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*S. G. Znamerovsky, I. V. Savytskyi, O. F. Dzygal*

## **DYNAMICS OF BIOCHEMICAL MARKERS IN THE PATHOGENESIS OF EXPERIMENTAL BILE PERTONITIS**

Odessa National Medical University, Odessa, Ukraine

**Summary.** Znamerovsky S. G., Savytskyi I. V., Dzygal O. F. **DYNAMICS OF BIOCHEMICAL MARKERS IN THE PATHOGENESIS OF EXPERIMENTAL BILE PERTONITIS.** - *Odessa National Medical University*. Biliary peritonitis (BP), which is a severe disease of the abdominal cavity in severity, prognosis and mortality rate depends on endogenous intoxication Systemic Inflammatory Reaction Syndrome (SIRS) is considered in surgical practice as a non-specific response to injury. The study was performed on 180 Wistar rats weighing 180-200 g. The animals were divided into 4 groups. As a result of the conducted researches it is proved that the level of leukocytes is an important diagnostic test in the pathogenesis of experimental peritonitis. When studying the dynamics of leukocyte levels on the first and third days of the experiment, its increase is noted against the background of experimental biliary peritonitis. The results of the 4th group indicate a decrease in this indicator against the background of the proposed method of remediation and are closer to the values of the norm in comparison with the 3rd group. On the 7th day of the experiment, data on the normalization of this marker in the 4th group were obtained.

**Key words:** biliary peritonitis, model, sanitation of abdominal cavity, leukocytes, correction.

**Реферат.** Знамеровский С. Г., Савицкий И. В., Дзигал А. Ф. **ДИНАМИКА БИОХИМИЧЕСКИХ МАРКЕРОВ В ПАТОГЕНЕЗЕ ЭКСПЕРИМЕНТАЛЬНОГО ЖЕЛЧНОГО ПЕРИТОНИТА.** – *Одесский национальный медицинский университет*. Желчный перитонит (ЖП) - тяжелое заболевание брюшной полости, исход которого зависит от степени эндогенной интоксикации. Синдром системной воспалительной реакции (ССЗР) в хирургической практике рассматривается как неспецифический ответ организма на повреждение. Исследование выполнено на 180 крысах линии Вистар весом 180-200 г.

Животные были разделены на 4 группы. Доказано, что уровень лейкоцитов является важным диагностическим тестом в патогенезе экспериментального перитонита. При исследовании динамики уровня лейкоцитов на 1 и 3 сутки эксперимента, отмечается ее повышение на фоне экспериментального желчного перитонита. Результаты 4 - й группы свидетельствуют о снижении данного показателя на фоне предложенного способа санации и больше приближаются к значениям нормы по сравнению с третьим группой. На 7-е сутки эксперимента были получены данные о нормализации данного маркера в 4-й группе.

**Ключевые слова:** желчный перитонит, модель, санация брюшной полости, лейкоциты, коррекция.

**Реферат. ДИНАМІКА БІОХІМІЧНИХ МАРКЕРІВ У ПАТОГЕНЕЗІ ЕКСПЕРИМЕНТАЛЬНОГО ЖОВЧНОГО ПЕРИТОНІТУ.** Жовчний перитоніт (ЖП), який є важким захворюванням черевної порожнини по ступеню важкості, прогнозу і відсотку летальності, залежить від ендогенної інтоксикації. Синдром системної запальної реакції (ССЗР) у хірургічній практиці розглядається як неспецифічна відповідь організму на пошкодження. Дослідження виконано на 180 щурах лінії Вістар вагою 180-200 г. Тварини були розподілені на 4 групи. В результаті проведених досліджень доведено, що рівень лейкоцитів є важливим діагностичним тестом у патогенезі експериментального перитоніту. При дослідженні динаміки рівнюлейкоцитів на першу та третю добу експерименту відмічається його підвищення на фоні експериментального жовчного перитоніту. Результати 4-ї групи свідчать про зниження даного показника на фоні запропонованого способу санатії і більше наближаються до значень норми в порівнянні з 3-ю групою. На 7-му добу експерименту були отримані дані про нормалізацію даного маркера у 4-й групі.

**Ключові слова:** жовчний перитоніт, модель, санатія черевної порожнини, лейкоцити, корекція.

**Introduction.** Bile peritonitis (BP), which is a severe abdominal disease in terms of severity, prognosis and mortality rate depends on endogenous intoxication [1]. Therefore, an important element of comprehensive treatment of biliary peritonitis is the effective rehabilitation of the abdominal cavity [2-4].

One of the effective ways to treat peritonitis, as noted, is the method of indirect electrochemical oxidation. Its use allows to reproduce the detoxification function of cytochrome P-450 of liver hepatocytes and bactericidal function of the enzyme myeloperoxidase of neutrophilic granulocytes [5]. Decamethoxine has also proven to be an effective means of detoxification therapy [6].

A number of studies have proven the effectiveness of hyaluronic acid as a prevention of adhesive disease [7]. Based on the above, our study was based on the task to analyze the effectiveness of the complex effect of sodium hypochlorite and decamethoxine in combination with hyaluronic acid on the pathogenetic links of experimental biliary peritonitis.

**The aim:** to determine the main pathophysiological mechanisms of changes in the number of leukocytes in experimental biliary peritonitis and pathogenetic substantiation of methods of correction of the pathology under study.

**Materials and methods:** The study was conducted on 180 Wistar rats, the average weight of which was 180-200 grams.

The animals were divided into 4 groups:

1st group - intact (20 animals).

2nd group - rats, which simulated biliary peritonitis without further correction (80 animals).

In the 3rd group rats correction of simulated biliary peritonitis was performed by rehabilitating the abdominal cavity with a solution of chlorhexidine bigluconate (0.02% solution), followed by the use of standard antibiotic therapy (40 animals).

In the 4<sup>th</sup> group rats simulated biliary peritonitis was corrected using a combined scheme of rehabilitation of the abdominal cavity.

1st remediation - 0.04% solution of sodium hypochlorite, 12 hours after the second injection of bile (Tereshchenko O. A. et al., 2001).

2nd remediation - a mixture consisting of a combination of decamethoxine (10 mg / 50 ml solution), sodium hyaluronate (250 mg / 50 ml solution) and succinate buffer - 6 hours after the

first remediation (40 animals).

Bile peritonitis was modeled according to E. A. Petrosyan's and V. I. Sergienko's scheme (2001): animals were injected intramuscularly with a sterile 10% solution of calcium chloride (1.0 ml / 100 g of animal weight), which created a focus of aseptic inflammation. Then, after 72 hours, a layered incision of soft tissues 2.5 cm long was performed under local infiltrative anesthesia (using 20 ml of 0.25% solution of novocaine) from the xiphoid process of the sternum along the white line of the abdomen. The rectus muscles were pulled to the sides. A puncture of the abdominal cavity was performed with a thick blunt needle, through which a conductor was inserted. Then the needle was removed and a catheter 8 cm long was inserted into the abdominal cavity through a conductor. 0.33 ml / 100 g of animal weight with an interval of 12 hours was injected twice intrauterinely through the catheter [8]. After the first injection, a rubber stopper was put on the catheter pavilion and the edges of the wound were sutured. The next injection was performed through a catheter, piercing the plug with a needle. It is known that eight hours after the first intraperitoneal injection of bile, the simulated peritonitis is aseptic. After 24 hours, it becomes bacterial, due to the translocation of microorganisms from the intestinal lumen into the abdominal cavity due to damage to the mucous membrane of the intestinal wall caused by hypoxia [9].

Twenty-four hours after the start of biliary peritonitis simulation, the catheter was removed. Anesthetized animals of groups 3 and 4 underwent upper median laparotomy and revision of the abdominal cavity. Inflammatory exudate was removed with an electroaspirator. Carried out the first rehabilitation of the abdominal cavity (in the 3rd group - chlorhexidine bigluconate 0.02% solution), in the 4th - 0.04% sodium hypochlorite solution). Then the wound was drained and sutured in layers (Povylyaeva T. L., 2004).

Animals of the fourth group underwent a two-stage remediation. The second step was performed by administering a solution consisting of decamethoxine and sodium hyaluronate. Sanitized as follows: in the ileocecal corner of the abdominal cavity was injected 5 ml of gel antiseptic solution (Feleshtynsky J. P., Demkovich O. P., Smishchuk V. V., Trilis O. L., 2018). Animals were anesthetized with sodium thiopental during the manipulations.

Blood sampling from the caudal vein was performed at the end of the 1st, 3rd and 7th days of modeling the BP.

The research was conducted in accordance with the "Rules of performance of works using experimental animals", approved by the Order of the Ministry of Health of Ukraine № 249 from 01.03.2012 and the Law of Ukraine № 3447-IV "On protection of animals from cruel treatment" (amended 15.12.2009 and 16.10.2012).

Blood sampling from the caudal vein was performed at the end of the 1st, 3rd and 7th days of modeling the BP.

Determination of the level of leukocytes during a general blood test was performed using an automated hematology analyzer BC-2800Vet (PRC) using MINDRAY (South Korea) reagents.

The following indicators and methods in the package of statistical analysis SPSS 19.0 were used as mathematical and statistical methods of processing the results.

Before using parametric, based on the normality of the statistical distribution, methods were used to verify the studied series of quantitative data for normality using the Shapiro-Wilk's test (Shapiro-Wilk's W test). Ensuring that the distribution of data in the samples did not differ from normal, used the parametric Student's t test with Bonferroni correction.

### **Research results and their discussion**

It is known from the literature that the pool of leukocytes is characterized by a strong autoaggressive potential [10]. In this regard, when decompensating buffer systems of anti-inflammatory resistance and reducing the functional reserves of organs and systems of the body, the inflammatory process goes beyond the protective reaction and inflammation leads to the development of systemic damage [10, 11].

A number of scientists note that the leukocyte formula is characterized by entropy [12, 13], which is considered as an integral characteristic of the leukocyte ratio [10].

The data obtained during our study confirm the information [10, 14] on the increase in the level of leukocytes on the background of simulated biliary peritonitis (Fig. 1-3). This is due to the fact that the systemic nature of the inflammatory process, which is associated with the development of peritonitis, leads to the mobilization of bone marrow reserve and parietal pool of peripheral blood neutrophils in response to endogenous intoxication [10].

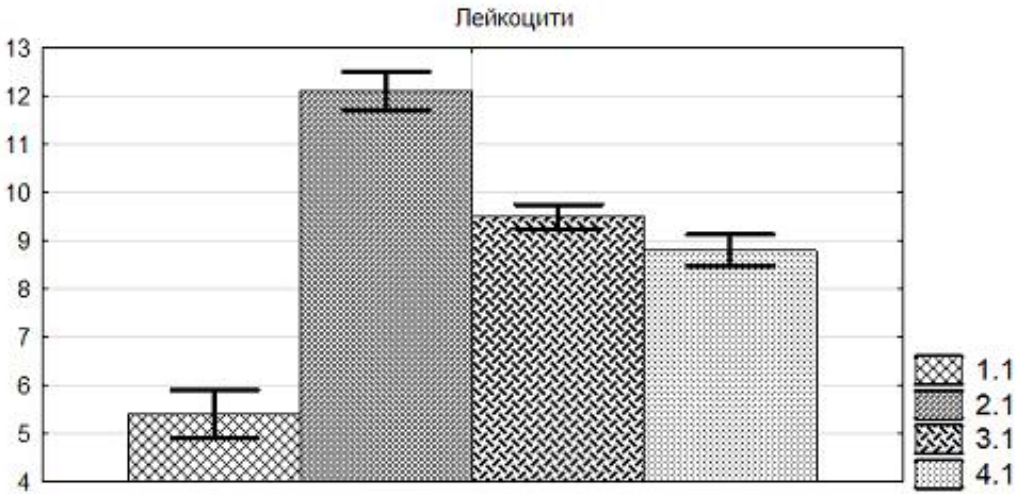


Fig.1. Leukocyte dynamics in experimental biliary peritonitis and its correction on the 1st day of the study.

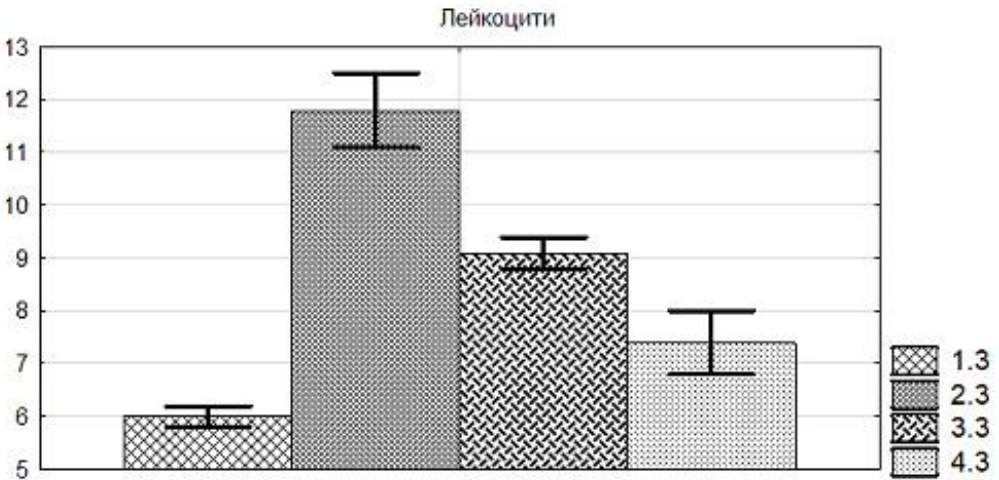


Fig.2. Leukocyte dynamics in experimental biliary peritonitis and its correction on the 3rd day of the study

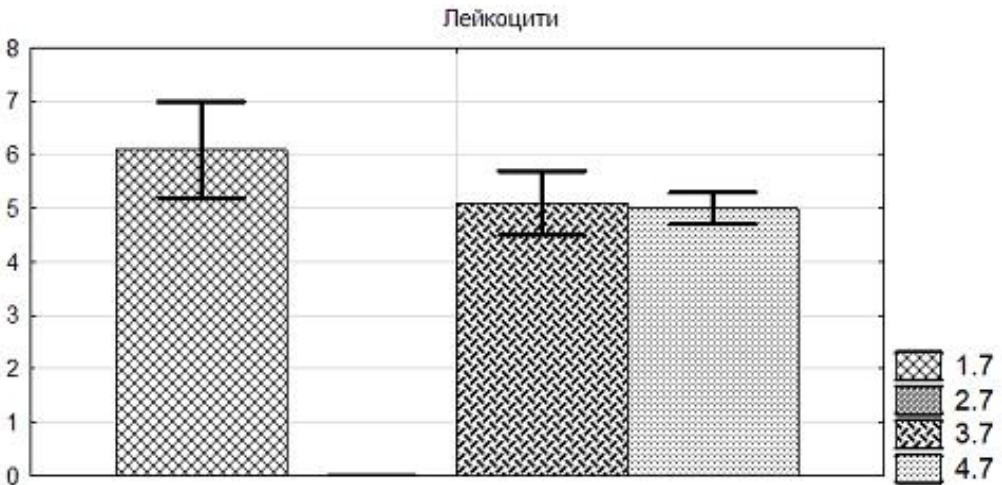


Fig.3. Leukocyte dynamics in experimental biliary peritonitis and its correction on the 7th day of the study

It is also noted that the increase in white blood cell count is a reflection of the severity of the inflammatory process. The combination of high LII and leukocytosis indicates the intensity of compensatory detoxification mechanisms [10, 14].

Systemic inflammatory reaction syndrome (SIRS), which underlies the development of peritonitis [15, 16], is associated with the spread of the inflammatory process from the primary focus of localization, entry into the bloodstream of proinflammatory mediators, impaired permeability of cell membranes and the development of multiorgan failure [14]. It is noted that the list of parameters on which the diagnosis of SIRSs is based, includes a change in the number of blood leukocytes and an increase in the content of rod-shaped forms [17].

It is also indicated that the main factor that leads to an increase in the leukocyte intoxication index is the relative content of rod-shaped neutrophils [8].

The results obtained during the experimental study indicate a significant increase in the number of leukocytes on the first day in all groups with simulated biliary peritonitis. At the same time already at this stage the positive influence of correction of the investigated pathology is shown. In the third and fourth groups, the level of the studied indicator is lower than in the group in which the BP was not adjusted. It is noteworthy that the use of sodium hypochlorite, decamethoxine and hyaluronic acid has a more pronounced positive effect: in group 4 (correction of pathology in which this method was performed) the least pronounced manifestations of systemic inflammatory reaction (Fig. 1).

On the third day of the study in the second group, whose animals were not corrected simulated pathology, found a more pronounced increase in the number of leukocytes, which indicates a critical activation of the inflammatory process. As for the third and fourth groups, the trend detected on the first day remains. The data obtained from the analysis of the number of leukocytes indicate that the two-stage rehabilitation proposed by us is more effective in comparison with antibiotic therapy and rehabilitation with chlorhexidine (Fig. 2).

Rats of the second group did not live to the seventh day of the experiment. The results of the number of leukocytes of the third group at this stage of the study are close to those of the fourth group, but the effectiveness of correction in the 4th remains more pronounced (Fig. 3).

#### **Conclusions:**

1. As a result of the researches conducted it is proved that the level of leukocytes is an important diagnostic test in the pathogenesis of experimental peritonitis.
2. When studying the dynamics of leukocyte levels on the first and third days of the experiment, its increase is noted against the background of experimental biliary peritonitis.
3. The results of the 4th group indicate a decrease in this indicator against the background of the proposed method of remediation and are closer to the values of the norm in comparison with the 3rd group.
4. On the 7th day of the experiment, data on the normalization of this marker in the 4th group were obtained.

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