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## INDICATORS OF BACTERIAL TRANSLOCATION INTENSITY IN EXPERIMENTAL ACUTE OBSTACLES OF THIN AND THICK INTESTINE

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### ABSTRACT

*The aim was to study the germination of microorganisms in the mesenteric lymph nodes, liver, spleen, lungs, peripheral and portal blood, peritoneal exudate to assess the intensity of bacterial translocation (BT). It was established that in experimental acute obstruction of the small and large intestines, the intensity of BT or the percentage of germination of microorganisms from the extraintestinal organs of animals was most pronounced in mesenteric lymph nodes and the liver. The intensity of BT was directly proportional to the duration of the experiment.*

**KEYWORDS:** *Bacterial Translocation, Microorganisms, Extra-Intestinal Organs, Experimental Studies.*

### INTRODUCTION

Bacterial translocation (BT) is the passage of bacteria through the mucous membrane of the gastrointestinal tract into the extraintestinal parts of the body [8].

The “BT phenomenon” is quite common [1, 5, 9]. Currently, this phenomenon is interpreted in two ways: supporters of the first believe that BT develops under the influence of stress, injuries or other external extreme influences and with a decrease in the activity of the body's immune system, while it is a pathogenetic link in some diseases; supporters of the second believe that BT is not only the transfer of pathogens of endogenous infections into the internal environment of the body, but also is a natural protective mechanism of the body [4, 6, 10].

It is known that most of the normal microflora are capable of translocation of *Escherichia coli*, *Proteus* spp., some other representatives of the Enterobacteriaceae family, transient strains of *Bacillus subtilis*, gram-positive aerobes, the ability to translocate obligate anaerobes is low [1, 7, 11].

Justification of the relevance and relevance of these studies shows that numerous scientific works are devoted to the clinical, pathogenetic, and diagnostic aspects of the problem, but studies related to the microbiological aspects of BT formation and their place in the development of endogenous infections have not been carried out sufficiently. In this regard, conducting experimental microbiological studies to solve this problem are relevant.

Purpose of the study. Studying and assessing the germination of microorganisms from mesenteric lymph nodes (MLN), liver, spleen, lungs, peripheral and portal blood, peritoneal exudate in the dynamics of the experiment to assess the intensity of BT in experimental acute obstruction of the small and large intestines.

## **MATERIALS AND METHODS**

When choosing an experimental material, the basis was the numerous studies on experimental microbiology, the convenience of working with it, cheapness and the high possibility of achieving the purity of the experiment in a methodological aspect. When working strictly observed all the ethical principles of working with experimental animals and the rules of biological safety.

For research, 240 white mongrel mice were used at the age of 2-3 months and weighing 18-25 g.

Before the experiments, all animals were divided into groups, then they were weighed for 3 days and thermometry was performed. During these days, a decrease in body weight and an increase in body temperature were not detected.

Identification and differentiation of seeded microorganisms was carried out by traditional bacteriological methods. For this, nutrient media of HiMedia firm (India) were used.

The results are processed by traditional methods of variation statistics. All studies were conducted on personal computers using the package of programs for biomedical research. The organization and conduct of research is based on the principles of evidence-based medicine.

### **The results obtained and their discussion**

In carrying out the studies, models of experimental acute obstruction of the small intestine (EAOSI) and large intestine (EAOLI) proposed by Kruglyanskiy Yu.M. [3] in our modification. Conducted 3 series of studies.

All laboratory animals are divided into 4 groups: 1 group - EAOSI, n = 72; Group 2 - EAOLI, n = 72; Group 3 - animals in which the abdominal cavity was opened, but did not perform obstruction (comparison group, n = 72); Group 4 - intact laboratory animals (control group, n = 24).

In turn, 1, 2, 3 groups were divided into subgroups: 1a, 2a and 3a - EAOSI and EAOLI lasting 24 hours (n = 8 each); 1b, 2b and 3b - EAOSI and EAOLI lasting 48 hours (n = 8 each); 1c, 2c and 3c - EAOSI and EAOLI lasting 72 hours (n = 8 each).

Considering the fact that during these periods the main clinical, pathological and morphological changes in the intestinal walls associated with obstruction are observed [2, 3], we chose these particular periods of the study.

Following aseptic rules, the abdominal cavity was opened with a sterile scalpel. For the formation of EAOSI, a ligature was performed along the edges of the ileum bridge, while they tried not to involve the breach into the pathological process. After the ligature, a purse string suture was applied and pulled to create an obturation. After this, the abdominal cavity was sutured with a surgical needle.

The same measures were taken to form EAOLI, but in contrast to EAOSI, obstruction was performed on the distal part of the large intestine.

The laboratory animals of the third group (comparison group) opened the abdominal cavity and sutured without applying a ligature to the small and large intestines.

In the control group (fourth group), surgery was not performed.

When opening the corpses of animals, precautionary measures were strictly observed to prevent the introduction of microorganisms from the surface into the depth of the tissue, as well as their transfer from one organ to another. Using sterilized instruments, the skin and subcutaneous tissue were opened, biological material was taken first from the chest organs (lungs), then from the abdominal organs (MLN, liver, spleen). However, with the help of a syringe, blood was taken from the portal vein (portal blood) and the abdominal aorta (peripheral blood), as well as peritoneal exudate from the abdominal cavity. To take the material from the organs of the laboratory animal, they were first cauterized, then they were cut using sterile scissors and grabbing a piece of the organ with tweezers they made an imprint on the surface of sterile pipette media.

Given that normally all extraintestinal organs of laboratory animals are sterile, the growth of any microorganism on the surface of culture media was evaluated as a bacterial translocation.

It was found that with EAOSI and EAOLI, the BT intensity was different depending on the duration of the experiment and its type.

We have identified the following microorganisms that are representatives of the normal intestinal microflora - *Escherichia* spp, *Enterobacter* spp, *Citrobacter* spp, *Klebsiella* spp, *Proteus* spp, *Staphylococcus* spp, *Enterococcus* spp, *Bacteroides* spp.

The sowing rate of these microorganisms is described by our proposed microbiological criterion that determines the intensity of BT - the percentage germination of microorganisms (PGM).

Studies have found that after EAOSI after a 24-hour period, the PGM for MLN was  $45.8 \pm 5.9\%$  ( $n = 33$ ). This indicator increased to  $91.7 \pm 3.3\%$  after 48 hours ( $n = 66$ ), and after 72 hours this parameter was 100% ( $n = 72$ ). The difference between the periods was significant ( $P < 0.05$ ).

The liver PGM index differed from the same MLN parameters, so if after 24 hours microorganisms from the liver were sown in  $29.2 \pm 5.4\%$  ( $n = 21$ ) cases, then after 48 and 72 hours these parameters were increased - to 56, respectively.  $9 \pm 5.8\%$  ( $n = 41$ ) and  $81.9 \pm 4.5\%$  ( $n = 59$ ). When comparing with the results of a 24-hour period, the degree of reliability, respectively, was  $P < 0.02$  and  $P < 0.001$ .

PGM from the spleen of animals sharply differed from the indicators of the previous organs described. If microorganisms were not identified 24 hours after the start of the experiment, then after 48 and 72 hours these indicators were  $29.2 \pm 5.4\%$  ( $n = 21$ ) and  $31.9 \pm 5.5\%$  ( $n = 23$ ), respectively .

A distinctive feature of the plating of microorganisms from the lung parenchyma was that the PGM was several times significantly low compared to other described organs. After the formation of EAOSI after 24 hours, the growth of microorganisms from lung tissue was not observed, while PGM after 48 and 72 hours was  $9.7 \pm 3.5\%$  ( $n = 7$ ) and  $15.3 \pm 4.2\%$  ( $n = eleven$ ). When studying the indicators of the comparison and control groups, positive bacteriological indicators were not obtained.

At the next stage of the studies, the intensity of BT on the extraintestinal organs of animals was studied at various times with EAOLI.

It was found that in subgroup 2a (EAOLI after 24 hours) the PGM in MNL was at the level of EAOSI -  $41.7 \pm 5.8\%$  ( $n = 30$ ) versus  $45.8 \pm 5.9\%$  ( $P > 0.05$ ) . But, 48 hours revealed significant differences between these parameters -  $59.7 \pm 5.8\%$  ( $n = 43$ ) versus  $91.7 \pm 3.3\%$ , ( $n = 66$ ) -  $P < 0.001$ . Results after 72 hours were identical for EAOSI and EAOLI.

The results of studies on the liver showed the following results: PGM after 24 hours  $18.1 \pm 4.5\%$  ( $n = 13$ ), after 48 hours  $51.3 \pm 5.9\%$  ( $n = 37$ ) and after 72 hours  $80.6 \pm 4.7\%$  ( $n = 58$ ). After 24 hours in the liver with EAOLI, PGM is 1.6 times reliably low compared with EAOSI, but after 48 hours there were no significant differences between the indicators ( $P < 0.05$ ).

The obtained results on PGM from the spleen differed from the results on MNL and liver. So after 24 hours, cultures from the spleen gave a negative bacteriological result, but after 48 hours, the growth of microorganisms was noted, where the PGM was  $19.4 \pm 4.7\%$  ( $n = 14$ ), after 72 hours the PGM was increased by 1.9 times compared with the previous result,  $37.5 \pm 5.7\%$  ( $n = 27$ ) -  $P < 0.001$ .

The trend of changes in the results of studies on lung tissue were similar to the data of PGM of the spleen. If it was not possible to identify microorganisms after 24 hours (0%), then after 48 hours this indicator was  $16.7 \pm 4.4\%$  ( $n = 12$ ), and after 72 hours the PGM significantly increased 2.2 times ( $P < 0.001$ ) compared with the previous indicator -  $36.1 \pm 5.7\%$  ( $n = 26$ ).

In the spleen, in all periods of the experiment, there were no statistically significant differences between the indicators, but the lung PGM parameters after 72 hours significantly differed 2.4 times between these models. As in studies with EAOSI with EAOLI, no growth of microorganisms was found in the comparison and control groups.

The next stage of the study was the study of PGM of portal, peripheral blood and peritoneal exudate in the same animals.

The results show that with EAOSI after 24 hours in the portal blood, the PGM was  $33.3 \pm 5.6\%$  ( $n = 24$ ), and with EAOLI this indicator was  $15.3 \pm 5.6\%$  ( $n = 11$ ), which significantly lower than the previous indicator. But, after 48 hours, these parameters significantly increased in relation to the previous indicators -  $56.9 \pm 5.8\%$  ( $n = 41$ ) and  $37.5 \pm 5.7\%$  ( $n = 27$ ), respectively,  $P < 0.001$ . In addition, significant differences also persisted ( $P < 0.001$ ). When studying the data of the next experimental period (72 hours) with EAOSI and EAOLI PGM was found in all

animals - 100%, respectively (n = 72). It was found that these indices were 3.0 and 6.5 times, respectively, significantly higher than the indices of the 24-hour period of the experiment, and also, respectively, 1.8 and 2.7 times reliably more than the indices of the 48-hour experiment ( $P < 0.001$ )

With these models and the timing of the experiment, microbiological studies were performed with the peripheral blood of animals. The results show that in both models after 24 hours it was not possible to identify microorganisms. But with an increase in the duration of the experiment (48 hours), the growth of microorganisms was noted. The PGM indicators in both models were  $19.4 \pm 4.7\%$  (n = 14) and  $25.0 \pm 5.1\%$  (n = 18), respectively. The results obtained through the 72-hour experiment were somewhat different from the previous period. With EAOSI, the result was not statistically different from the previous period ( $23.6 \pm 5.0\%$ , n = 17), but with EAOLI the percentage of positive bacteriological samples was 1.9 times significantly higher ( $P < 0.001$ ) than with 48 hourly experiment -  $47.2 \pm 5.9\%$  (n = 34).

The PVM parameters of peritoneal fluid differed sharply from the parameters of peripheral blood, but were close to portal blood data. The research results depending on the duration of the experiment (24, 48, 72 hours) were as follows: with EAOSI, respectively -  $48.6 \pm 5.9\%$  (n = 35),  $65.2 \pm 5.6\%$  (n = 47) and  $94.4 \pm 2.7\%$  (n = 68); EAOLI, respectively -  $34.7 \pm 5.6\%$  (n = 25),  $58.3 \pm 5.8\%$  (n = 42) and  $97.2 \pm 1.9\%$  (n = 70).

It should be emphasized that in both models only with a 24-hour period there were significant differences between the figures obtained ( $P < 0.05$ ), with other periods the indicators did not significantly differ from each other ( $P > 0.05$ ).

Negative bacteriological results were obtained in the comparison and control groups during microbiological studies of portal and peripheral blood. But, in the comparison group, microorganisms were seeded from the peritoneal fluid after 48 and 72 hours, the PGM was equal to  $2.8 \pm 1.9\%$  (n = 2) and  $4.2 \pm 2.4\%$  (n = 3), respectively. The control group data were identical with other biological samples.

## CONCLUSIONS

1. With EAOSI and EAOLI, the intensities of BT or PGM from the extraintestinal organs of laboratory animals at different times of the experiment differed.
2. The intensity of BT was most pronounced in MLN and liver than in the spleen and lungs. The intensity of this phenomenon was directly proportional to the duration of the experiment.
3. PGM from MLN and liver are recommended as an experimental microbiological criterion for assessing the intensity of bacterial translocation in an experiment.

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