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Morphological features of compensatory and reparative processes of the cecum in the long term of the experiment using polycaprolactone thread modified with L-arginine

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When performing surgery, the surgeon should be aware of the direct impact of the suture material on the structure of the sutured tissues and the condition of the postoperative scar, as the right surgical material is the key to rapid and successful wound healing. Therefore, searching for the safest surgical material, especially in pediatric abdominal surgery, is an urgent problem today.

The aim is to determine the morphological changes in the tissues of the cecum over the long term of the experiment using polycaprolactone thread modified with L-arginine.

Materials and methods. Colotomy was performed on 35 sexually mature outbred domestic rabbits (of different sexes) aged 8–10 months and weighing (2.6±0.2) kg. The experimental animals were divided into two groups: control and experimental. The first group was control (5 animals), and the second group was experimental (30 animals), where a monofilament absorbable thread with polycaprolactone modified with L-arginine was used to close the wound defect. The following research methods were used: histological, method of semi-thin serial sections, morphometric, and statistical methods.

Results. Fibroblasts play the most crucial role in the process of reorganization of the postoperative scar among cellular elements. Their activation and increase occur by day 14 of the experiment. On the 30th day of the study, the structural organization and restoration of connective tissue are determined. The reorganization of the muscle component changes: a layer of young myocytes is formed in the gap between the connective tissue capsule around the surgical thread and the fibrous component of the connective tissue fibrillar framework. By the 30th day of the study, the inflammatory reaction involving plasmocytes ceases, and single plasmocytes already perform the function of local immune surveillance. Also, the role of lymphocytes that actively migrate to the focus of the inflammatory process is suppressed by day 30 of the study, and single lymphocytes perform the function of local immune surveillance.

Conclusions. Thus, the dynamic changes in the cellular composition of the tissues of the perivulnar area of the cecum demonstrate the acceleration of the healing process and the formation of a loose scar when using polycaprolactone thread modified with L-arginine.

No conflict of interests was declared by the authors.

Keywords: morphology, compensatory and reparative processes, greater omentum, cecum, suture material.

Морфологічні особливості компенсаторно-репаративних процесів сліпої кишки у віддалені терміни експерименту при використанні полікапролактонової нитки, модифікованої L-аргініном**О.М. Проніна¹, С.М. Білаш¹, І.В. Ксьонз¹, М.М. Кобеняк², А.В. Пирог-Заказникова¹, Я.О. Олійніченко¹, М.М. Коптев¹, С.В. Донченко¹, Б.С. Кононов¹, В.В. Олексієнко¹**¹Полтавський державний медичний університет, Україна²Департамент охорони здоров'я Полтавської обласної державної адміністрації, Україна

Під час проведення оперативного втручання хірург повинен пам'ятати про безпосередній вплив хірургічного шовного матеріалу на структуру зшитих тканин та вираженість післяопераційного рубця, оскільки правильно обраний хірургічний матеріал є запорукою швидкого та успішного загоєння ран. Тому пошук найбезпечнішого хірургічного матеріалу, особливо в дитячій абдомінальній хірургії, є актуальною проблемою сьогодення.

Мета – визначити морфологічні зміни у тканинах сліпої кишки у віддалені терміни експерименту з використанням полікапролактонової нитки, модифікованої L-аргініном.

Матеріали та методи. Колотомію проведено на 35 статевозрілих безпородних свійських кролях (різної статі) віком 8–10 місяців, масою (2,6±0,2) кг. Піддослідних тварин було розподілено на дві групи: контрольну та експериментальну. Перша група – контрольна (5 тварин), друга група – експериментальна (30 тварин), де для зашивання ранового дефекту було використано монофіламентну розсмоктувальну полікапролактонову нитку, модифіковану L-аргініном. Було застосовано наступні методи дослідження: гістологічний; метод напівтонких серійних зрізів; морфометричний та статистичний метод.

Результати. У процесі реорганізації післяопераційного рубця серед клітинних елементів найважливішу роль відіграють фібробласти. Активація та їхнє збільшення відбувається до 14 доби експерименту. На 30 добу дослідження визначається структурна організація і відновлення сполучної тканини. Реорганізація м'язового компонента змінюється: утворюється шар молодих міоцитів у проміжку між сполучнотканинною капсулою навколо хірургічної нитки та волокнистим компонентом сполучнотканинного фібрилярного каркаса. До 30 доби дослідження запальна реакція за участю плазмочитів, припиняється, а поодинокі плазмочити вже виконують функцію місцевого імунного нагляду. Також роль лімфоцитів, які активно мігрують у вогнище запального процесу до 30 доби дослідження, пригнічується, а поодинокі лімфоцити виконують функцію місцевого імунного нагляду.

Висновки. Таким чином, динамічні зміни клітинного складу тканин перивульнарної зони сліпої кишки демонструють прискорення процесу загоєння і формування пухкого рубця при використанні полікапролактонової нитки, модифікованої L-аргініном.

Автори заявляють про відсутність конфлікту інтересів.

Ключові слова: морфологія, компенсаторно-репаративні процеси, великий чепець, сліпа кишка, шовний матеріал.

Introduction

Today, surgical interventions in surgical practice require not only the high professionalism of the doctor, but also the use of high-quality surgical suture material, considering the basic requirements, such as antigenic properties, sterility, versatility, biodegradation, strength, and others [6].

One of the factors leading to the inflammatory process during surgical interventions may be the suture material, which remains in the human body as a foreign agent and may subsequently cause postoperative septic complications [7,8,9].

The correct choice of surgical suture material is the key to rapid and successful wound healing after surgical interventions on the large intestine, including the cecum. Considering only the manipulative and technical conveniences of the suture material, such as strength, smoothness, elasticity, and ability to hold the knot, the surgeon forgets about the direct effect of the suture material on the structure of the sutured tissues, the degree of inflammation in the wound, the toxic effect of the suture on the body, and the severity of the postoperative scar [2,11].

In view of the above, scientific research on the study of morphological and functional features of the tissues of the

cecum when sutured with different sutures in the experiment is promising, timely and relevant for both practical and theoretical medicine, and the creation of new competitive, modified domestic sutures solves several problems of modern surgery [3,10,12]. A new modification of the domestic suture material polycaprolactone modified with L-arginine (PCL-MA) has been proposed, which does not have a negative effect on the hemomicrocirculatory tissue bed, stimulates the activity of vasculature in the process of reparative regeneration, reduces the risk of allergic reactions, and, as a result, improves oxygenation of the scar, which leads to the maturation of connective tissue and decreases [1,4,5,13,14].

The **aim** of the study – to determine the morphological changes in the tissues of the cecum over the long term of the experiment using polycaprolactone thread modified with L-arginine.

Materials and methods of the research

Colotomy was performed on 35 sexually mature outbred domestic rabbits (of different sexes) aged 8–10 months, weighing (2.6±0.2) kg. The experimental animals were divided into two groups: control and experimental. The first group was control (5 animals),

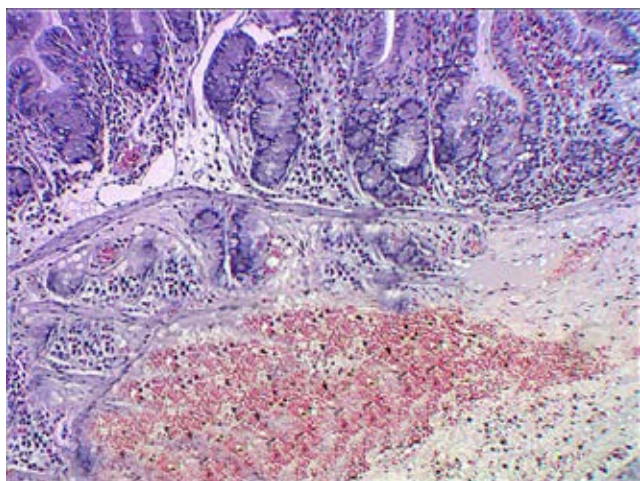


Fig. 1. Morphological changes in the perivulnar area of the rabbit's cecum wall on the 21st day after suturing the wound defect with PCL-MA suture. Paraffin section. Staining: haematoxylin and eosin. Magnification: okh. 10; obh. 40

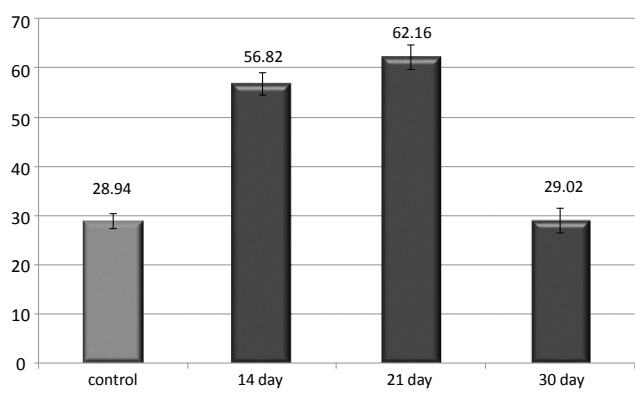


Fig. 2. Dynamics of changes in the average number of fibroblasts in the perivulnar area during suturing of a wound defect with PCL-MA suture in the dynamics of the experimental study

where access to the large intestine was performed, and intact material was taken, and the second group was experimental (30 animals), where a monofilament absorbable suture with polycaprolactone modified with L-arginine was used to close the wound defect. The animals were not euthanized. Material was collected on days 14, 21, and 30 of the experiment. The following research methods were used: histological – to determine the morphological changes in the wall of the cecum both normally and in the perivulnar area in the dynamics of the experiment; the method of semi-thin serial sections – to detail the histological structures of the intestinal wall in the dynamics of the experiment; morphometric method – to analyze the quantitative changes in structural components in the perivulnar area of the cecum in the dynamics of the experiment; statistical method – to establish the reliability of the dynamics of quantitative changes in structural components in the perivulnar area of the cecum in the dynamics of the experimental study.

The statistical analysis of the study results was carried out on a computer using the InStat software package for statistical processing of data from biomedical and epidemiological studies. The program allows to obtain research results in the form of the following predicted values: M – mean value; σ – standard deviation; m – standard error of the mean. The Student's criterion was calculated using tables. The results of the calculations were presented graphically in the form of histograms using Microsoft Office Excel software, indicating reliable intervals at a confidence level of 95%. Differences at $p < 0.05$ were considered significant.

The experimental studies were conducted in compliance with the requirements for humane treatment of experimental animals as regulated by the Law of Ukraine «On Protection of Animals from Cruelty» (No. 3447-IV of 21.02.2006) and the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes (Strasbourg, 18.03.1986).

Results of the study

The study showed that wound suturing with surgical suture material (polycaprolactone modified with L-arginine (PCL-MA)) not only restores the integrity of the intestinal wall but also leads to structural changes in the surrounding tissues aimed at resorbing the thread as a foreign body and forming a connective tissue scar at the incision site.

It was found that the average number of fibroblasts, myocytes, macrophages, lymphocytes, and plasma cells in the perivulnar area changed dynamically at different periods of the experimental study (Fig. 1).

Thus, the average number of fibroblasts increased after 14 days of the experiment, which was statistically significant at $p < 0.05$ compared to the control values.

The same tendency was observed after 21 days of the experiment, and the average number of fibroblasts increased compared to the control values and the values of the previous period of the experimental study: 2.22 and 1.11 times, respectively.

Analyzing the changes in the average number of fibroblasts after 30 days, it was found that, compared to the control, this indicator did not differ significantly and was within the statistical error. Compared to the previous period, it was 2.14 times lower at $p < 0.05$ (Fig. 2).

We also analyzed the number of myocytes in the area of the wound defect, which included both the muscular lamina propria and smooth muscle cells of other layers of the cecum wall. The morphological and morphometric analysis of myocytes of the perivulnar area of the cecum showed that the average number of myocytes varied after 14 days of the experiment. Thus, myocytes in-

creased statistically significantly by 1.12 times compared to the control values and 2.22 times compared to the previous period.

On the 21st day of the experiment, the number of myocytes increased by 1.36 and 1.21 times, respectively, compared with the control values and the previous period of the experiment.

On the 30th day of observation, it should be noted that this indicator was not statistically different from the control group at $p < 0.05$, and compared to the previous period, a decrease in the number of myocytes by 1.36 times was observed (Fig. 3).

Plasmocytes are known to be directly involved in the inflammatory process. Their reaction is very important, as they were visualized in the histological specimens of the control and the experimental groups and actively reacted in the dynamics of the experimental study.

After 14 days of the experiment, dynamic changes in the average number of plasmocytes in the perivulnar area were observed. Thus, compared to the control group, this indicator increased statistically significantly by 1.85 times at $p < 0.05$, and compared to the previous observation period, it also increased statistically significantly by 1.09 times at $p < 0.05$.

After 21 days of the experimental study, there is a different reaction of plasmocytes of the perivulnar area to wound defect suturing with PCL-MA suture. Thus, compared to the previous observation period, the average number of plasmocytes statistically significantly decreased by 1.67 times at $p < 0.05$, and compared to the control group, it remained 1.09 times higher at $p < 0.05$. Thus, it can be stated that after 21 days of experiment, the intensity of the inflammatory process decreased sharply.

After 30 days of the experiment, the cessation of the inflammatory process of the perivulnar area is determined, as evidenced by changes in the average number of plasmocytes. Thus, compared to the control group, this indicator did not differ statistically significantly at $p < 0.05$; compared with the previous observation period, it decreased by 1.06 times (Fig. 4).

Macrophages, as active participants in the regeneration of wound healing processes, immune and inflammatory processes, specific and nonspecific defense, and regulation of fibroblasts, mastocytes, lymphocytes, and endothelial cells, were directly involved in the implementation of the inflammatory process of the perivulnar area of the cecum after the implantation of PCL-MA suture.

After 14 days, the average number of macrophages in the perivulnar area compared to the control group was 7.16 times higher, and compared to the previous

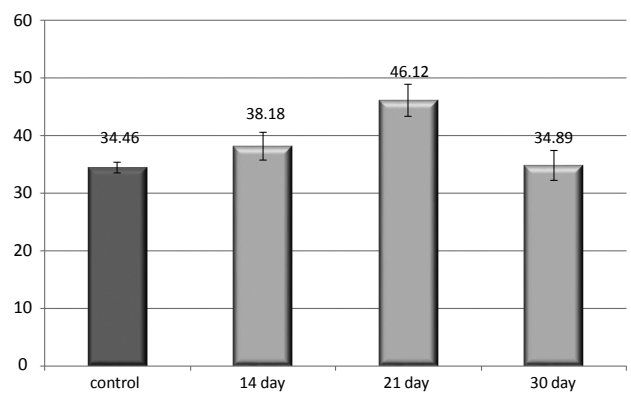


Fig. 3. Dynamics of changes in the average number of myocytes in the perivulnar area during suturing of a wound defect with PCL-MA thread in the dynamics of the experimental study

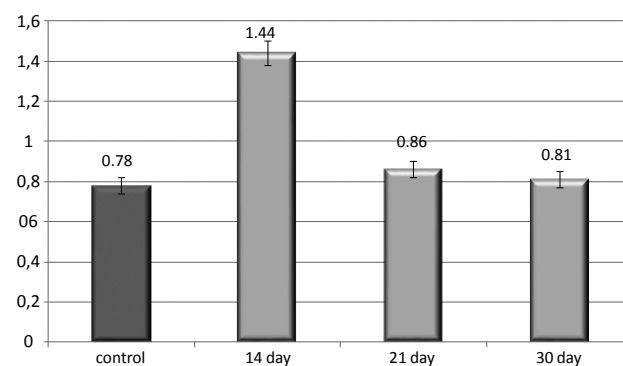


Fig. 4. Dynamics of changes in the average number of plasmocytes in the perivulnar area during suturing of a wound defect with PCL-MA suture in the dynamics of the experimental study

observation period, it decreased by 1.21 times, indicating the beginning of inhibition of macrophage activity in the perivulnar area after 14 days of the experimental study.

After 21 days of the experimental study, it was morphometrically determined that, compared to the control group, the average number of macrophages in the perivulnar area was statistically significantly higher by 4.05 times at $p < 0.05$. Compared with the previous observation period, the above indicator was statistically significantly reduced by 1.77 times at $p < 0.05$, indicating the continuation of the active inflammation focus after 21 days of the experiment.

After analyzing the results of the morphometric analysis of the average number of macrophages in the perivulnar area after 30 days of experimental study, we concluded that macrophages' participation in regulating the activity of the inflammatory process in the perivulnar area decreased. Thus, the average number of macrophages did not differ statistically significantly at $p < 0.05$ compared to the control group, and compared to the previous observation period, it decreased signifi-

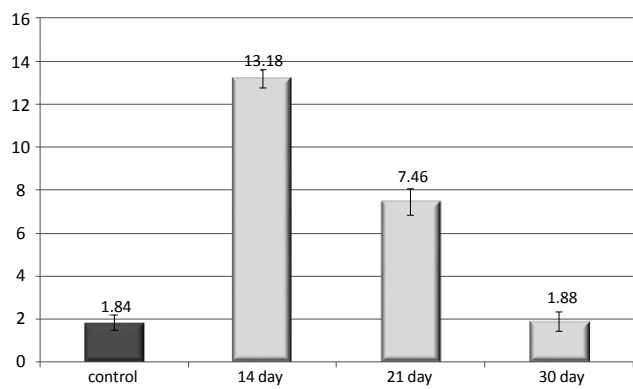


Fig. 5. Dynamics of changes in the average number of macrophages in the perivulnar area during suturing of a wound defect with PCL-MA suture in the dynamics of the experimental study

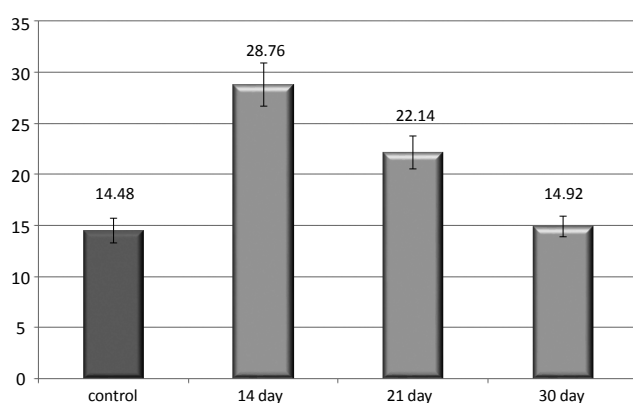


Fig. 6. Dynamics of changes in the average number of lymphocytes in the perivulnar area after suturing a wound defect with PCL-MA suture in the dynamics of the experimental study

cantly and very sharply statistically significantly by 3.97 times at $p < 0.05$ (Fig. 5).

In our study, we also identified and analyzed the dynamic changes in lymphocytes in the area of the wound defect sutured with PCL-MA sutures.

After 14 days of the experimental study, the average number of lymphocytes in the perivulnar area was 2.61 times higher than in the control group, and compared to the previous observation period, it decreased by 1.31 times, indicating a decrease in the activity of the inflammatory process in the perivulnar area after 14 days of the experimental study.

On the 21st day of the experimental study, an increase in the number of lymphocytes was morphometrically determined. Compared with the control group, the average number of lymphocytes in the perivulnar area was statistically significantly higher by 1.53 times at $p < 0.05$. There was a sharp decrease in lymphocytes compared to the previous observation period. The above indicator was statistically significant at $p < 0.05$ and sharply decreased by 1.29 times, indicating

the continuation of the inflammation focus after 21 days of the experiment.

According to the results of morphometric analysis of the average number of lymphocytes in the perivulnar area after 30 days of experimental study, we concluded that the participation of lymphocytes in regulating the activity of the inflammatory process in the perivulnar area decreases. Thus, the average number of lymphocytes did not differ statistically significantly at $p < 0.05$ compared to the control group, and compared with the previous observation period, it decreased significantly and sharply statistically significantly by 1.29 times at $p < 0.05$ (Fig. 6).

Thus, it can be concluded that surgical polycaprolactone thread containing L-arginine on its surface accelerates the realization of the inflammation focus, and by the 30th day of the experimental study, inflammatory processes cease.

Conclusions

1. Fibroblasts play the most crucial role in reorganizing the postoperative scar among cellular elements. Their activation and increase occurred by the 14th day of the experiment. On the 30th day of the study, the structural organization and restoration of connective tissue were determined, which did not differ from the control group.

2. The reorganization of the muscular component changes by the 14th day of the experiment. A layer of young myocytes is formed in the gap between the connective tissue capsule around the surgical thread and the fibrous component of the connective tissue fibrillar framework.

3. Plasmocytes are directly involved in the implementation of the inflammatory process in the perivulnar area. They reacted actively to the dynamics of the experimental study. Their participation in local immunity peaked between 14th and 21st days of the research. By the 30th day of the study, the inflammatory reaction involving plasmocytes ceases, and single plasmocytes already perform the function of local immune surveillance.

4. Lymphocytes, which actively migrate to the inflammatory focus in the early stages of the experiment. On the 14th day of the study, their average number was quite high compared to the control group. By the 30th day of the study, the inflammatory reaction involving lymphocytes was suppressed, and single lymphocytes already performed the function of local immune surveillance.

No conflict of interests was declared by the authors.

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