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DYNAMICS OF MICROBIOTA CHANGES IN THE REGION OF INTESTINAL SUTURES UNDER EXPERIMENTAL CONDITIONS OF THEIR LEAKAGE

ДИНАМІКА ЗМІН МІКРОБІОТИ ЛІНІЇ ШВІВ КИШОК ЗА ЕКСПЕРИМЕНТАЛЬНИХ УМОВ ЇХ НЕСПРОМОЖНОСТІ

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Abstract. Chronological dynamics of changes of quantitative and qualitative composition of microflora in the intestinal sutures region has been researched on the anastomotic leaks model in 75 experimental rats. It has been established that an exponential increase of total number of microorganisms with the changes of population and typical composition of intestinal microflora occurs in the region of applied sutures under the conditions of their leakage. In 12-24 hours a MC index rising is mainly due to autochthonous flora, and after 3-5 days – by increasing the number of obligate anaerobes, along with decreasing of facultative ones concentrations.

Keywords: intestinal microbiota, anastomotic leaks, experiment.

Анотація. Хронологічна динаміка змін кількісного та якісного складу мікрофлори в ділянці кишкових швів досліджена на моделі їх неспроможності у 75 експериментальних щурів. Доведено, що в ділянці накладених швів за умов їх неспроможності відбувається експоненціальне зростання загальної кількості мікроорганізмів зі зміною їх популяційно-видового складу. У перші 12-24 год. зростання мікробного числа зумовлено переважно автохтонною мікрофлорою, а пізніше (через 3-5 діб) – за рахунок збільшення кількості облигатних анаеробів поряд із зменшенням концентрації факультативних анаеробів.

Ключові слова: кишкова мікрофлора, неспроможність швів, експеримент.

Introduction. Anastomotic leak (AL) is a threatening complication in abdominal surgery. Despite the improvement of surgical techniques and active introduction of modern methods of surgical treatment, the incidence of this complication ranges from 1,5 to 32% of cases depending on the urgency, site and technical conditions of surgical operations, the immune status and comorbidity of patients [2, 3, 5]. Most unfavorable outcomes registered in colorectal surgery, and in cases of widespread postoperative peritonitis the mortality rate is up to 50% [6].

A microbial contamination of the sutured tissues of gastrointestinal tract is one of the most constant among the various conditions of this pathology [4]. Numerous methods of intestinal sutures protection including

through adhesive compositions and bioexplants are not always effective, especially in the site of edematous and inflamed tissues of the intestinal walls [8]. Biological leakage in site of stitches caused by the translocation of intestinal microflora is often a trigger factor for the formation of the mechanical anastomotic leak, particularly in the distal colon. It is reasonable that a particular attention is devoted to research the changes of colonization resistance of intestinal wall in current publications on the issues to improve the prevention of intra-peritoneal infectious complications after operations on hollow organs of the digestive system [1, 10]. A known role of intestinal flora in causing the biological anastomotic leakage allows to improve and elaborate new curative methods both inside and outside the

line of suturing [9]. Despite this some patterns of microbiota changes on the surface of the sutured intestinal tissues during all process of anastomosis healing remain obscure. Traditional selective decontamination of intestine are not always conducive to the achievement of expected results that manifests the formation of separate intra-abdominal fluid accumulation, fistulas or adhesions in some patients after surgery on the organs of the gastrointestinal tract [1, 8]. An irrational antibiotic prophylaxis of postoperative septic complications can lead to disturbances of normal intestinal microbiota, colonization the proximal part by the allochthonous microflora that in the conditions of postoperative immunosuppression may induce the uncontrolled growth of pathogenic and opportunistic antibiotic-resistant strains with the following initiation the enterogenous component of endotoxemia [7]. So, chronological research of local changes of microbiota in anastomotic area of intestine is relevant today.

The aim of research: to find out patterns of changes of quantitative and qualitative composition of microflora in the region of applied intestinal sutures under the experimental conditions of their leakage.

Material and methods. The investigation has been carried out on 75 albino nonlinear male rats, weighting 180±20g. All the animals underwent a resection of caecum with suturing the defect of intestinal wall

by means of interrupted stitches (vicryl 5-0). AL was modelled by way of excessive mobilization of the area of junction and a rare application of stitches in the animals of the experimental group. In 12, 24, 48, 72 hours and 5 days following a surgical interference an euthanasia of the animals was performed under ether anesthesia. In aseptic conditions the samples of smears from line of the sutures were taken for bacteriological study. Indices of microbial count (MC) and numbers of the most significant types of intestinal microflora such as *E. coli*, *Enterococcus* spp., *Bacteroides* spp., *Clostridium* spp. were determined in concentrations equal to Lg CFU/ml. Statistical processing of results of the investigation was performed on PC both the Mann-Whitney's t-test and Pearson's r-coefficient calculating by means of app. "Primer of Biostatistics" (4th ed.) and "Analysis ToolPak" of app. MS® Excell. To reject the null hypothesis the significance level was used equal to p<0,05. The experiments were carried out in compliance with the European convention as to the protection of vertebrate animals (Strasbourg, 1986) and "General Principles of animal experiments" approved by 4th National Congress on Bioethics (Kyiv, 2010).

Results of the research and its discussion. Results of investigation are presented in the table.

Table
Quantitative and population-typical composition of microflora (Lg CFU/ml) in the region of suture line of caecum rats (x±Sx)

Periods	Group	<i>E.coli</i>	<i>Enterococcus</i> spp.	<i>Bacteroides</i> spp.	<i>Clostridium</i> spp.	Microbial count
12 h.	C (n=5)	3,73±0,078	3,10±0,059	0*	0*	3,84±0,056
	E (n=10)	4,53±0,053 P<0,001 r=0,98	3,45±0,093 P<0,05 r=0,18	0*	0*	4,57±0,048 P<0,001
24 h.	C (n=5)	4,04±0,036	3,82±0,047	0*	0*	4,24±0,039
	E (n=10)	4,84±0,059 P<0,001 r=0,97	3,77±0,139 r=-0,3	2,07±0,589 r=0,29	0*	4,92±0,035 P<0,001
48 h.	C (n=5)	3,67±0,081	2,46±0,438	0*	0*	3,76±0,073
	E (n=10)	,62±0,0401 P<0,001 r=0,97	3,62±0,612 P<0,001 r=-0,28	3,01±0,66 r=0,37	0*	5,68±0,032 P<0,001
72 h.	C (n=5)	3,05±0,021	2,58±0,087	0*	0*	3,19±0,015
	E (n=10)	5,79±0,12 P<0,001 r=0,32	4,67±0,795 P<0,001 r=-0,08	5,00±0,839 r=-0,38	0*	6,55±0,037 P<0,001
5 days	C (n=5)	0*	0*	0*	0*	0*
	E (n=10)	5,06±0,062 r=-0,49	2,80±0,934 r=-0,53	6,47±0,143 r=0,39	6,21±0,692 r=0,41	7,10±0,038

Notes: C – control group; E – experimental group;
0* – concentration of microorganisms less than 2 Lg CFU/ml;
P – degree of evidence of difference between control and experimental data;
r – coefficient of group correlation between MC and concentration of certain types of microorganisms.

It has been established that the MC indices were reliable higher (p<0,001) in the animals of experimental group as compared with the control one during entire period of the observation. Etiologically significant concentrations of microorganisms (>5 Lg CFU/ml) were detected in the sutured tissues of intestine in the animals with AL since 24 h. after surgery with increasing of their total number up to 7,10±0,038 Lg CFU/ml until 5th day. In the animals of control group the highest indices of MC (4,24±0,039 Lg CFU/ml) have been registered in 24 h. after the sutures were applied, however it was reliably lower than minimal infected dose. Only trace concentrations (<2 Lg CFU/ml) of microorganisms remained on surface of the junction area in animals this group

until period of observation was finished.

An analysis of a chronological dynamics of MC changes in the junction zone of intestinal wall shows the increase of this index in the animals with the AL as to an exponential relation (fig.). Along with this, a reliable tendency to reduce microbial contamination of the suture line was detected in the animals of control group since 48 h. till the end of period of observation.

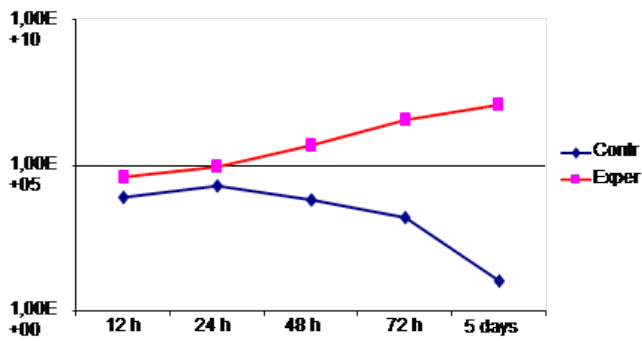


Fig. Comparative dynamics of microbial count changes in the region of suture line of caecum rats (Lg KYO/ml)

When analysing the dynamics of changes of the population structure of microorganisms it has been established that in 12 h. following the ISI was modeled a rising of MC index in the region of sutures applied is mainly due to increasing the number of *E. coli* ($r = 0,98$). This testifies to the manifestation of biological leakage due to translocation of intestinal microflora. In 24 h. a powerful positive relationship was detected between the increasing MC and the number *E. coli* ($r=0,97$) and a moderate negative one – between the index MC and the number *Enterococcus spp.* ($r=-0,37$). In 48 h. increasing MC was correlated with increasing the numbers *E. coli* ($r=0,97$) and *Bacteroides spp.* ($r=0,37$). In 72 h. a moderate positive relationship was detected between the

index MC and the number *E. coli* ($r=0,32$) and negative one – to the number *Bacteroides spp.* ($r=-0,38$). In 5 days following the AL was modeled, an increasing of index MC was in a moderate positive relationship with the numbers *Bacteroides spp.* ($r=0,39$) and *Clostridium spp.* ($r=0,41$) and in a negative one with the numbers *E. coli* ($r=-0,49$) and *Enterococcus spp.* ($r=-0,53$). Such dynamics of changes of qualitative and quantitative composition of microflora in the junction region of intestine testify about the disturbances of colonization resistance of intestinal wall under the conditions of AL development.

Conclusions: 1. An exponential increasing of total quantity of microorganisms with the specific chronologically dependent changes of the population structure of intestinal microflora occurs in the sutured tissues of intestine under the experimental conditions of anastomotic leakage. 2. In the early terms (12-24 hrs. following the sutures application) an increase the microbial count above the etiologically significant concentrations (>5 Lg CFU/ml) is mainly caused by the number of an autochthonous microflora (*E. coli*, $r=0,97$). 3. At a later stage (after 3-5 days of the anastomotic leakage initiation) the changes of microbial contamination of junction zone correspond to increased number of obligate anaerobes (*Bacteroides spp.*, $r=0,39$ and *Clostridium spp.*, $r=0,41$) along with the decreasing of concentration of facultative anaerobic microorganisms (*E. coli*, $r=-0,49$ and *Enterococcus spp.*, $r=-0,53$).

Prospects of scientific research. We consider it expedient to study correlations between the fibrinolytic activity of intestinal tissues and their microbial contamination in the region of applied stitches in case of their leakage.

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