithm of quantitative indicators of microflora (lg CFU/g).

Aimed to discover mechanism of contamination and colonization of biotope by the microorganisms, author used ecological method, that allowed realizing characteristics of microbial co-existance and traced up the direction of microecology changes of large intestinal cavity in case of destabilization of microbioma.

Constancy index (CI) served for evaluation of typology of dominants: dominant microorganism assumed if CI was 50 % and more, additional - from 25-50 %, and random microflora - if CI less 25 % [1].

Margalef index used for characteristics of microbiocenosis diversity that is specific "biotope rating", which described space and nutrition resources and conditions of environment for microorganisms [2].

Just to know a level of domination of certain microbe in biotope it was calculated an index of domination by Berger-Parker and by Simpson as well as coefficient of quantitative domination and meaningless [9]. Statistical analysis performed using the MS® Excel software [3].

Results and discussion

The first stage of the experiment was simulation of acute peritonitis. Microbiome of any biotope of human and animal body characterized by species composition and population level. These characteristics are generally stable in the normal functioning of the body for each biotope. However, disruption of physiological state and changes in other biotopes (peritonitis) can cause changes in a microecology of large intestine cavity. The first stage of the investigation was to establish the species composition of the microbiota of the colon content in rats with experimental acute peritonitis (AEP) after 6 hours from the start of the simulation (Table 1).

Even after 6 hours of acute experimental peritonitis (AEP) there are coming changes in species composition and microecological characteristics of cavitary microbiota of large intestine. In intact

Table 1

Species composition of large intestine content microflora in albino rats with AEP after 6 hours of its initiation

Microorganisms	Basic group (n=10)						Control group (n=15)					
	Isolated strains	CI	OF	MI	BPI	SI	Isolated strains	CI	OF	MI	BPI	SI
Obligate anaerobic microorganisms												
Bifidobacteria	9	90,0	0,13	0,12	0,13	0,016	14	93,33	0,14	0,13	0,14	0,016
Lactobacteria	9	90,0	0,13	0,12	0,13	0,016	15	10,00	0,15	0,14	0,15	0,020
Bacteroids	10	100,0	0,15	0,13	0,15	0,020	15	10,00	0,15	0,14	0,15	0,020
Peptostreptococci	5	50,0	0,07	0,06	0,07	0,004	12	80,00*	0,12*	0,11*	0,12*	0,013
Peptococcus	6	60,0	0,09	0,07	0,09	0,007	5	33,33*	0,05*	0,04*	0,05*	0,002
Clostridia	2	20,0	0,93	0,01	0,03	<0,001	3	20,00	0,03	0,02	0,03	0,001
Facultative anaerobic and aerobic microorganisms												
E.coli	10	100,0	0,15	0,13	0,15	0,020	15	100,00	0,15	0,14	0,15	0,020
Proteus	9	90,0	0,13	0,12	0,13	0,016	12	80,00	0,12	0,11	0,12	0,013
Klebsiella	3	30,0	0,04	0,03	0,04	0,001	0	-	-	-	-	-
Edwardsiella	2	20,0	0,03	0,01	0,03	<0,001	0	-	-	-	-	-
Enterococci	3	30,0	0,04	0,03	0,04	0,001	11	73,33*	0,11*	0,10*	0,11*	0,011

Notes. CI – constancy index; OF – occurrence frequency; MI – Margalef index; BPI – Berger-Parker index; SI – Simpson index; * – corresponding degree of evidence by $p<0,05$.

animals (control group) the dominant microflora is presented with obligate anaerobic bacteria of genera Bifidobacterium, Lactobacillus, Bacteroides, Peptostreptococcus, and among facultative anaerobic and aerobic - bacteria of genera Escherichia, Proteus, Enterococcus. Peptococci belongs to the additional microorganisms and bacteria of Clostridium - to casual microorganisms

In animals with AEP main microbiome is presented by obligate anaerobic bacteria of the genus Bifidobacterium, Lactobacillus, Bacteroides, Peptostreptococcus, and among facultative anaerobic and aerobic - bacteria of genera Escherichia, Proteus; additional microbiome is presented by Enterococci and conditionally pathogenic Enterobacteria (Klebsiella), casual - bacteria of the genus Clostridium, Edwardsiella.

These changes prove that the development of AEP after 6 hours cause in a certain number of animals the elimination of Enterococci, Peptostreptococci, Lactobacilli from large intestine cavity and colonization and contamination of biotope with opportunistic Enterobacteria (Klebsiella and Edwardsiella), Peptococci.

Meanwhile investigation of microbiome of any ecological niche must be based on the availability of different species in biotope: permanent (typical, dominant, major, resident etc.). Number of characteristic (main microbiome) species is relatively small but numerically they are always presented in biotope in large amount (90% or more). So the next step was to study the quantitative composition of the cavitary microbiome of large intestine of albino rats with AEP after 6 hours from the beginning of the simulation (Table 2).

The development of AEP after 6 hours had accompanied with the formation of microbiota disorders: Bifidobacteria decreased by 35.56% (by 3-4 orders), lactic acid bacteria - by 47.79% (in 3 orders) and reduction occurs in the population level of Peptostreptococci - 51.71% (2 orders), Enterococci - 16.13% and Escherichia coli - 10.51%. On a background of deficit of autochthonous, obligate anaerobic, facultative anaerobic and aerobic bacteria, there increases number of opportunistic Enterobacteriaceae generally and Proteus particularly by 17.43%.

In the large intestine cavity of rats with AEP after 6 hours there significantly reduced the dominant activity of Bifidobacteria by 33.5%, Lactobacilli - by 36.72%, Peptostreptococci - by 2 times, Enterococci

Table 2

Populational level of microbiome of content of large intestine in albino rats with AEP after 6 hours of its modelling

Microorgnisms	Basic group (n=10)			Control group (n=15)		
	PL	CQD	CM	PL	CQD	CM
Obligate anaerobic microorganisms						
Bifidobacteria	5,89±0,93	100,40	0,15	9,14±1,03*	133,91*	0,20*
Lactobacteria	6,11±0,93	104,0	0,15	9,03±0,80*	142,39*	0,21*
Bacteroids	7,04±0,91	133,33	0,20	6,13±0,22	96,23*	0,14*
Peptostreptococci	3,81±0,22	36,08	0,05	5,78±0,38*	72,59*	0,11*
Peptococcus	5,21±0,39	59,20	0,09	3,81±0,15*	19,94*	0,03*
Clostridia	3,69±0,13	13,98	0,01	3,81±0,15	11,96	0,02
Facultative anaerobic and aerobic microorganisms						
E.coli	7,42±1,25	140,53	0,21	8,20±0,30	128,73	0,19
Proteus	4,11±1,05	70,06	0,10	3,50±0,57	43,96*	0,07
Klebsiella	5,66±0,10	32,16	0,04	0	-	-
Edwardsiella	4,69±0,13	17,77	0,04	0	-	-
Enterococci	6,82±0,07	38,75	0,05	7,92±0,63	91,17	0,14

Note. PL – populational level as lg CFU/g; CQD – coefficient of quantitative domination; CM – coefficient of meaningfullness; * – corresponding degree of evidence by $p<0,05$

reduced by 2.5 times. However, quantitative dominance in microbiocenosis of large intestine cavity increases in opportunistic Bacteroides by 38.55%, Peptococci - in 2.97 times, E. coli - by 9,17%, Proteus - by 59.37%.

There is reduction of role of Bifidobacteria in the formation of microbiota by 33.33%, Lactobacilli - by 40.0%, Peptostreptococci - in 2.2 times, Enterococci - in 2,8 times. At the same time there significantly increased role of opportunistic Enterobacteriaceae in the formation of microbiota of large intestine cavity in animals with AEP after 6 hours: Bacteroides - by 42.86%, Peptococci - in 3 times, Escherichia coli - by 10.53%. Opportunistic pathogenic Enterobacteriacea (genus Klebsiella, Edwardsiella), which contaminate and colonize the large intestine cavity, reach a moderate population level and microecological indexes.

It was established the degree of dysbacteriosis of large intestine cavity in experimental and control groups animals on the background of species composition and populational level and mentioned above microecological indexes after 6 hours of AEP initiation (table 3).

In the majority of experimental animals with AEP after 6 hours the dysbacteriosis I-II degrees is

Table 3

Degrees of dysbacteriosis of large intestine cavity in albino rats with AEP after 6 hours of its initiation

Degree of dysbacteriosis	Basic group (n=10)		Control group (n=15)		P
	abs.	%	abs.	%	
Norm flora	0		14	93,33	-
First degree dysbacteriosis	3	30,0	1	6,67	<0,05
Second degree dysbacteriosis	6	60,0	0	-	-
Third degree dysbacteriosis	0	-	0	-	-
Fourth degree dysbacteriosis	1	10,0	0	-	-

formed, and in one animal - IV degree, that proved abnormality ecological interactions of species composition and populational level of autochthonous obligate and facultative microbiota.

Conclusions

1. After 6 hours of initiation of acute experimental peritonitis in albino rats the mild disorders of species composition of large intestine cavity microflora due to elimination in certain part of basic group animals of Enterococcus, Peptostreptococcus and contamination and colonization in biotope of opportunistic pathogenic enterobacteria (Klebsiella, Edwardsiella, Proteus).

2. In the cavity of large intestine of albino rats with AEP within 6 hours the expressed quantative deficit (on 2-3 levels) of bacteria of genera Bifidobacterium, Lactobacillus, Peptostreptococcus had formed, and quantity of opportunistic pathogenic eneterobacteria, peptococcus had increased.

3. By constancy index, the frequency of occurrence, index of species variety by Margalef, Berger-Parker index and Simpson index, and by two coefficients (quantity domination and meaningless) the role of bifidobacteria, lactobacteria, peptostreptococci and enterococci in formation of microbiocenosis had evidently decreased; meanwhile domination role of opportunistic pathogenic enterobacteria, Escherichia, bacteroides and peptococcus had evidently increased.

4. The development of AEP after 6 hours of initiation had accompanied with formation of dysbacteriosis in cavity of large intestine in 30% as first degree, in 60,0% as second degree and in 10,0% of experimental albino rats as fourth degree disorders of large intestinal microbiota.

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ОСТРЫЙ ЭКСПЕРИМЕНТАЛЬНЫЙ ПЕРИТОНИТ: МИКРОЭКОЛОГИЧЕСКИЕ ПОКАЗАТЕЛИ, ВИДОВОЙ СОСТАВ И ПОПУЛЯЦИОННЫЙ УРОВЕНЬ МИКРОБИОТЫ ПОЛОСТИ ТОЛСТОЙ КИШКИ ЭКСПЕРИМЕНТАЛЬНЫХ ЖИВОТНЫХ ЧЕРЕЗ 6 ЧАСОВ ИНИЦИАЦИИ

Л.И. Сидорчук

Резюме. Изучение качественного, количественного состава и микроэкологических показателей микрофлоры толстого кишечника белых крыс с острым экспериментальшения видового состава через 6 часов от начала заболевания и выраженный дефицит бифидо-, лактобактерий, пептострептококка на фоне роста количества условно-патогенных бактерий. Развитие острого экспериментального перитонита через 6 часов сопровождается формированием дисбактериоза полости толстого кишечника первой (30,0%), второй (60,0%) и четвертой (10,0%) степеней.

Ключевые слова: микробиота толстого кишечника, контаминация, эксперимент, острый перитонит.

ГОСТРИЙ ЕКСПЕРИМЕНТАЛЬНИЙ ПЕРИТОНІТ: МІКРОЕКОЛОГІЧНІ ПОКАЗНИКИ, ВИДОВИЙ СКЛАД І ПОПУЛЯЦІЙНИЙ РІВЕНЬ МІКРОБІОТИ ПОРОЖНИНИ ТОВСТОЇ КИШКИ ЕКСПЕРИМЕНТАЛЬНИХ ТВАРИН ЧЕРЕЗ 6 ГОДИН ІНІЦІАЦІЇ

Л.І.Сидорчук

Резюме. Вивчення якісного, кількісного складу та мікроекологічних показників мікрофлори товстої кишки білих щурів з гострим експериментальним перитонітом (ГЕП) встановило незначні порушення видового складу через 6 годин від початку захворювання та виражений дефіцит біфідо-, лактобактерій, пептострептококу на фоні зростання кількості умовно-патогенних бактерій. Розвиток гострого експериментального перитоніту через 6 годин супроводжується формуванням дисбактеріозу у порожнині товстої кишки першого (30,0%), другого (60,0%) та четвертого (10,0%) ступеня.

Ключові слова: мікробіота товстої кишки, контамінація, експеримент, гострий перитоніт.

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