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Types and Localization of Abdominal Adhesions after Open Operations (Experimental Study)

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There is a great need for more efficient and more widely used preventive treatment of post-operative abdominal adhesions.

Purpose – to study the type of adhesions in the abdominal cavity after open operations in the experiment.

Materials and methods. The study was conducted on 90 white outbred rats divided into three groups. We mechanically damaged the surface of the small bowel segment in all rats. The control group (group 1) included 30 rats not administered any drugs into the abdominal cavity after the small bowel segment damage. The comparison group (group 2) consisted of 30 rats, which were introduced one mL of mezogel. The study group comprised 30 rats, which were administered a 1 mL mixture of metronidazole, dextran, contrykal (in a ratio of 1:1:0.1, respectively) + O₂ into the abdominal cavity; the wound of the latter was closed using a layered suture technique. On days 5, 10 and 21, the tissues taken from the suture site, peritoneum, damaged small bowel segment, liver, and omentum were analysed histologically.

Results. On day 10 in groups 1 and 2, compared to day 5, the proportion of filmy adhesions decreased by 45.9% ($p=0.004$), but the proportion of dense adhesions increased by 64.7%. The comparative between-group analysis on day 5 in groups 1 and 2 revealed no difference in the proportion of filmy adhesions, whereas in group 3 the proportion of filmy adhesions was 4% higher and the proportion of dense adhesions was 16.5% lower. Planar adhesions predominated in all groups.

Conclusions. There were more filmy adhesions seen on day 1, and the complete dense adhesion formation was observed by day 21. Planar adhesions predominated (54.5–60.0%). The area of postoperative sutures was involved in the adhesion formation. The adhesions in the sutures – peritoneum area as well as the omental adhesions were detected in all animals. Introduction of drugs, mezogel or a mixture of metronidazole, dextran, contrykal + O₂, into the abdominal cavity contributed to the reduction of adhesions between the small bowel loops.

The experiments with laboratory animals were provided in accordance with all bioethical norms and guidelines. No conflict of interests was declared by the author.

Keywords: peritoneum, omentum, adhesion modelling, anti-adhesion agents, type of adhesions, localization.

Види і локалізація спайок у черевній порожнині після відкритих операцій (експериментальне дослідження)

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Існує велика потреба в ефективнішому і ширшому застосуванні профілактичного лікування післяопераційних спайок черевної порожнини.

Мета – вивчити характер спайок у черевній порожнині після відкритих операцій в експерименті.

Матеріали та методи. Дослідження проводили на 90 білих безпородних щурах, поділених на 3 групи. Усім щурам механічно пошкоджували поверхню тонкої кишки. Контрольна група (група 1) включала 30 щурів, яким після ушкодження тонкої кишки в черевну порожнину нічого не вводили. У групі порівняння (група 2), що включала 30 щурів, вводили препарат мезогель у кількості 1 мл. У дослідній групі (група 3), що складалася з 30 щурів, вводили по 1 мл суміші метронідазолу, декстрану і контрикалу (у співвідношенні 1:1:0,1 відповідно) + O₂ у черевну порожнину і пошарово зашивали рану черевної стінки. На 5, 10 і 21-шу добу проводили гістологічний аналіз тканин, узятих у ділянці швів, очеревини, ушкодженого сегмента тонкої кишки, печінки, сальника.

Результати. На 10-ту добу в групах 1 і 2 частка м'яких спайок порівняно з 5-ю добою знизилася на 45,9% ($p=0,004$), а частка щільних спайок збільшилася на 64,7%. За результатами порівняльного міжгрупового аналізу на 5-ту добу частка м'яких спайок у групах 1 і 2 не відрізнялася, у групі 3 їхня частка була вищою на 4%, а частка щільних спайок – нижчою на 16,5%. У всіх групах переважали плоскоподібні спайки.

Висновки. У 1-шу добу відзначалося більше спайок м'якого типу, а повне формування щільних спайок спостерігалось до 21-ї доби. Переважали плоскоподібні спайки (54,5–60,0%). У розвиток спайок залучалася ділянка післяопераційних швів. У всіх тварин відзначалися спайки в ділянці «шви – очеревина» і спайки сальника. Введення в черевну порожнину препарату мезогель і суміші метронідазол + декстран + контрикал + O_2 сприяло зменшенню утворення спайок у петлях тонкої кишки.

Під час проведення експериментів із лабораторними тваринами дотримано всіх біоетичних норм і рекомендацій.

Автор заявляє про відсутність конфлікту інтересів.

Ключові слова: очеревина, сальник, моделювання спайок, протиспайкові препарати, тип спайок, локалізація.

Adhesions are the most common factor of long-term morbidity after abdominal surgery. Postoperative adhesions are pathological bonds formed between organs or tissues within a virtual space, such as the peritoneal cavity. It is one of the causes of postoperative morbidity, leading to various complications [3,6]. Abdominal adhesions occur in 79–93% of patients who have undergone extensive abdominal or pelvic interventions [4,10]. They can cause pain and distress or lead to more severe complications, such as bowel obstruction and female infertility [11]. The only efficient treatment for postoperative adhesions once developed is corrective surgery [4], but this procedure is invasive and often leads to recurrent adhesions [4], indicating the importance of their prevention. Currently, the most common preventive approach to the postoperative abdominal adhesive process is the implantation or introduction of barrier products made of biomaterials, including artificial films, liquid, or gel, separating the surface of the damaged tissue from the adjacent tissue/organ [9]. However, these barrier products are effective in only half of patients and have limitations in practical application. Therefore, there is a great need for more effective and more widely applicable preventive treatment of postoperative abdominal adhesions. For this purpose, it is essential to better understand the mechanism underlying the formation of postoperative adhesions.

Considering that adhesions do not have characteristic laboratory signs and are poorly visible with currently available imaging techniques, many cases of peritoneal adhesions remain undiagnosed for long periods of time, leaving healthcare professionals in an awkward diagnostic and therapeutic situation. Consequently, after extensive non-diagnostic testing and empiric treatment patients may not only have prolonged symptoms and adverse medical consequences, but also suffer from significant emotional distress or demoralization, which in turn may be misdiagnosed as depression, anxiety, or functional bowel disorder. In this regard, experimental studies appear especially relevant.

The purpose of the study is to investigate the adhesion types in the abdominal cavity after open operations in the experiment.

Materials and Methods

The study was conducted on the white outbred rats kept in the vivarium of the Research and Development Centre of the Azerbaijan Medical University. Keeping of rats and experimental studies in animal models were performed according to the Guide for the Care and Use of Laboratory Animals [2,8]. Before the experiment, the animals were placed in a special room in turns for one month. There was a health check of the animals before the operative procedure began. A total of 90 rats divided into three groups were used. Group 1 was the control group included 30 rats kept in three cages with 10 animals in each. After calyptol anaesthesia under sterile conditions, they were fixed dorsally on a special wooden board. Hair on the abdominal surface was shaved with a sharp razor. Then the abdominal cavity was opened using a midline access 3–4 cm long. The surface of the small bowel segment found carefully in the abdominal cavity on the left was mechanically damaged with a clean toothbrush until bleeding appeared. Then the abdominal wound was closed using a layered suture technique. The operation time on each animal was up to 15–20 minutes. Group 2 was the comparison group: 30 rats were fed and housed in three cages with 10 animals in each. Under sterile conditions, the animals were anaesthetised with calyptol, fixed dorsally on a special wooden board; hair on the abdominal surface was shaved with a sharp razor. Then the abdominal cavity was opened with a 3–4 cm midline access. The surface of the small bowel segment found in the left half of the abdominal cavity was mechanically damaged with a toothbrush (until bleeding appeared). Afterwards, one mL of mezogel was introduced. The preparation was administered into the abdominal cavity to prevent adhesions. The abdominal wound was closed with a layered suture technique. The operation on each animal lasted 15–20 minutes. Group 3 was the study group com-

Оригінальні дослідження. Абдомінальна хірургія

Table 1

Proportions of the abdominal adhesion types in white rats in the experimental groups, abs. (%)

Group	Type of adhesions					
	Day 5		Day 10		Day 21	
	filmy	dense	filmy	dense	filmy	dense
Group 1 (n=30)	24 (80.0)	6 (20.0)	13 (43.3)	17 (56.7)	0	30 (100)
Group 2 (n=30)	24 (80.0)	6 (20.0)	13 (43.3)	17 (56.7)	0	30 (100)
Group 3 (n=30)	25 (83.3)	5 (16.7)	13 (43.3)	17 (56.7)	0	30 (100)

Table 2

Types of adhesions in the abdominal cavity of white rats according to the prevention technique, abs. (%)

Type of adhesions	Group		
	Group 1, n=5	Group 2, n=5	Group 3, n=5
On day 5			
Wedge-shaped	5 (26.3)	1 (11.1)	0 (0)
Planar	9 (47.4)	5 (55.6)	4 (57.1)
Laminar	5 (26.3)	3 (33.3)	3 (42.9)
Total	19 (100)	9 (100)	7 (100)
On day 10			
Wedge-shaped	3 (21.4)	0 (0)	0 (0)
Planar	7 (50.0)	5 (71.4)	3 (60.0)
Laminar	4 (28.6)	2 (28.6)	2 (40.0)
Total	14 (100)	7 (100)	5 (100)
On day 21			
Wedge-shaped	1 (9.1)	0 (0)	0 (0)
Planar	6 (54.5)	4 (66.6)	3 (60.0)
Laminar	4 (36.4)	2 (33.4)	2 (40.0)
Total	11 (100)	6 (100)	5 (100)

prised 30 fattened rats housed in three cages, 10 animals in each. Under sterile conditions, the experimental animals were anaesthetised with calypsol, fixed on a special wooden board. Hair on the abdominal surface was shaved with a sharp razor. Then the abdominal cavity was opened with a midline access 3–4 cm long. A surface of the small bowel segment, found in the left half of the abdominal cavity, was mechanically damaged with a toothbrush until bleeding appeared. After that one mL of a specially prepared mixture of metronidazole, dextran, contrykal (in a ratio of 1:1:0.1, respectively) + O₂ was introduced into the abdominal cavity; and the abdominal wound was closed with a layered suture technique. Each surgical intervention lasted 15–20 minutes.

The experimental animals were kept in a separate animal facility under normal temperature and free-access feeding conditions. Rats that died of various reasons were subjected to autopsies and internal organ examinations to determine the possible causes of their deaths during the experiments. The macroscopic analysis results showed that most of the operated animals died due to severe purulent inflammation or haemorrhage from the abdominal organs. The surviving rats from all (control,

comparison, and study) groups were decapitated in the laboratory conditions according to the conventional rules on days 5, 10, and 21. Tissues taken from the sutural area, peritoneum, damaged small bowel segment, liver, and omentum were analysed histologically. The collected material was fixed in 10% formalin buffer for further microscopic examination. Histological tissue processing was provided in 75%, 85%, 95% and 99.9% concentrations of alcohol solution. Then specimens were contained in xylene solution and paraffinized. Then we formed and prepared histological blocks. The blocks obtained were sliced with a microtome to 3–5 microns thick. The sections were stained with standard haematoxylin-eosin, and the prepared microslides were microscoped using a light microscope (Leica DM 750, Germany). We recorded all changes observed during the microscopic examination using a camera attached to a microscope (Leica ICC 50, Germany). Statistical analysis was performed using SPSS for Windows software (version 12.0, SPSS Inc., Chicago, IL, USA). The parameters were expressed as numbers and percentages. We compared all main characteristics between groups using Mann–Whitney test of variance analysis and chi-square test.

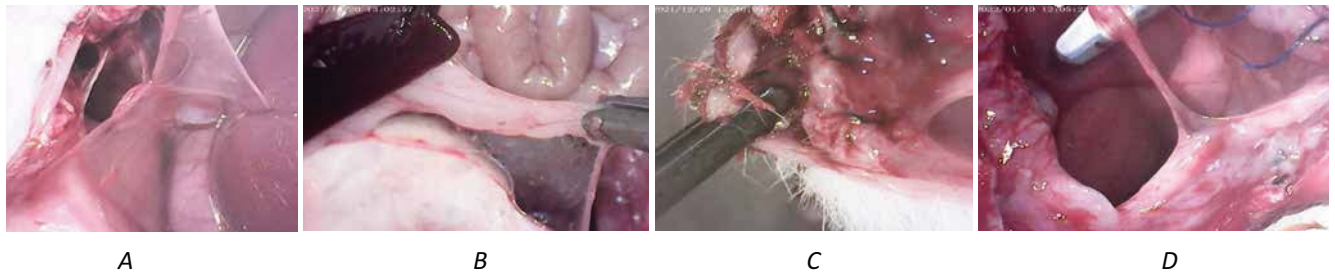


Fig. 1. Types of adhesions: A – planar, B – laminar, C, D – wedge-shaped ($\times 200$ magnification, haematoxylin-eosin staining)

Results and Discussion

The character of the abdominal adhesions appeared after the open operation was visually and macroscopically examined in all groups during the experiments (Table 1).

The results showed that on day 5 after the open surgery in all (control, comparison, and study) groups the proportion of filmy adhesions predominated and made up 80–83.3%, while the proportion of dense adhesions was lower, 16.7–20%. On day 10, the proportion of filmy adhesions was 43.3% and of dense adhesions was 56.7% in all groups. On day 21, only dense adhesions were formed in all animals, whereas no filmy adhesions were detected in all groups. The comparative between-group analysis of the adhesion type in groups 1 and 2 revealed that the proportion of filmy adhesions decreased significantly by 45.9% ($\chi^2=8.531$, $p=0.004$) compared to day 5, while the proportion of dense adhesions increased by 64.7%, respectively. The comparative between-group analysis of the adhesion type in group 3 showed that the proportion of filmy adhesions decreased significantly by 48.0% on day 10 ($\chi^2=10.335$, $p=0.002$), while, in contrast, the proportion of dense adhesions increased by 70.5%. The comparative between-group analysis demonstrated that in groups 1 and 2 on day 5, there was no difference in the proportion of filmy adhesions; in group 3, its proportion was slightly higher (by 4%), and the proportion of dense adhesions was lower (by 16.5%). There were no differences in the proportions of filmy and dense adhesions between the groups on day 10.

Thus, on day 1 after the open surgery, the experimental animals had more filmy adhesions, while the dense adhesion formation started later and were completely formed by day 21.

The types of abdominal adhesions formed in rats during the postoperative period were also investigated in all groups. Visual macroscopic analysis showed that three types of adhesions were predominantly formed in the abdominal cavity of rats in the postoperative period, namely, wedge-shaped, laminar, and planar (Fig. 1).

In our study, laminar and planar adhesions predominated in most animals (Table 2).

Table 2 shows that a total of 19 cases of adhesions were recorded in the control group (group 1) on day 5. Of these, 47.4% were planar, and 26.3% each were laminar and wedge-shaped, respectively. There was a decrease in the total number of adhesions, with a total of 14 adhesions on day 10. Planar adhesions accounted for half of them (50.0%), laminar adhesions for 28.6%, and wedge-shaped adhesions for 21.2%. On day 21, the total number of adhesions in animals of group 1 was only 11, of which planar made up 54.5%, laminar – 36.4%, and wedge-shaped – 9.1%. In the comparison group (group 2) on day 5, there were nine adhesions, of which planar – 55.5%, laminar – 33.3%, and wedge-shaped – 11.1%. On day 10 after the surgical operation the number of adhesions decreased to seven; besides, more than half of them were planar (71.4%), the remaining adhesions were laminar (28.6%), and there were no wedge-shaped ones. On day 21 of the experimental study in this group, we detected only six adhesions, of which planar adhesions made up 66.6% and laminar ones – 33.4%. No wedge-shaped adhesions were observed on day 10.

In the animals of the study group, there were only seven adhesions on day 5 of the experiment. Of these, planar composed 57.1%, laminar – 42.9%, and no wedge-shaped adhesions were detected. On day 10 in the animals ($n=5$), there were 3 cases (60.0%) of planar lesions and 2 (40.0%) laminar adhesions. There were no wedge-shaped adhesions. On day 21, a total of 5 cases of adhesions were seen in this group, and no wedge-shaped adhesions were detected either.

The experimental results demonstrated that planar adhesions predominated in all (control, comparison, and study) groups. This type of adhesions was most common in the control group. In the comparison and study groups, the introduction of anti-adhesion agents into the abdominal cavity reduced the total number of adhesions and their types. Although the number of laminar adhesions was relatively small, they occurred in all study groups. This adhesion type was also more frequent in the control group. The highest number of wedge-shaped adhesions was detected in the control group and the lowest in the study group.

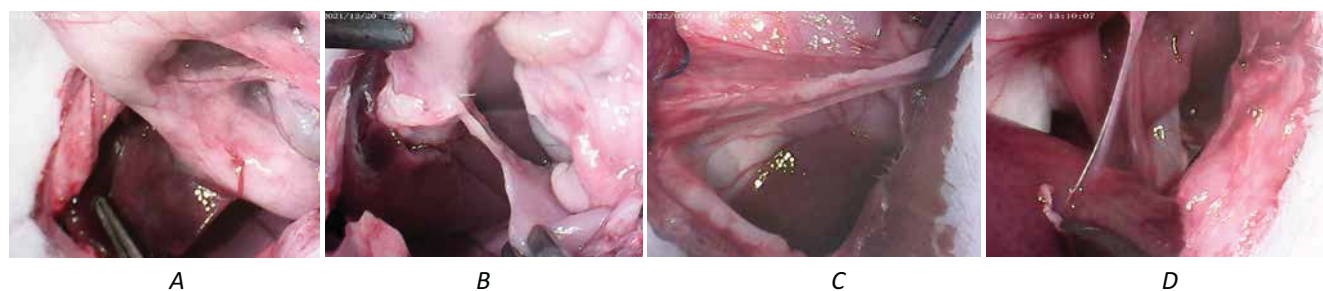


Fig. 2. Localization of adhesions formed in white rats during the postoperative period: A) peritoneum – omentum, B) peritoneum – small bowel, C) suture site – omentum, D) omentum – liver ($\times 200$ magnification, haematoxylin-eosin staining)

The macroscopic analysis results of the animals of all three groups showed that the damaged peritoneum was involved in most cases of adhesion formation (88.22%). Adhesions between the peritoneum and the omentum were recorded more frequently (64.70%) and between the peritoneum and the stomach less often (5.88%) (Fig. 2).

Adhesions between the damaged peritoneum and the damaged segment of the small bowel composed 17.64%. The highest number of those adhesions was detected in the control group (29.41%) and the lowest in the study group (14.70%). In the comparison group, the number of adhesions between the peritoneum and the omentum and between the peritoneum and the damaged small bowel segment was significantly lower than in the control group and slightly higher than in the study group (Table 3).

As shown in Table 3, the abdominal adhesions occurred mainly in the control group (group 1). In the comparison and study groups, these adhesion types were not detected or were very rare. This seems to be due to the effects of medications introduced into the abdominal cavity for therapeutic purposes. The adhesion formation also actively involved the site of postoperative abdominal sutures. All animals had adhesions in the sutures – peritoneum area and the omental adhesions. A positive effect on adhesion formation has the suture material used, mechanical damage, bleeding, and inflammation caused by microorganisms. The damaged small bowel segment is also actively involved in the adhesion formation in the abdominal cavity. The adhesions between the damaged small bowel and the liver, between the damaged small bowel and the stomach, and between the small bowel loops were most likely caused by the sequelae of intestinal damage, bleeding, and infection, and were particularly pronounced in the control group.

We presented the models capable of consistent formation of adhesions in the abdominal cavity of rats. Creating an animal model of abdominal adhesions with consistent, reliable and reproducible results for positive control is a major challenge. Currently, researches are

ongoing to find more efficient anti-adhesion agents and components [7,13,15]. In this experimental study, anti-adhesion agents, such as mezogel, metronidazole, dextran, and contrykal, were used. We showed that administration of a mixture of metronidazole, dextran, contrykal (in a ratio of 1:1:0.1 respectively) + O_2 into the rat's abdomen after a surgical procedure causing adhesions leads to fewer and less frequent adhesion formation. Our results are comparable with the literature data [1,13,14,16]. For example, M. A. Karpov et al. [5] presented the results of the study of adhesion formation in the rat's abdominal cavity after laparotomy followed by a single intraperitoneal administration of 5% aqueous oxidized dextran (OD) solution 2 mL with the molecular mass of 40 kDa (oxidation degree – 10%). On days 7 and 21 after laparotomy, the number of adhesions in the rats treated with OD was 7.5 and 4 times lower than in the animals not administered OD. The number of neutrophils in the adhesions on day 21 was many times lower in the rats receiving OD. On days 7 and 21 after laparotomy, the number of fibroblasts in the adhesions of rats treated and not treated with OD was similar but 2-fold higher than in the peritoneum of non-operated rats. The collagen content in the adhesions on day 21 after laparotomy was 10 times lower in the rats administered OD than in the animals not treated with OD.

Conclusions

After open laparotomy, more filmy adhesions form on day 1, and the complete dense adhesion formation occurs by day 21.

Planar adhesions predominate after open surgery (54.5–60.0%).

The site of postoperative sutures is actively involved in the adhesion formation. The sutures – peritoneum adhesions and omental adhesions are identified in all animals.

Administration into the abdominal cavity of mezogel as well as the mixture of metronidazole, dextran, contrykal (in a ratio of 1:1:0.1, respectively) + O_2 helps to reduce adhesions between the small bowel loops.

Table 3

Localization of abdominal adhesions in white rats according to prevention techniques, abs. (%)

Site of adhesion localization	Group			Adhesions (total number)
	Group 1 (n=30)	Group 2 (n=30)	Group 3 (n=30)	
Peritoneum – omentum	20 (29.41)	14 (20.58)	10 (14.70)	44 (64.70)
Peritoneum – small bowel (damaged segment)	7 (10.29)	3 (4.41)	2 (2.94)	12 (17.64)
Peritoneum – stomach	3 (4.41)	1 (1.47)	0	4 (5.88)
Damaged small bowel – liver	4 (5.88)	0	1 (1.47)	5 (7.35)
Small bowel loops	3 (4.41)	0	0	3 (4.41)
Total	37 (54.40)	18 (26.57)	13 (19.1)	68 (100)

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