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ABSTRACT

of the dissertation for the degree of Doctor of Philosophy

ROLE OF MULTICOMPONENT SOLUTION IN THE PREVENTION OF ADHESION DISEASE AFTER ABDOMINAL OPERATIONS

Specialty: 3213.01 – Surgery

Field of science: Medicine

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The work was performed at Scientific Center of Surgery PLE named after acad. M.A. Topchubashev, at the Main Clinical Hospital of the Ministry of Defense of the Republic of Azerbaijan and at the Scientific Research Center of the Azerbaijan Medical University.

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GENERAL DESCRIPTION OF WORK

The relevance of the topic and the degree of its development.

Postoperative intra-abdominal adhesions may occur in 90-95% of patients undergoing abdominal surgery[1]. They cause complications such as intestinal obstruction, severe chronic pain, and organ dysfunction, increasing the risk of repeated operations, including operations to remove the adhesions themselves. Surgical adhesions during repeated operations increase the risk of bleeding, perforation, and also contribute to an increase in the duration of the operation[2].

Postoperative adhesions can range from thin films of connective tissue to thick fibrous bridges that are vascularized and innervated[3]. When adhesions form, inflammation is the initial reaction to peritoneal damage and plays an important role in the formation of intra-abdominal adhesions. It has been found that inhibition of inflammation caused by surgical trauma can prevent the occurrence of abdominal adhesions[4].

Surgical adhesions are pathological fibrous connections that form between the surfaces of organs and the walls of hollow organs after tissue trauma and ischemia. The formation of intra-abdominal adhesions is caused by exudation of effusion and fibrin deposition due to inflammation in the damaged peritoneum, therefore, a decrease in

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[2] Foster, D.S. Elucidating the fundamental fibrotic processes driving abdominal adhesion formation / D.S.Foster, C.D.Marshall, G.S.Gulati [et al] // Nat Commun, - 2020. 11, - 4061

[3] Şahin, H. Effects of sugammadex on the prevention of postoperative peritoneal adhesions / H.Şahin, H.Toman, H.A.Kiraz [et al.] // Kaohsiung J Med Sci, - 2015. 31(9), - p. 463–467.

[4] Wei, G. Effect of Resveratrol on the Prevention of Intra-Abdominal Adhesion Formation in a Rat Model / G.Wei, X.Chen, G.Wang [et al.] // Cell Physiol Biochem, - 2016. 39, - p. 33-46.

the ability to break down fibrin at sites of injury can also lead to the formation of adhesions[5].

Many strategies have been recommended to prevent peritoneal adhesions, but none are widely used due to low effectiveness or risk of side effects. It is important to have an animal model that can be used to test new anti-adhesion strategies in abdominal surgery, as well as to test adhesion prevention agents and strategies[6].

To date, a total of 75 potential antiadhesive agents have been tested in rat and rabbit models, including various fucoidan preparations. The fundamental finding was that fucoidans were the most effective anti-adhesive agents among the many candidates tested[7]. Fucoidans were incorporated into a hyaluronic glycerol film, which was placed between the injured cecum and the lateral wall defect, and thus served as an additional mechanical barrier.

It has been suggested that resveratrol, a natural extract with anti-inflammatory effects, may reduce the occurrence of adhesions. The anti-inflammatory mechanism of resveratrol and its antioxidant activity are associated with the suppression of key inflammatory cytokines[8].

Numerous supramolecular hydrogels containing substances of natural origin have been successfully prepared and used to relieve inflammation, wound healing, and resistance to bacteria[9]. There is no doubt that any pharmacological and technological proposal aimed at

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[5] Wei, G. Effect of Emodin on Preventing Postoperative Intra-Abdominal Adhesion Formation / G.Wei, Y.Wu, Q.Gao [et al.] // Oxidative Medicine and Cellular Longevity, - 2017. 2017, Article ID 1740317, 12 p.

[6] Charboneau, A.J., Beilman, G., Delaney, J.P. Correction: Fucoidans inhibit the formation of post-operative abdominal adhesions in a rat model. PLoS ONE, - 2019. 14(1), - p. e0211371.

[7] Charboneau, A.J., Delaney, J.P., Beilman, G. Fucoidans inhibit the formation of post-operative abdominal adhesions in a rat model // PLoS ONE, - 2018. 13(11), - p. e0207797.

[8] Pektaş, S.G., Akar, F. Long-Term Dietary Fructose Causes Gender-Different Metabolic and Vascular Dysfunction in Rats: Modulatory Effects of Resveratrol // Cell Physiol Biochem, - 2015. 37, - p. 1407-1420.

[9] Zou, L. Degradable carrier-free spray hydrogel based on self-assembly of natural small molecule for prevention of postoperative adhesion / L.Zou, Y.Hou, J.Zhang [et al.] // Mater Today Bio, – 2023. 22, - 100755.

limiting and controlling peritoneal adhesion formation must incorporate the latest advances in understanding both the pathogenesis and pathophysiology of adhesion formation. It is generally accepted that the best prevention of peritoneal adhesions is based on careful surgical technique, which aims to minimize peritoneal trauma through gentle maneuvers, constant tissue lavage with saline or lactated ringer's, and maintenance of hemostasis, including appropriate sutures and prostheses, preventing infection and tissue ischemia, as well as seam tension[10].

Numerous drugs are used that affect coagulation, inflammation, fibrinolysis, and associated cytokines. However, side effects, short duration of action and low therapeutic effect contribute to disappointment in the treatment of abdominal adhesions. Currently existing physical barriers isolate the wound from peripheral tissues, thereby blocking the formation of fibrin bridges and preventing the formation of intra-abdominal adhesions. Advances in hydrogels have led to renewed interest in the physical barrier and have eliminated most problems, but foreign implanted materials, especially those made from nondegradable components, may further prolong the inflammatory response and thereby promote the development of postoperative peritoneal adhesions.

The following strategies for preventing postoperative intra-abdominal adhesions are currently being considered: 1) minimizing surgical trauma; 2) reduction of the inflammatory response; 3) reducing fibrin exudation and promoting its absorption. However, there is still no generally accepted method that can safely and effectively prevent intra-abdominal adhesions. Therefore, identifying the ideal method or agent to prevent intra-abdominal adhesions remains a relevant and urgent need.

Object and subject of research

The object of the study was experimental models of abdominal adhesions, modeled on 90 white rats and 70 postoperative patients,

[10] Нейматов, И. Пути улучшение лечения результатов профилактики спаечной болезни. Scientific Collection «InterConf»,13th ISPC «Science and Practice: Implementation to Modern Society» (October 16-18, 2022; Manchester, Great Britain). - 2022. (128), - p. 192–194.

divided into 2 groups. The subject of the study was the prevention of adhesions through the use of intra-abdominal administration of a solution of Metronidazole-Dextran-Contrical, enriched with oxygen, tested in experimental models. To determine the effectiveness of the drug, the antioxidant activity and cytokine profile in experimental animals were determined.

Purpose of the study

To develop in an experimental form a more effective, easily applied and inexpensive method for the prevention of adhesive disease that can occur after intra-abdominal operations, and apply it in clinical practice.

Research objectives

1. Investigate the anti-adhesive effects of intra-abdominal administration of an oxygen-enriched mixture of Metronidazole + Dextran + Contrical and compare with the anti-adhesive effect of the drug "Mesogel" in a rat model.
2. Conduct a macro- and microscopic analysis of adhesions (number, types, localization, histological characteristics) simulated in the experiment.
3. Assess the effect of anti-adhesion agents on the indicators of the antioxidant system in the experiment.
4. Determine the effect of the drug "Mesogel" and a mixture of Metronidazole, Dextran and Contrical, enriched with oxygen, in terms of the concentration of pro- and anti-inflammatory cytokines in rats with simulated post-operative peritoneal adhesions.
5. Propose a method for complex prevention of adhesive disease based on the experimental results obtained and apply it in clinical practice.

Research methods

During the study, 3 experimental models of adhesions formation were developed: Group 1 (control) - n=30 rats, after mechanical damage to the small intestine and peritoneum, no drug was administered; Group 2 (comparison) - n=30 rats, after mechanical damage to the small intestine and peritoneum, the drug "Mesogel" was injected into the abdominal cavity; Group 3 (main) - n=30 rats, after mechanical damage to the small intestine and peritoneum, an oxygen-enriched mixture of Metronidazole, Dextran, Contrical was administered in a ratio of 1:1:0.1. On the 5th, 10th

and 21st days of the experiment, the abdominal cavity of 10 animals from each group was opened, the condition of the internal organs was visually assessed, a macroscopic analysis of the number, type and location of adhesions was carried out, as well as a microscopic analysis, the indicators of oxidant and cytokine levels were determined. blood profile. The clinical study included 70 postoperative patients, divided into 2 groups: a comparative group, 35 patients who underwent abdominal surgery, after which no preventive measures were taken to prevent adhesions; main group - 35 patients who underwent abdominal operations, after which an oxygen-enriched mixture of Metronidazole, Dextran, and Contrical was injected into the abdominal cavity. All patients were re-examined after 3-6 months, examined based on existing complaints, and all routinely underwent ultrasound diagnostics of the abdominal cavity.

Main provisions submitted for defense:

- the introduction of an oxygen-enriched mixture of Metronidazole, Dextran, Contrical in a ratio of 1:1:0.1 into the abdominal cavity of experimental animals helps to reduce adhesions and weaken the inflammatory process.
- the introduction of the drug "Mesogel" and the mixture Metronidazole + Dextran + Contrical in a ratio of 1: 1: 0.1, have a positive effect on the performance of the antioxidant system
- anti-adhesion drugs "Mesogel" and a mixture of Metronida-zol + Dextran + Contrical, enriched with oxygen, act as inhibitors of inflammation, which is manifested by a decrease in the level of pro-inflammatory cytokines IL-6 and TNF-α and an increase in the concentration of anti-inflammatory cytokines IL-4 and IL-10.
- the introduction of an oxygen-enriched mixture of Metronidazole, Dextran, Contrical in a ratio of 1:1:0.1 for the purpose of prophylaxis helped reduce the formation of adhesions by 55.2% ($p < 0.001$).

Scientific novelty.

1. In order to prevent adhesions, a new composition of a multicomponent solution (Metronidazole, Dextran and Contrical, enriched with oxygen) was used in the experiment with adequate results (verified by histological studies)

2. It has been proven that the introduction of the proposed composition of the medicinal mixture into the abdominal cavity leads to a significant increase in the concentration of anti-inflammatory cytokines in the blood, a decrease in the total antioxidant factor, increasing the activity of superoxide dismutase, which significantly reduces the likelihood of an adhesive process in the abdominal cavity after abdominal operations.

Theoretical and practical significance of the research.

Based on the results of an experimental study, a new approach to the prevention of adhesions in patients after abdominal surgery has been proposed.

It is justified and recommended for use in the prevention of an oxygen-enriched mixture of Metronidazole + Dextran + Contrical in a ratio of 1: 1: 0.1 and its effectiveness has been shown. The use of this complex mixture allows to reduce the development of the adhesive process.

Approbation of work.

The results were reported at the all-Ukrainian scientific and practical conference with international participation, dedicated to the memory of corresponding member of the National Academy of Medical Sciences of Ukraine, Professor Yu.B. Tchaikovsky "Tissue resections in normal conditions, experiments and clinics." The initial discussion of the dissertation was held at a meeting with the joint participation of employees of the Scientific Center of Surgery (SCS) named after academician M.A. Topchubashev, Department of Surgical Diseases II of the Azerbaijan Medical University, as well as the Main Clinical Hospital of the Ministry of Defense of the Azerbaijan Republic (Protocol No. 06, October 11, 2023). Approbation of the dissertation was carried out at a scientific seminar operating under the dissertation council (Protocol No. 03, April 4, 2024).

Publications.

6 articles and 6 theses have been published on this topic, 3 articles and 1 theses of which have been published in foreign journals.

Scope and structure of the dissertation.

The dissertation is written on 164 pages, contains 18 tables, 6 graphs, 13 photos, includes an introduction (15,059 characters), a

literature review (49,998 pp.), a chapter on materials and methods (17,270 pp.), 2 chapters of own research (60,989 pp. .; 23.443 pp.), conclusion (30.858 pp.), conclusions (2.677 pp.), practical recommendations (572 pp.), list of references containing 197 sources and a list of abbreviations.

THE CONTENT OF THE WORK

Material and research methods

Experimental studies were carried out on white outbred rats kept under normal conditions in the vivarium of the Research Center of the Azerbaijan Medical University. Modeling of the adhesion process was carried out on outbred white rats. A total of 90 outbred rats were used and divided into three groups.

The first group (group 1) is the control group: 30 white rats, placed in 3 cages of 10 animals each. Then, after anesthesia with calypsol under sterile conditions, they were fixed on their backs on a special wooden board, the fur on the anterior abdominal wall was cleaned with a sharp razor, after which, following the rules of asepsis and antiseptics, the skin of the anterior abdominal wall was cut 3-4 cm along the midline . Carefully, the stomach was found in the abdominal cavity, at a distance of 2-3 cm from which a section of the small intestine was isolated, the surface of which was mechanically damaged with a sterile toothbrush until traces of bruising appeared. After this, the skin of the abdomen was sutured in layers. The operation on each animal lasted up to 15-20 minutes.

The second group (group 2) is a comparison group: 30 white rats were fed and placed in 3 cages of 10 animals each. The animals were anesthetized under sterile conditions with Calypsol, fixed on their backs on a wooden board, and the hair on the anterior abdominal wall was removed with a sharp razor. Afterwards, observing the rules of asepsis, a midline laparotomy was performed. In the abdominal cavity, at a distance of 2-3 cm from the stomach, a section of the small intestine was isolated, mechanically damaged with a sterile toothbrush (until traces of blood appeared), after which the drug “Mesogel” was injected in an amount of 1 ml. The drug was injected into the abdominal cavity in order to prevent adhesions in the abdominal

cavity. After this, the skin of the abdomen was sutured in layers. The operation on each animal lasted 15-20 minutes.

The third group (group 3) is the experimental group: 30 white rats were placed in 3 cages of 10 animals each. Experimental animals under sterile conditions were anesthetized with calypsol and fixed on a special wooden board, the surface of the skin in the area of the anterior abdominal wall was cleared of hair with a sharp razor, the abdominal cavity was opened 3-4 cm along the midline, the same as in the previous groups, retreating by A segment of the small intestine was isolated 2-3 cm from the stomach, and mechanical damage to its wall was performed with a sterile toothbrush. At the end of the operation, 1 ml of a specially prepared mixture of Metronidazole, Dextran and Contrical, enriched with oxygen (in a ratio of 1:1:0.1) was injected into the abdominal cavity and the skin was sutured in layers. Each surgical intervention lasted 15-20 minutes.

When keeping rats and conducting experimental studies, the rules for the care and use of laboratory animals were followed[11]. Experimental animals were kept in a separate room of the vivarium at normal temperature and free feeding conditions.

All groups were scored as follows:

- Quantitative characteristics of the adhesion process
- Types of adhesion
- Localization of adhesions

Macro- and microscopic analysis of damaged tissues and adhesions was carried out. The studies were carried out dynamically: on the 5th, 10th and 21st days of the experiment, the abdominal cavity of each animal was opened, the condition of the internal organs, the number, type and location of adhesions were visually determined.

For morphological characteristics, a histological and light microscopic examination of the formed adhesions was carried out. On the 5th, 10th and 21st days of the study, 10 rats from all groups (control, comparison and experimental) were decapitated in laboratory conditions according to generally accepted rules. For histological analysis, tissues involved in the

[11] Руководство по содержанию и использованию лабораторных животных. Пер. с англ. под ред. И.В.Белозерцевой, Д.В.Блинова, М.С.Красильщиковой - Москва: ИРБИС, - 2017. - 336 с.

adhesive process were taken, namely in the area of sutures, peritoneum, damaged segment of the small intestine, internal organs - liver, omentum. The taken material was fixed in 10% formaldehyde buffer for further microscopic examination and the material was processed according to generally accepted methods. Then the collected tissues were impregnated with paraffin and cut into 6 microns. The sections were stained with standard hematoxylin-eosin staining and the prepared microslides were microscoped using a light microscope (Leica DM 750, Germany). All changes observed during microscopic examination were recorded using a camera attached to a microscope (Leica ICC 50, Germany).

The following anti-adhesion drugs were used: Mesogel, Metronidazole, Dextran, Contrical.

To determine the antioxidant status on the 5th, 10th and 21st days, catalase (CT) in the blood was studied by the spectrophotometric method using ammonium molybdate; superoxide dismutase (SOD) using an indirect spectrophotometric method, the total antioxidant factor (AOF) was calculated, which was calculated using the formula:

OAF = SOD/SODcontrol. + CAT/CATcontrol)/4)

Cytokine profile indicators were assessed by the concentration of pro-inflammatory and anti-inflammatory cytokines in the blood serum: tumor necrosis factor (TNF-α), interleukins IL-4, IL-6, IL-10. The determination was carried out using a solid-phase sandwich version of an enzyme-linked immunosorbent assay (ELISA) using reagent kits from Vector Best (Russia).

The clinical part of the dissertation work consisted of 70 patients who underwent surgical interventions at the Main Clinical Hospital of the Ministry of Defense of the Republic of Azerbaijan. The criteria for inclusion in the study were: patients after surgical interventions in the abdominal cavity using open access (median laparotomy, Kocher, McBurney incisions, pararectal incisions, etc.), as well as laparoscopically; patients after gynecological operations performed using the Pfannenstiel approach; patients of both sexes; patients who expressed written consent to participate in the study. Exclusion criteria: patients who did not sign written consent to participate in the study.

Patients were aged from 18 to 73 years, mean age 36.73±12.62 years. Of the 70 patients, 45 (64.3%) were men, 25 (35.7%) were women. 23 (32.85%) patients suffered from cholelithiasis and 10

(14.3%) from appendicitis. A gunshot wound penetrating the abdominal cavity with damage to the loops of the small or large intestine was diagnosed in 8 (11.4%), gynecological pathologies - 11 (15.7%), of which uterine fibroids - in 7 (10 %), as well as ovarian cysts – in 4 (5.7%) patients. Shrapnel wounds penetrating the abdominal cavity, accompanied by damage to the intestines against the background of a blast injury, were diagnosed in 10 (14.3%) patients, 2 (2.85%) patients had an hydatid cyst of the liver, 1 (1.45%) patient with inguinal, 2 (2.85%) patients with an umbilical hernia, 1 (1.45%) patient had esophageal achalasia, 2 (2.85%) patients had a colon tumor. 21 laparoscopic operations (30%), 38 open access operations (54%), and 11 gynecological operations with Pfannenstiel access (16%) were performed.

All surgical interventions were carried out according to generally accepted international standards and technologies. After 3-6 months, all patients were routinely examined, examined, and all underwent ultrasound examination of the abdominal organs using a GE Voluson S8 device (GE Healthcare, USA).

For statistical processing of the obtained results, SPSS software for Windows (version 12.0, SPSS Inc., Chicago, IL, USA) was used. Rates were expressed as mean ± standard deviation (SD) and as numbers and percentages.

Indicators between groups were compared using the Mann-Whitney test of variance. To compare the average values between groups, the Student's t-test and chi-square ($\chi 2$) were calculated, and the odds ratio (OR) was calculated with a 95% confidence interval (CI). The severity of correlations between individual indicators was calculated using the Spearman correlation coefficient. Statistical estimates were considered significant at a p value <0.05.

Results of our own research. The severity of postoperative abdominal spa process and its prevention (experimental study)

In group 1 of 30 animals, during the entire period of the experiment, adhesions were formed in 23 rats, which amounted to 76.7%, in group 2 - in 13 rats, which amounted to 43.3%, and in group 3, adhesions were formed in 11 rats, which amounted to 36 .7% of the total number of animals in the group. the number of rats with spikes in group 3 was significantly less than in group 1 ($\chi 2=9.774$, $p=0.002$), but with group

2 the difference was insignificant ($\chi2$=0.278, p=0.599). A comparison of the number of animals with spikes in group 2 with the number of rats with spikes in group 1 also showed a statistically significant difference ($\chi2$=6.944, p=0.009). The total number of adhesions formed during the observation period was 83 adhesions. A total of 44 adhesions were formed in group 1 during the study period, in group 2 - 22 adhesions, and in group 3 - 17 adhesions. In group 3, adhesions formed less frequently, which was significantly different compared to group 1 ($\chi2$=18.894, p<0.001), with group 2 the difference was not significant ($\chi2$=0.838, p=0.361). The difference in the adhesions that occurred in group 2 compared to group 1 was also significant ($\chi2$=12.173, p<0.001). On the 5th day of the study, in group 1, adhesions formed in 8 animals, in group 2 there were 6 such animals, in group 3 – 5 animals. On the 10th day of the experimental study, there were 7 animals with spikes in group 1, 4 rats in group 2 and group 3. On the 21st day of the study, the number of animals with spikes in group 1 was 8, in group 2 – 3, and in group 3 – 2 rats. A dynamic comparison revealed that in group 1 the number of rats with spikes by day 21 compared to the beginning of the study (5 days) practically did not change. At the same time, the number of adhesions decreased from 19 to 11 adhesions, but the difference was not significant ($\chi2$=3.237, p=0.073). In group 2, the number of animals with spikes decreased from 6 rats on day 5 to 4 rats on day 10 and to 3 animals on day 21. Therefore, the difference was not significant ($\chi2$=1.529, p=0.217). In this group, the number of adhesions decreased from 9, determined on the 5th day, to 7 adhesions on the 10th day, and to 6 adhesions, determined on the 21st day. A comparative analysis showed an insignificant difference in the number of adhesions between days 5 and 21 ($\chi2$=0.910, p=0.341). In group 3, the number of animals with spikes decreased from 5 rats on day 5 to 4 rats on day 10 and to 2 animals on day 21. Therefore, the difference was not significant ($\chi2$=1.619, p=0.204). In this group, on the 5th day of the study, 7 adhesions were determined, on the 10th and 21st days, 5 adhesions were determined, respectively. As you can see, the difference was not significant ($\chi2$=0.515, p=0.473). It can be noted that in groups 2 and 3 there was a tendency towards a decrease in the number of animals with

adhesions, while in group 1 such a trend was not noted; the number of animals with adhesions remained stable during the study period.

On the 5th day after open surgery, in all groups (control, comparison and experimental), soft-type adhesions predominated - up to 80-83.3%, and there were fewer dense-type adhesions - 16.7-20%. On the 10th day of the study, the number of soft adhesions in all groups was 43.3%, dense adhesions were 56.7%. On the 21st day of the study, only dense adhesions were formed in all animals; soft adhesions were not detected in all groups. A comparative intragroup analysis of the nature of adhesions showed that in groups 1 and 2, the number of soft adhesions compared to the 5th day decreased significantly on the 10th day of the study by 45.9% ($\chi 2 = 8.531$, $p = 0.004$), respectively, and the number of dense adhesions increased by 64.7% ($\chi 2=8.531$, $p=0.004$), respectively. A comparative intragroup analysis of the nature of adhesions in group 3 showed that on the 10th day the number of soft adhesions decreased significantly by 48.0% ($\chi 2 = 10.335$, $p = 0.002$), in turn, the number of dense adhesions increased by 70. 5% ($\chi 2=10.335$, $p=0.002$). A comparative intergroup analysis showed that on the 5th day in groups 1 and 2 the number of soft adhesions did not differ; in group 3 their number was slightly higher (by 4%), respectively, and, therefore, the number of dense adhesions was lower (by 16.5%).). At days 10 and 21, there were no differences in the number of soft and hard adhesions between groups.

Consequently, on the first day after open surgery, soft adhesions were more often detected in experimental animals, and the formation of dense adhesions began later, and by the 21st day they were already fully formed.

In most animals, lamellar and planar adhesions predominated (Table 1). A comparative analysis of the types of adhesions of the experimental group with the comparison group showed a slight difference. Thus, on days 10 and 21 in the experimental group there were 16.0% ($\chi 2=0.171$, $p=0.679$) and 9.9% ($\chi 2=0.052$, $p=0.819$) fewer flat-shaped adhesions, respectively, and more lamellar adhesions by 28.5% ($\chi 2=0.171$, $p=0.679$) and by 16.5% ($\chi 2=0.171$, $p=0.679$).

Almost all animals had adhesions in the area of the suture-peritoneum and suture-omentum. The suture material used, mechanical damage and bleeding, inflammation caused by microorganisms, have a positive effect on the formation of

adhesions. The damaged segment of the intestine is also actively involved in the formation of adhesions in the abdominal cavity. Especially in the control group, adhesions between the injured intestine and the liver and between the injured intestine and the stomach, as well as between intestinal loops, were most likely caused by the consequences of intestinal injury, bleeding and infection.

Table 1

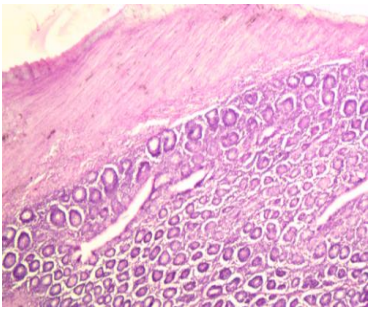
Types of adhesions that occur in the abdominal cavity of white rats, depending on the method of prevention

Types of adhesions	Experimental groups		
	1 group	2 group	3 group
On the 5th day			
Wedge-shaped, n (%)	5 (26,3)	1 (11,1)	0 (0)
Flat-shaped, n (%)	9 (47,4)	5 (55,6)	4 (57,1)
Lamellar, n (%)	5 (26,3)	3 (33,3)	3 (42,9)
Total	19 (100)	9 (100)	7 (100)
On the 10th day			
Wedge-shaped, n (%)	3 (21,4)	0 (0)	0 (0)
Flat-shaped, n (%)	7 (50,0)	5 (71,4)	3 (60,0)
Lamellar, n (%)	4 (28,6)	2 (28,6)	2 (40,0)
Total	14 (100)	7 (100)	5 (100)
On the 21st day			
Wedge-shaped	1 (9,1)	0 (0)	0 (0)
Flat-shaped, n (%)	6 (54,5)	4 (66,6)	3 (60,0)
Lamellar, n (%)	4 (36,4)	2 (33,4)	2 (40,0)
Total	11 (100)	6 (100)	5 (100)

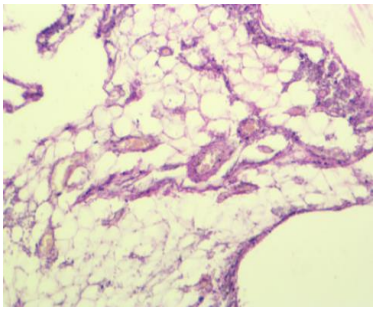
In the animals of the experimental group, on the 5th day of the experiment, there were significantly fewer adhesions in the abdominal cavity when killed than in the control group, and they differed little from the comparison group. Adhesions were mainly formed between the peritoneum and the omentum near the suture or between the

peritoneum and the damaged segment of the intestine. On the 10th day of the experiment, a small number of adhesions formed between the suture area, the peritoneum, the omentum and the damaged segment of the intestine. There were no adhesions between internal organs - intestinal loops, intestines and other organs. On day 21, a small number of adhesions appeared between the suture area, the peritoneum, the omentum and the damaged segment of the intestine. No adhesions between the damaged intestine and internal organs were registered. Microscopic analysis of samples taken from animals of this group showed that only dense type adhesions are formed from developed collagen fibers. Individual macrophages and leukocyte cells are found on the fibers, and blood vessels have developed between the fibers. There is an accumulation of red blood cells in some blood vessels. Unlike previous days, the swelling has subsided significantly (photo).

According to the results obtained, the greatest adhesive changes after modeling were observed in the peritoneum-omentum area (Table 2).



A



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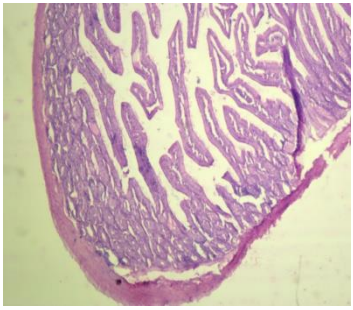

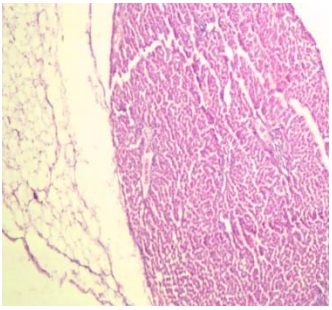

C D

Photo. Changes in the internal organs of white rats in the experimental group 21 days after surgery: A – adhesions and affected intestine; B – oil seal; C – intact intestine; D – omentum and liver (magnification x100, hematoxylin-eosin staining)

Indicators of the antioxidant system and the level of cytokines in experimental models of postoperative intra-abdominal adhesion

Catalase activity in group 1 (control) increased by 5.8% 10 days after the intervention (t=0.40, p=0.702) and decreased on day 21 compared to the value on day 5 (by 6.2%, t =0.67, p=0.524) and with activity on day 10 (by 11.7%, t=0.75, p=0.476).

Catalase activity in group 2 (comparison group) decreased during the study period. In comparison with enzyme activity on day 10, the decrease was 15.1% (t=1.40, p=0.204), compared with activity on day 21 – by 19.8% (t=2.62, p=0.035) . When comparing enzyme activity on days 10 and 21 of the experiment, a statistically insignificant difference was revealed (t=0.52, p=0.617).

Table 2

Histological characteristics of adhesions and surrounding tissues in animal models over time

Experiments days	Type of adhesions analysis	Experimental groups		
		Control (group 1, n=30; after injury to the small intestine no drug was administered)	Comparison group (group 2, n=30; mesogel was introduced after damage to the small intestine)	Experienced group (group 3, n=30; after damage to the small intestine, a mixture of metronidazole, dextran and contrical + O2 was administered in a ratio of 1:1:0.1)
5-th	Macroscopically	Developed adhesions. In the area of the peritoneum, sutures, damaged intestines, omentum	There are fewer adhesions in the area of the damaged peritoneum	There are fewer adhesions, a weak inflammatory process. Between the peritoneum and the omentum near the suture, between the peritoneum and the damaged segment of the intestine
	Microscopically	Soft fibrin and collagen fibers (predominant), macrophages, fibroblasts, lymphocytes. Inflammation, swelling, poorly developed	Scattered collagen and collagen fibers, mild edema, macrophages, leukocytes, destruction of collagen fibers. Surrounding tissues: edema, leukocyte	Thin fibrin, collagen fibers, macrophages, a small number of fibroblasts, lymphocytes. Mild swelling and inflammation

		blood capillaries. Surrounding tissues: edema, dilated vessels, congestion in venous vessels, weak fibrin layer	infiltration, slight vasodilation, minor hemorrhages, rarely stagnation of blood in the veins	
10-th	Macro-scopi-cally	The number of adhesions decreased. Between the area of sutures, the peritoneum and the damaged segment of the intestine, between the peritoneum and the omentum.	The number of adhesions has decreased. Between the suture site, the peritoneum, the omentum and the damaged intestine	There are few adhesions between the suture area, the peritoneum, the omentum and the damaged segment of the intestine
	Micro-scopi-cally	Fibrin, collagen fibers, macrophages, fibroblasts, lymphocytes. Surrounding tissues: mild swelling, inflammation, small areas of hemorrhage	Collagen fibers, macrophages, fibroblasts, a small number of lymphocytes	Soft collagen fibers. Mild swelling
21-st	Macro-scopi-cally	Adhesions in the area of the damaged peritoneum - the area of the sutures - the damaged intestine, between the peritoneum - the omentum, between the internal organs - the damaged intestine - liver, peritoneum - stomach, etc.	Adhesions in the area of sutures, visceral peritoneum, omentum, damaged intestinal segment	Rare adhesions between the suture area, peritoneum, omentum and damaged intestinal segment.
	Micro-scopi-cally	Collagen fibers, rare macrophages, slight swelling, inflammatory process	Collagen fibers, a small number of macro-phages, leukocyte cells. Surrounding tissue: edema, leukocyte infiltration	Developed collagen fibers, rare macrophages, leukocytes. Surrounding tissue: mild swelling and inflammation

The activity of catalase in the blood of animals of group 3 (experimental group), as well as in group 2, dynamically decreased. On days 10 and 21, compared to day 5, enzyme activity decreased by 15.3% ($t=1.19$, $p=0.273$) and 29.5% ($t=2.29$, $p=0.055$),

respectively. The difference between catalase activity on days 10 and 21 was 16.7% (t=1.49, p=0.181) and 16.7% (t=0.93, p=0.376), respectively.

A comparative intergroup analysis of catalase activity on day 5 between groups 1 and 2, as well as groups 1 and 3 revealed a decrease by 19.1% (t=2.70, p=0.030) and by 11.1% (t=1.49, p=0.181) respectively. Enzyme activity in group 2 was higher than in group 3 by 8.9% (t=0.65, p=0.538). On the 10th day, the level of catalase in groups 2 and 3 compared to group 1 decreased by 35.3% (t=2.29, p=0.056) and by 29.1% (t=2.03, p=0.082), respectively. When comparing enzyme activity in group 2 compared with group 3, there was a decrease of 8.8% (t=0.88, p=0.406). On day 21, catalase activity in groups 2 and 3 compared to group 1 was also reduced by 30.8% (t=3.38, p=0.012) and by 33.2% (t=2.75, p=0.028). During the same period of the study, catalase activity in group 3 compared to group 2 was slightly lower - by 3.4% (t = 0.27, p = 0.795).

SOD activity in group 1 on day 5 was lower than on day 10, by 10.7% (t=1.06, p=0.326) and slightly higher - by 5.3% (t=0.33, p= 0.752) than at 21 days. The difference in SOD activity in this group on days 10 and 21 was 15.5% (t=1.12, p=0.299).

In group 2, SOD activity gradually increased. On days 10 and 21, compared with activity on day 5, it increased by 4.0% (t=0.51, p=0.627) and by 8.1% (t=0.98, p=0.361), respectively. SOD activity on day 21 compared to enzyme activity on day 10 was higher by 12.6% (t=0.50, p=0.630).

In group 3, SOD activity, as well as in group 2, increased on days 10 and 21 compared to day 5 by 4.0% (t=0.69, p=0.513), by 7.1% (t= 0.78, p=0.460) respectively. SOD activity in animals of this group on day 21 compared with activity on day 10 increased by 3.2% (t=0.36, p=0.726).

On day 5, the highest SOD activity was observed in animals of group 3, which was 33.5% higher (t=4.53, p=0.003) than in group 1 and 4.1% higher than in group 2 (t =0.58, p=0.578). SOD activity in group 2 was higher than in group 1 by 30.6% (t=3.70, p=0.008). On day 10, SOD activity was also high in group 3, which was 28.4%

higher relative to group 1 (t=4.70, p=0.002) and 4.1% higher relative to group 2 (t=0.61) , p=0.560). The difference in SOD activity in groups 1 and 2 was 25.4% (t=3.36, p=0.012). On day 21, the activity of SOD in animals of group 3 exceeded the activity of this enzyme in group 1 by 41.5% (t=3.55, p=0.009) and in group 2 by 3.0% (t=0.30, p= 0.775). The difference in enzyme activity in group 2 compared to group 1 was 39.7% (t=3.64, p=0.008).

The value of TAF in the control group (group 1) dynamically increased. The TAF indicator on days 10 and 21 compared to the indicator on day 5 was higher by 5.0% (t=0.47, p=0.652) and 17.7% (t=1.25, p=0.252), respectively. The difference in TAF between the values on days 10 and 21 was 13.4% (t=0.97, p=0.362).

In group 2, there was a decrease in TAF on days 10 and 21 compared to the value on day 5 by 2.4% (t=0.15, p=0.884) and 8.4% (t=0.66, p=0.530) respectively. In this experimental group, TAF on day 21 was lower than on day 10 by 6.2% (t=0.43, p=0.683).

In group 3, as well as in group 2, the TAF value decreased on day 10 compared to day 5 by 7.7% (t=0.49, p=0.639), on day 21 compared to day 5 - by 11.3% (t=0.69, p=0.514). The TAF indicator decreased on day 21 compared to the indicator noted on day 10 by 3.9% (t=0.21, p=0.841).

On day 5, the TAF value in groups 2 and 3 was higher than in group 1 by 12.0% (t=0.94, p=0.378) and by 10.0% (t=0.77, p=0.464) respectively. The OAF rate during this study period in group 2 was slightly higher than in group 3 by 2.1% (t=0.15, p=0.886). On the 10th day of the experiment, the TAF value in group 2 was higher than in group 1 by 5.0% (t=0.36, p=0.729), and in group 3 by 2.5% (t=0.18, p =0.862) below. On day 21, there was an increase in TAF in group 1 and a decrease in groups 2 and 3. During this period of the study, there was a decrease in TAF in groups 2 and 3, which compared to group 1 was 14.5% (t=1.01, p=0.345) and 18.9% (t=1.12, p=0.301).

The results obtained allow us to state an increase in SOD activity in the comparison group and the experimental group, while catalase activity in these groups tended to decrease. The dynamics of TAF tended to decrease on the 21st day of the experiment, especially in group 3: from 51.34±5.35 units on the 5th day of the experiment to

45.56±6.49 units on the 21st day, t .e. the decrease was 11.3%. (Table 3).

Table 3

AOS indicators in animals during the study period

Groups	Study period	Catalase, mcat/l	SOD, unit/q	TAF
	5 days			
1 (n=5)		20,62±0,91	38,18±3,34	46,19±3,96
2 (n=5)		16,68±1,14*	55,02±3,10*	52,47±5,37
3 (n=5)		18,32±2,26	57,38±2,62*	51,34±5,35
	10 days			
1 (n=5)		21,90±3,08	42,78±2,79	48,64±3,37
2 (n=5)		14,16±1,39	57,32±3,30*	51,22±6,30
3 (n=5)		15,52±0,66	59,78±2,30*	47,40±6,0
	21 days			
1 (n=5)		19,34±1,68	36,14±5,23	56,16±6,94
2 (n=5)		13,38±0,54#,*	59,90±3,92*	48,04±4,03
3 (n=5)		12,92±1,62#,*	61,78±4,98*	45,56±6,49

Note: # - statistical significance of the differences between the group indicators on days 10 and 21 with the indicator on day 5; * - statistical significance of differences with the control group indicator

Table 4

Level of cytokines in experimental models of adhesions

Groups	IL-4, pg/ml	IL-10, pg/ml	IL-6, pg/ml	TNF-alpha, pg/ml
	5 days			
1	16,3±1,0	37,84±3,53	220,64±21,67	38,54±1,97
2	17,04±3,33	40,98±4,90	158,02±7,46*	32,78±5,58
3	17,88±3,18	45,38±4,26	130,22±8,50*,**	22,38±1,74*
	10 days			
1	14,22±1,42	30,74±2,53	252,06±23,55	43,96±5,51
2	17,88±1,46	45,54±5,89*	150,72±12,82*	30,98±4,74
3	19,14±1,41*	46,42±5,10*	128,48±4,70*	22,52±2,30*
	21 days			
1	16,3±1,0	37,84±3,53	220,64±21,67	38,54±1,97
2	18,64±1,15	47,38±3,30	137,56±6,87*	28,40±3,76*
3	20,0±1,76	48,92±2,34*	125,7±4,28*	20,32±1,58*

Note: * - statistical significance of differences with the control group; ** - statistical significance of differences in indicators between groups 2 and 3

The influence of anti-adhesive agents on the cytokine profile.

In the comparison group and the experimental group, there was an increase in anti-inflammatory IL-4 and IL-10 and a decrease in pro-inflammatory cytokines: IL-6 and TNF-α (Table 4).

Statistically significant strong connections were determined between IL-10 and IL-6 in all groups on day 5 of the study, and if in groups 1 and 2 the relationship was direct ($r=0.866$, $p<0.001$, $r=0.937$, $p<0.001$, respectively) , then in group 3 it was reversed ($r = -0.835$, $p<0.001$). Also during this period of the study, a statistically significant, average relationship was noted in group 2 between IL-10 and TNF-α, which further intensified on day 10 ($r=0.658$, $p<0.05$). On day 10, there was a strong, inverse, statistically significant relationship between IL-10 and IL-6 in group 3 ($r=-0.921$, $p<0.001$). In this group, there was a moderately significant relationship between IL-10 and TNF-α ($r=0.579$, $p<0.05$). On day 21, a strong, significant direct relationship was determined between the anti-inflammatory cytokines IL-4 and IL-10 in groups 2 and 3 ($r=0.802$, $p<0.001$, $r=0.805$, $p<0.001$, respectively), while in the control group these cytokines correlated with each other with a weak connection ($r=0.459$, $p>0.05$). A high correlation was observed in group 3 between IL-4 and TNF-α ($r=0.794$, $p<0.01$), IL-10 and with TNF-α an average direct significant relationship ($r=0.524$, $p<0.05$), and IL-6 correlated with TNF-α by an inverse mean significant relationship ($r=-0.677$, $p<0.05$).

Assessment of the severity of the adhesive process in the abdominal cavity (clinical study).

The patients were randomized into 2 groups: Group I included 35 postoperative patients who did not receive anti-adhesion drugs into the abdominal cavity at the end of surgery; Group II consisted of 35 postoperative patients who, at the end of the operation, in order to prevent adhesive disease, were injected into the abdominal cavity with a solution of an oxygen-enriched mixture of Metronidazole + Contrical + Dextran (in a ratio of 1:1:0.1). The average age of patients

in groups I and II was 30.91±9.21 and 42.54±14.76 years, respectively (t=0.67, p=0.506). In group I there were 26 (74.3%) men, 9 (25.7%) women, in group II 19 (54.3%) and 16 (45.7%) respectively (t=3.049, p=0.081).

Patients in both groups more often suffered from cholelithiasis (22.9% and 31.4% in groups I and II, respectively); 19 patients were operated on laparoscopically, 4 patients – using the Kocher approach. Patients with gunshot wounds penetrating the abdominal cavity, for whom a colostomy was removed during the first stage of surgical treatment, and the colostomy was removed and a colonic anastomosis was performed at the second stage, were included in group I ($\chi 2 = 24.231$, $p < 0.001$), patients with appendicitis were included in group II ($\chi 2 = 11.667$, $p < 0.001$). 1 patient of the first group underwent a laparoscopic Heller operation for achalasia of the esophagus, 2 patients underwent removal of an hydatid cyst of the liver. The main group also included 3 patients with hernias, 1 patient of them underwent laparoscopic TAPP hernioplasty, 2 patients were operated on with an open approach for an umbilical hernia, and 2 patients in this group underwent right-sided hemicolectomy for a tumor colon. 11 gynecological operations with the Pfannenstiel approach were performed, of which 6 patients were in the comparison group, 5 in the main group (Table 5; Table 6).

Table 5

Patients of the 1st (comparative) group who underwent surgical interventions (n=35)

Laparoscopy			Laparotomy			Gynecology		
Operation	n	adhesions	Operation	n	adhesions	Operation	n	adhesions
Cholecystectomy	8	4	Echinococcus of the liver	2	2	Tubectomy	2	1
Heller operation	1	1	Coloplasty	18	18	Hysterectomy	4	3
Total	9	5		20	20		6	4
Percentage of adhesions (%)		55,5			100			66,6

Table 6

Patients of the 2nd (main) group who underwent surgical interventions (n=35)

Laparoscopy			Laparotomy			Gynecology		
Operation	n	adhesions	Operation	n	adhesions	Operation	n	adhesions
Cholecystectomy	11	2	Umbilical hernia repair	2	2	Myomectomy	1	1
TAPP hernia repair	1	0	Appendectomy	10	3	Hysterectomy	2	2
-			Hemicolectomy	2	1	Cystectomy	2	0
-			Cholecystectomy	4	2			
	12	2	Total	18	8	Total	5	3
Percentage of adhesions (%)		16,7%			44,4%			60%

In both groups, 3-6 months after surgery, when examining patients, complications such as intestinal obstruction and chronic constipation were not observed. 5 patients of group 1 had periodic pain in the abdominal cavity in the area of the postoperative suture. A study on the presence of postoperative adhesions using abdominal ultrasound showed that adhesions in 70 examined postoperative patients occurred in 60.0% of cases (n=42). Thus, in group I, postoperative adhesions were visualized in 29 (82.8%), in group II - in 13 (37.1%) patients ($\chi 2 = 15.238$, $p < 0.001$) (Table 7).

Table 7

Frequency of diagnosed adhesions in patients during various operations

Operation name	I group (n=35)	II group (n=35)	$\chi 2$	p
Laparoscopy, n (%)	9 (55,5)	12 (16,7)	3500	=0,062
Laparotomy, n (%)	20 (100)	18 (44,4)	15,079	<0,001
Gynecology, n (%)	6 (66,6)	5 (60)	0,052	=0,819

Thus, in patients of group II, who were administered a mixture of Metronidazole + Dextran + Contrical in a ratio of 1:1:0.1, enriched with oxygen, for the purpose of prevention, the adhesive process was observed less frequently - in 37.1% of cases, versus 82.8% cases in the group that did not receive this mixture. The analysis showed that the chance of detecting postoperative adhesions was significantly higher in patients who were not administered an anti-adhesive mixture for prophylaxis – OR=8.179 (95% CI 2.683-24.940, $p<0.01$).

CONCLUSIONS

1. In the group of rats with a simulated adhesive process, after administration of the anti-adhesive mixture of Metronidazole, Dextran and Contrical + O2, abdominal adhesions developed in 20.5% of cases, while in the group of rats after administration of the drug "Mesogel" - in 26.5% of cases, and in the group with a simulated adhesive process without the introduction of anti-adhesive drugs - in 53.0% of cases, which indicates the anti-adhesive effect of the multicomponent solution.

2. Intraperitoneal administration of an oxygen-enriched mixture of Metronidazole + Dextran + Contrical in a ratio of 1:1:0.1 allowed us to obtain positive results. During the dynamic observation of histological changes in adhesions and surrounding tissues, 21 days from the start of the experiment, rare adhesions between the suture area, peritoneum, omentum and damaged intestinal segment were macroscopically observed; microscopically - developed collagen fibers, rare macrophages, leukocytes, mild edema, and inflammation of surrounding tissues.

3. Administration of the drug “Mesogel” and a mixture of Metronidazole + Dextran + Contrical in a ratio of 1:1:0.1, enriched with oxygen, to experimental animals led to an increase in SOD activity, while catalase activity tended to decrease. An oxygen-enriched mixture of Metronidazole, Dextran, Contrical in a ratio of

1:1:0.1 contributed to a decrease in the total antioxidant factor on the 21st day of the experiment by 11.3% ($p = 0.514$).

4. The administration of anti-adhesion drugs “Mesogel” and a mixture of Metronidazole + Dextran + Contrical + O2 inhibits inflammation, which was expressed by a decrease in the concentration of pro-inflammatory IL-6 and TNF-α and an increase in the concentration of anti-inflammatory cytokines IL-4 and IL-10 .

5. The introduction of an oxygen-enriched mixture of Metronidazole + Dextran + Contrical in a ratio of 1:1:0.1 in order to prevent adhesive disease in the abdominal cavity helped reduce the formation of adhesions by 55.2% ($p < 0.001$). The likelihood of developing postoperative adhesions is higher in patients who were not administered an anti-adhesion mixture for prophylaxis - OR=8.179 8.179 (95% CI 2.683-24.940, $p<0.01$).

PRACTICAL RECOMMENDATIONS

1. Patients after abdominal operations are recommended to undergo a follow-up examination, examinations based on complaints, as well as abdominal ultrasound diagnostics after 3-6 months to determine the development of adhesions.

2. An oxygen-enriched combination mixture of Metronidazole + Dextran + Contrical in a ratio of 1:1:0.1 showed itself as an inhibitor of the formation of postoperative adhesions by reducing inflammation, reducing the response to oxidative stress and stimulating the restoration of mesothelial cells peritoneum.

3. In order to prevent adhesion, it is advisable for postoperative patients to administer an oxygen-enriched mixture of Metronidazole + Dextran + Contrical in a ratio of 1:1:0.1.

List of published works on the topic of the dissertation

1. Определение основных факторов, способствующих образованию спаечного процесса в брюшной полости у женщин. // Georgian Medical News, Июль-Август 2017, №7-8, с. 94-98
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12. Role of multicomponent solution in prevention of adhesion disease after abdominal operations // Abstracts of the 20th international Eurasian congress of hepatogastroenterology & surgery, 25-27 april, 2024, c. 111-112 (соавтор Исаев Г.Б.)

List of abbreviations

IL - interleukin
CT - catalase
TAF - total antioxidant factor
SOD - superoxide dismutase
USE - ultrasound examination
AOS - antioxidant system

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