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Possibilities of local effect the healing of small bowel sutures in a rat model of acute intra-abdominal infection

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Abstract

Aims: Investigate the regeneration of the small bowel suture line regeneration after suturing with a material with antioxidant properties.

Methodology: 7 intact rats, 63 with simulated intra-abdominal infection (IAI). A laparotomy was performed in 12 h after simulating. The small bowel was cut and sutured in 56 rats (2 groups of 28) after an abdominal lavage. Standard Kapron was used in the control group (CG). Kapron impregnated with a 5% solution of ethylmethylhydroxypyridine succinate was used in the research group (RG). Sections of the small bowel were collected from a total of 21 rats (7 intact rats, 7 rats with intra-abdominal infection (IAI) models, and 7 rats at different time points (1, 3, 5, and 7 days) post suturing) for subsequent histological examination. The preparations were stained with hematoxylin-eosin and by Mikel Calvo. The R/B coefficient (R/BC) was determined. The experiment's time duration was 7.5 days in December 2022.

Results: R/BC increased ($p<0.05$) in the connective tissue fibers of the small bowel submucosal base in 12 h after the simulating of IAI. R/BC increased ($p<0.05$) in both groups in 1 day after suturing. R/BC increased in CG, decreased ($p<0.05$) in RG in 3 days. Fibrin was found between the ligature's fibers in RG. R/BC decreased ($p<0.05$) in both groups in 5 days, indicators were smaller ($p<0.01$) in RG. Fibrin was found between the ligature's fibers in CG, granulation tissue was in RG. R/BC decreased in both groups in 7 days, but indicators were smaller in RG ($p<0.01$). Unfilled areas in the granulation tissue between the ligature fibers were in CG.

Conclusion: 1. The use of ligatures impregnated with the 5% ethylmethylhydroxypyridine succinate solution for suturing the small bowel in rats with intra-abdominal infection models is associated with the significant decrease in the activity of local proteins oxidation and the significant acceleration of local regeneration processes. 2. The creation of a suture material with antioxidant properties may be a promising direction for the prevention of suture leakage.

Keywords: Anastomotic Leak; Sutures; Protein Oxidation; Antioxidants; Regeneration; Wound Healing.

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Introduction

Bowel suture leakage is a severe complication [1-3]. The main risks for this, apart from technical errors, are regeneration disorders. The process of repairing damage to the bowels, known as reparative regeneration, is a

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highly intricate process that is governed by numerous factors. Different bowel tissues regenerate in different ways after damage. Particularly, the mucous membrane regenerates by restitution, that is, a tissue identical to the damaged one is formed. Other bowel layers are regenerated by substitution, that is, connective tissue and a scar are formed. It is the substitution disorders that causes bowel sutures leaking [4].

There are various factors that can contribute to the development of bowel suture leakage. Different causes have leading importance at different stages of bowel sutures healing. At first, the processes of primary sealing of the suture line and ligature channels may be disrupted, which is due to a violation of the fibrin formation processes. Gut microflora penetrates through the leaky ligature channels and the space between the tissues from the bowel cavity, which infects the tissues and causes their inflammation. In addition, this microflora infects the peritoneum and causes its inflammation, which additionally damages the bowel walls and disrupts regeneration [5].

Such processes are facilitated by mechanical damage to the bowel walls with ligatures. This becomes crucial, particularly when utilizing outdated ligatures. The bowel suture technique is also important. The rough handling increases the damage. Excessive ligatures tightening and tissues compression causes their ischemia, which increases the damage. Conversely, insufficient ligatures tightening leaves larger gaps between the tissues, causing leakage of bowel contents [6].

Later, 2-3 days after suturing, the causes of suture leaking may be disruptions of regeneration regulation mechanisms. These mechanisms are very numerous. The main molecular mechanisms are proteolysis, fibrinolysis, oxidation and reduction. These mechanisms are regulated by numerous factors, namely, immunocompetent cells, liver cells, endothelial cells, mucous membrane cells, microorganisms, etc. [4,6,7]. Therefore, a violation of these regulators state disrupts regeneration. Further on, the processes of connective tissue formation, based on the participation of cells, may be disrupted. All processes of regeneration disruption are interrelated. So, the violation of one violates the rest. Different types of vicious cycles lead to the emergence of such violations, as various negative processes mutually reinforce one another.

Main reasons of regeneration disorders are the presence of malignant neoplasms, comorbid diseases, bowel ischemia, bowel obstruction, acute intra-abdominal infections [1-3,8,9]. Various ways have been proposed for the prevention of sutures leakage, such as the use of intestinal tubes [10,11], preventive stomas [12,13], modification of the suture application technique [3,14-16], sutures strengthening [17-19]. However, their effectiveness does not meet the urgent needs [20,21].

The use of intestinal tubes or preventive stomas is primarily aimed at reducing pressure in the cavity of the bowels and eliminating bowel contents. Lowering the pressure in the cavity of the bowel really has a good effect. This effect is due to the improvement of microcirculation in the bowel wall. It helps the regeneration of the bowel wound. But this does not preclude other factors that disrupt regeneration. Outputting to the outside of the bowel contents reduces its negative affect on the suture line. But it is impossible to eliminate all the content. First of all, it is about microorganisms whose vital activity is activated as a result of the disease. Microorganisms synthesize various substances, namely, those capable of disrupting regeneration – enzymes, toxins, etc., in the process of vital activity. So, such ways do not preclude the disruption of regeneration mechanisms caused by the inflammatory process. In addition, the use of stomas causes significant anatomical deviations of the organs and structures in the abdominal cavity. Adhesions are more often formed in the abdominal cavity. These methods also necessitate repairing the stoma through rep-operations. The bowel contents with water, proteins, etc. are lost through stomas and tubes. This can cause deterioration of metabolism and, as a result, regenerative disorders. The deterioration of metabolism requires appropriate treatment, use of additional medications. The condition of patients deteriorates the duration of treatment increases. This causes the treatment to become more expensive. It should also be noted that the use of stomas or tubes is only a temporary procedure. During second surgical interventions, problems with regeneration arise again and there is again a threat of the leaking sutures development.

The modification of the suturing technique contributes to a better matching of the bowel wound juxtaposition, which improves the conditions for regeneration. But this does not eliminate the main cause of the suture leakage, namely, the disruption of regeneration mechanisms [4,6,7]. In addition, modified sutures, especially mechanical sutures, cannot always be used for severe inflammatory disorders of the bowel wall (oedema, thickening, dilatation etc.), when it is necessary to anastomose loops of bowels that differ significantly in size [8,13,15,20]. This reduces the possibility of using modified sutures in emergency surgery.

The use of suture strengthening ways has limitations. Particularly, the great omentum can be used only if it does not have inflammatory disorders. This, for example, cannot be done with generalised peritonitis. At the same time, generalised peritonitis is one of the main causes of suture leakage and there is often a need to prevent suture leakage [4,6,8].

Muscles, namely the sternocleidomastoid, can only be used in thoracic surgery. Abdominal surgery does not allow for this possibility. In addition, fixing the muscles over the sutures does not improve regeneration in any way. At the same time, this procedure can cause severe anatomical deviations and, conversely, cause complications [8,18].

The use of glue application on the suture line seals the suture and channels of ligatures in the bowel wall. This reduces the possibility of leakage of bowel contents. But it does not affect the bowel wound regeneration, which is disturbed due to excessive inflammation, hypoxia, oedema, etc [1,4,8].

An important area of research is the use of precision suturing technologies using robotic surgery, particularly, robotic intestinal suture [22-24]. The use of robotic surgery makes it possible to achieve minimal traumatisation of tissues, almost perfect tissues matching, controlled tightening of ligatures, etc. [23,24] That is, such technologies reduce to a minimum the technical problems of bowel suture leaking. However, these technologies have a number of limitations. Their use in emergency abdominal surgery is quite problematic due to technical reasons. First, this is a limitation of the robot program [22]. In an unconventional scenario, only a human can determine the optimal solution. But the problems of bowel sutures arise precisely in non-standard situations [4,6]. In addition, even the most advanced suturing technique does not eliminate the problem of regeneration disorders. Eventually, the robotic technology is extremely expensive. Due to these problems, many are currently taking it critically, although, of course, these technologies have a great future.

It is worth noting that the efforts of researchers are focused, for the most part, on external influences on the area of applied sutures, namely, their sealing, reduction of intra-bowel pressure, removal of bowel contents, etc. Currently, several studies touch on the possibility of influencing the regulation of regeneration mechanisms, which, particularly, can be disturbed under the conditions of the inflammatory process in the walls of organs caused by intra-abdominal infections (IAI) [8].

It is not surprising that many researchers are dedicated to exploring alternative methods for enhancing bowel suture regeneration. Such research primarily concerns the modification of the ligature's material using nanotechnology [25,26]. The modification of ligatures makes it possible to deliver various components affecting regeneration directly to the suture zone. These are, in particular, ligatures coated with silver nanoparticles, the effect of which reduces the inflammation intensity [27]. A number of studies are devoted to ligatures with antimicrobial properties [28-30]. Such ligatures deliver antimicrobial drugs to the zone of the ligature channel, which stop the negative influence of gut microflora on regeneration. Another aspect is the use of ligatures with zinc oxide, which is a powerful stimulator of regeneration [31].

To optimise the local effect, bioabsorbable and biodegradable ligature technologies are proposed [32-34]. In the process of bioresorption and biodegradation, such ligatures release some substances that affect some mechanisms of regeneration [34]. Then, these ligatures are gradually replaced by connective tissue, which improves the result of their use.

There is a significant focus on researching technologies that control the mechanisms of cellular regeneration. This is, namely, the use of stem cells, autologous cells, growth factors, that potentiate the activity of cell regeneration processes [35-37]. Researchers are exploring the potential applications of the collagen extracellular matrix [38,39], inhibitors of matrix metalloproteinases [40], the use of which significantly improves regeneration.

Research aimed at monitoring wound healing using modern nanotechnology is very interesting. For this, researchers suggest measuring local temperature, pH, and oxygenation [41,42]. However, these studies currently concern soft tissue wounds. The use of such technologies for bowel sutures is, for now, a matter of the future. However, their perspective is clear, since it would make it possible to monitor the healing process, detect violations and apply the necessary means.

Several researchers propose to manipulate fundamental processes of regeneration. Specifically, hyperbaric oxygen therapy, activating sympathetic neurons, inducing the hypoxic adaptive response, and neutralising gut microorganisms [43-46]. But these experimental approaches are still in the preclinical phase. It should be noted that these approaches are certainly rational. But these are means of indiscriminate action. There are no clear data on the state of local regeneration mechanisms under the influence of these means. In addition, these means can negatively affect the mechanisms that regulate other vital processes. It is worth noting that these approaches are certainly rational. But these are means of indiscriminate action. There are no clear data on the state of local regeneration mechanisms under the influence of these means. In addition, such general effects can negatively affect the mechanisms that regulate other vital processes. Some means have contraindications for use. Specifically, hyperbaric oxygenation is not recommended for certain lung diseases, and microbial decontamination of the bowel should be avoided in cases of dysbiosis, certain types of colitis, and similar conditions. Consequently, alternative approaches that involve localized action appear to hold greater promise.

Indeed, regeneration is a very complex process that depends on many factors. Therefore, additional studies on the possibility of influencing these factors to stimulate regeneration processes are appropriate.

Redox reactions (ROR) are one of the important metabolism regulation links. This is due to their participation in the processes of adaptive restructuring in various physiological and pathological conditions [47]. Components of ROR are involved in the regulation of the inflammatory process, immune response, regeneration, etc. Researchers of these problems often recommend antioxidant drugs to correct ROR, given the activation of oxidation reactions

[48]. The activity of POR is determined by numerous genetic factors. The violation of these factors can cause a violation of the balance of POR [49,50].

It is generally known that excessive activation of oxidative reactions is one of the important factors of impaired regeneration [51]. At the same time, excessive activity of oxidative reactions is compensated by antioxidant mechanisms [52]. However, with the development of IAI, there is a deficiency of antioxidant mechanisms, which requires correction [48,53]. Excessive activation of oxidative processes can cause leakage of bowel sutures and anastomoses [54,55]. This is a consequence of the free radicals' negative effect both on the systemic mechanisms of regeneration regulation and on local processes, particularly on the formation of granulation and connective tissue in the area of the bowel wound.

Exploring the genetic factors influencing ROR regulation holds great promise for future research. For this purpose, changes in a number of genes that determine the activity of ROR were studied [49,50]. Determining the genetic features of these reactions would make it possible to apply individualised exposure to correct changes in ROR. However, the authors note that ROR processes are coded by a very large number of genes [49,50]. Because of this, it is currently almost impossible to individualise treatment. Therefore, researchers suggest determining biochemical markers of ROR activity and prescribing treatment based on them [53].

So, one of the promising directions for improving the ways of preventing sutures leakage is the study of the possibility of redox reactions local regulation in order to normalise regeneration processes. At the same time, we did not find any studies aimed at local correction of these processes in the area of bowel sutures.

Research Problem

The research problem, which was the basis of this study, consisted in a comparative study of the regeneration of the small bowel suture line after suturing with different type of suture materials: standard suture material and suture material pre-soaked with an antioxidant agent. The research also aimed to study the oxidation activity of the bowel wall proteins, as excessive oxidation disrupts the structure of proteins and their biological activity [56]. At the same time, proteins, particularly, fibrin, collagen, etc., were the basis of regeneration processes [8,51].

Research Focus

The research focus was the experimental analysis of the protein's oxidation activity and the regeneration processes of the bowel sutures line after the small bowel suturing in animals with IAI models. This study aimed to evaluate the results of using suture material impregnated with an antioxidant agent. This research could become the basis for the development of suture nanomaterials with antioxidant properties in the future. By integrating genetic, morphological, and molecular analyses of different aspects related to bowel suture leakage, a personalised prevention approach can be developed as the foundation for tackling this issue.

Research Aim and Research Questions

1. To evaluate indicators in the bowel wall proteins oxidation activity after simulating of IAI.

2. To study the differences between the protein's oxidation activity indicators in the bowel suture line after suturing with standard material and material impregnated with an antioxidant agent.

3. To study in the small bowel suture the line regeneration after suturing with standard material and material impregnated with an antioxidant agent.

4. To study the differences of the regeneration processes in the bowel sutures line after suturing with standard material and material impregnated with an antioxidant agent.

Research Methodology

General Background

Histological examination is essential for accurately determining the regeneration processes. Therefore, it is crucial to conduct these studies as part of an experimental approach.

Sample / Participants / Group

70 white non pedigree female rats. All rats were sexually mature (age 6 months). The weight of the rats was 180-200 g. Before the start of the experiment, the rats were in a vivarium. All rats experienced the same conditions for their stay and food. IAI was simulated in 63 animals. 7 intact rats were used to study the initial data. 7 rats were used to study the data after IAI simulation. Suturing of the small bowel was performed in 56 rats with IAI models: 28 rats were the control group, and 28 rats were the experimental group. Throughout the experiment, the rats were placed under identical conditions and provided with the same food and water. The experiment lasted 7.5 days. On the first day, IAI was simulated. Subsequently, the small bowel underwent suturing. Over the course of seven days, meticulous observations were conducted, while samples were collected for further research.

The criteria for selecting rats for each group were the same age, weight of 180-200 g, the absence of diseases in rats. Rats for each group were randomly selected from the 70 rats taken for the experiment.

Each rats group was homogeneous in terms of age, sex, animal weight, and living conditions. The average weight of intact rats was 191.35±2.15. The average weight of the rats with IAI models was 192.12±1.29. The average weight of the rats in the control group was 192.7±3.43. The average weight of the rats in the research group was 191.59±3.76. Therefore, all groups were comparable by these criteria ($p>0.05$).

Instrument and Procedures

IAI was simulated by puncture intraperitoneal injection of 20% autofecal mixture. A laparotomy was performed in 7 intact rats and in 63 rats with IAI models in 12 h after IAI simulating. The peritoneal cavity was flushed with a 0.9% solution. NaCl solution in 56 rats with IAI models. After that, in the middle part of the small bowel an enterotomy with a length of 0.7 cm was performed and the incision was sutured transversely with two separate knotted through sutures. Standard Kapron was used in the control group. Kapron, pre-soaked for 24 h with the 5% solution of ethylmethylhydroxypyridine succinate, that is an inhibitor of oxidative reactions [57], was used in the research group. The laparotomy wound was sutured in rats of the control and research groups. Kapron was selected due to its excellent wicking capabilities and effective absorption of liquids.

Sections of the small bowel in the middle part were taken for histological examination in 7 intact rats and 7 rats with IAI models during the laparotomy. A laparotomy was performed and sections of the sutured parts of the small bowel were taken for histological examination in rats with sutured bowel, in 1, 3, 5, and 7 days after suturing (7 rats each).

Histological sections were stained with hematoxylin-eosin and bromophenol blue by Mikel Calvo. Stained preparations were studied in a light optical microscope Delta Optical Evolution Pro 100. The R/B coefficient (R/BC) was determined by computer microspectrophotometry [58] on digital copies of images in the ImageJ 1.52 environment (free license, W. Rasband, National Institute of Health, USA, 2019). R/BC shows the ratio of acidic and basic proteins. If the R/BC value is equal to "1" – the ratio between basic and acidic proteins is equal, and an increase in the R/BC value indicates the predominance of acidic proteins [58].

Inhalational sevoflurane anesthesia was used to anesthetise the animals. Animals were removed from the experiment by an overdose of anesthetic.

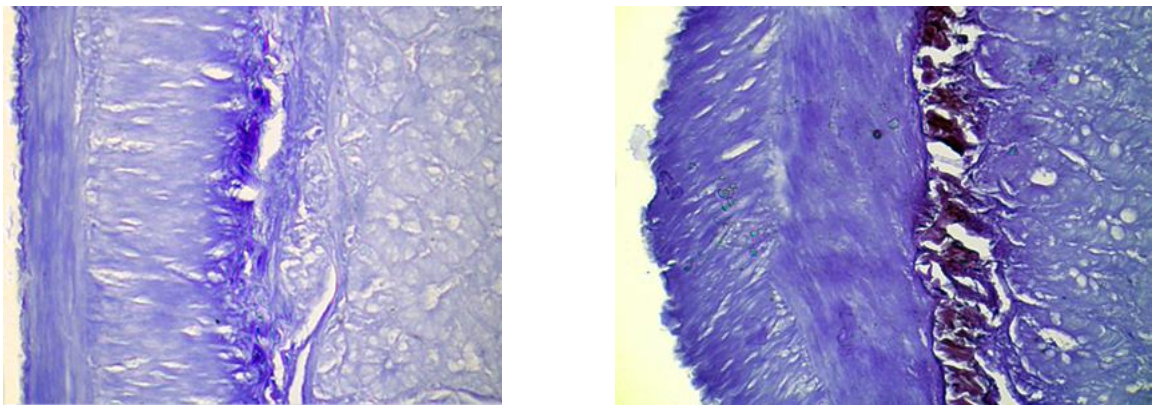
While performing the work, the norms of conducting research in the field of biology and medicine were observed: the Vancouver Conventions on Biomedical Research (1979, 1994), the Council of Europe Convention on the Protection of Vertebrate Animals Used in Experiments and for Other Scientific Purposes (1986).

Data Analysis

The testing of the law of distribution of samples for normality was carried out using the Shapiro-Wilk test. The null-hypothesis of the Shapiro-Wilk test is that the population is normally distributed. Thus, if the p value is less than the chosen alpha level, then the null hypothesis is rejected and there is evidence that the data tested are not normally distributed. On the other hand, if the p value is greater than the chosen alpha level, then the null hypothesis (that the data came from a normally distributed population) cannot be rejected. To test the hypothesis of equality of means, the Wilcoxon test was used, since the data distribution in the samples differed from normal. The null hypothesis assumed that there is no significant difference between the data that were being compared. The alternate hypothesis posited that there exists a substantial discrepancy. If the p-value is greater than alpha, then we did not reject the null hypothesis and assumed that there is no significant difference between the two samples. We have set the level of significance 0.05.

Research Results

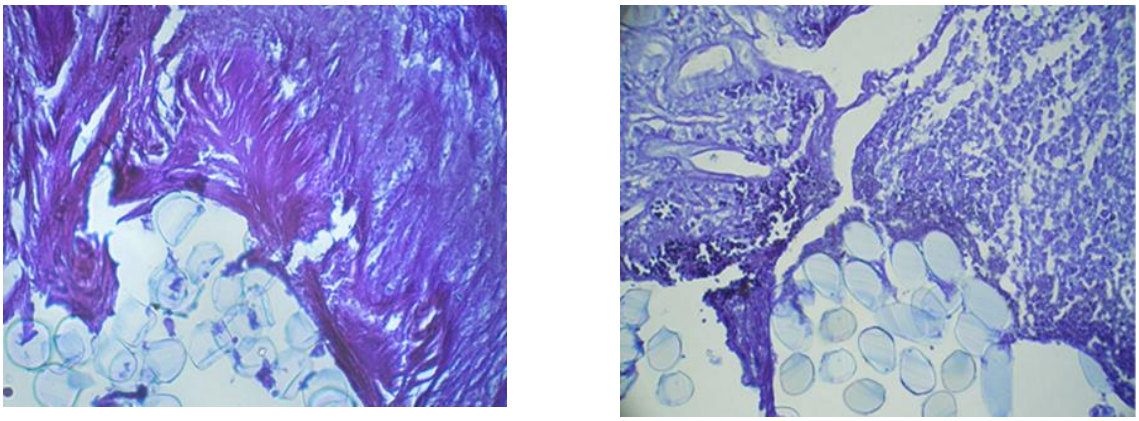
In intact rats, the value of R/BC (Fig. 1 A) in the connective tissue fibbers of the small bowel submucosal base was 1.02±0.01. The value of R/BC (Fig. 1 B) increased to 1.85±0.01 ($p<0.05$) in 12 h after IAI simulating.



A B

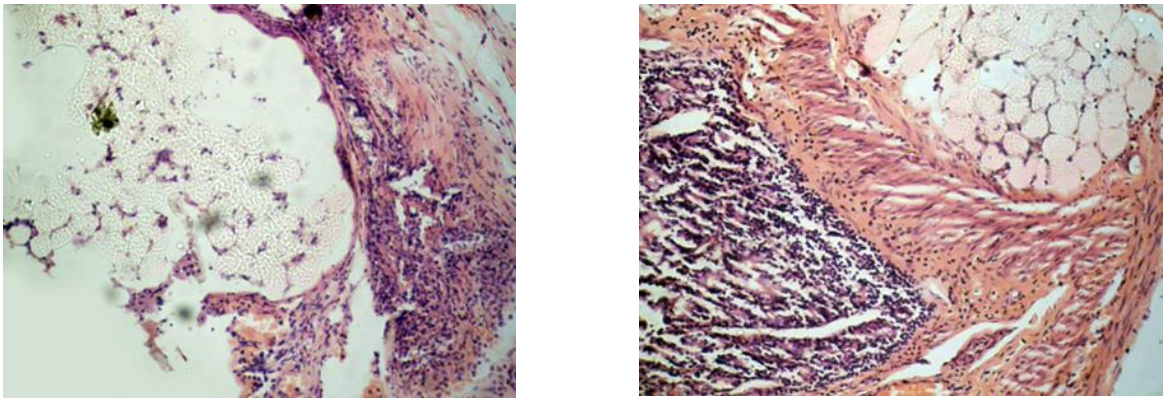
Fig. 1 Histological preparations of the rats' small bowel walls, Mikel Calvo staining (objective 20X, eyepiece 10X, total optical magnification 200X): A – intact animal, B – in 12 h after IAI simulating

The value of R/BC in the control group (Fig. 2 A) increased to 2.21±0.08 ($p<0.05$) in 1 day after suturing the bowels. The value of R/BC in the research group (Fig. 2 B) increased to 2.13± 0.07 ($p<0.05$). There was no significant difference between the indicators of the groups. At the same time, signs of an inflammatory process (oedema, infiltration by leukocytes, local hemorrhages) were found in the bowel walls around the ligatures (Fig. 3). Inflammatory deviations were greater in the control group, as evidenced by a significant infiltration of polymorphonuclear leukocytes, a greater degree of oedema (Fig. 3 A).



A B

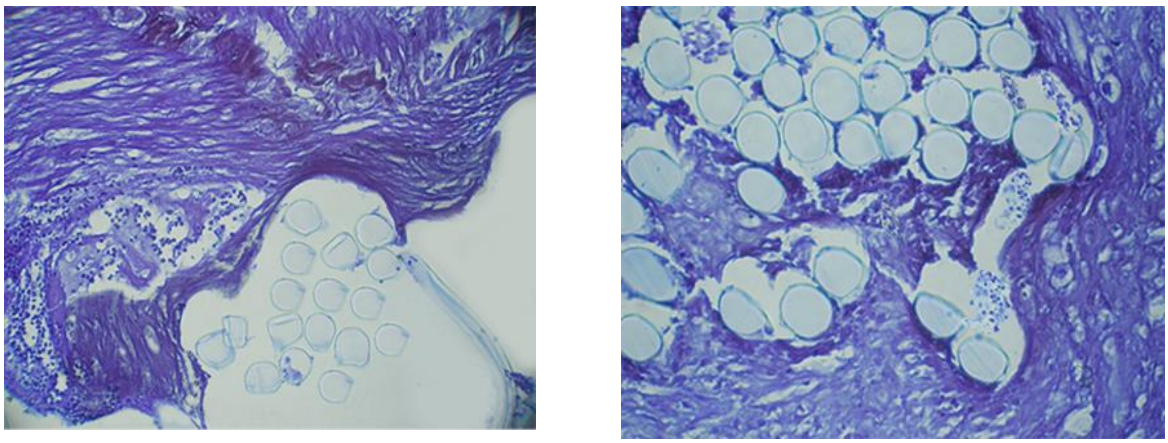
Fig. 2 Histological preparations of the rats' small bowel walls in 1 day after suturing, Mikel Calvo staining (objective 20X, eyepiece 10X, total optical magnification 200X): A – control group, B – research group



A B

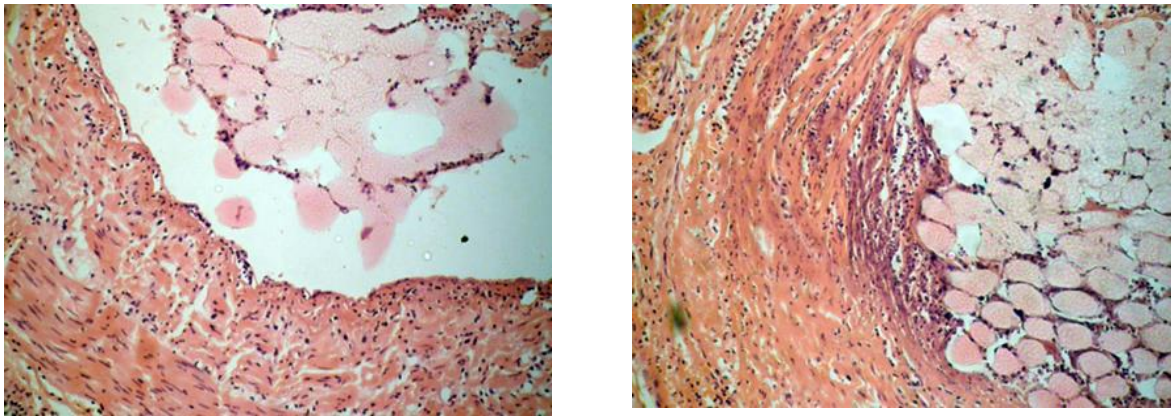
Fig. 3 Histological preparations of the rats' small bowel walls in 1 day after suturing, hematoxylin-eosin staining (objective 20X, eyepiece 10X, total optical magnification 200X): A – control group, B – research group

The value of R/BC in the control group in 3 days after suturing (Fig. 4 A) was 2.3±0.08. It was slightly more than in 1 day. At the same time the value of R/BC in the research group (Fig. 4 B) decreased to 1.91±0.05. It was statistically significantly smaller than both from the control group ($p<0.01$) and from the indicators for 1 day ($p<0.05$). The gaps were observed between the fibers of the ligatures and the channel formed by the ligatures in animals of the control group (Fig. 5 A). Such gaps were almost not detected in the research group (Fig. 5 B), and fibrin deposition was noted between the fibers of the ligatures (Fig. 4 B).



A B

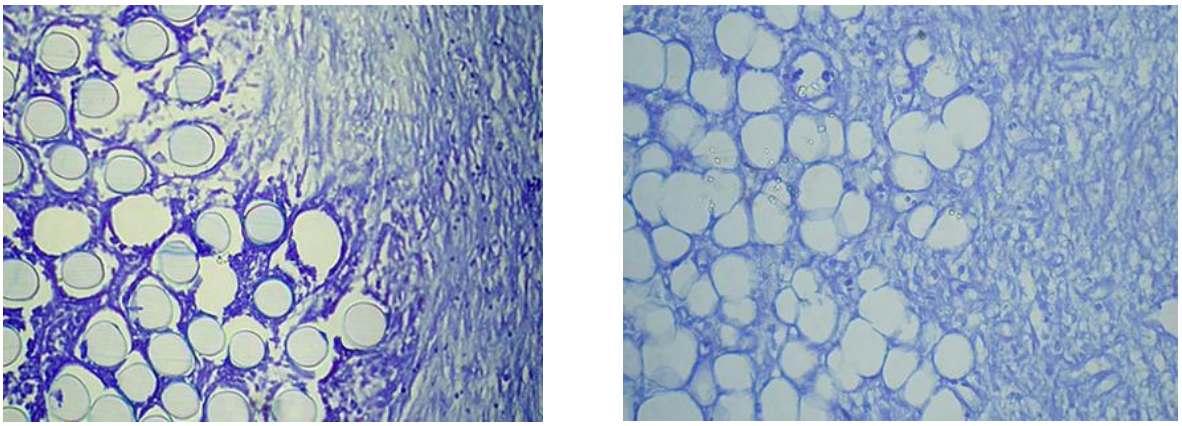
Fig. 4 Histological preparations of the rats' small bowel walls in 3 days after suturing, Mikel Calvo staining (objective 20X, eyepiece 10X, total optical magnification 200X): A – control group, B – research group



A B

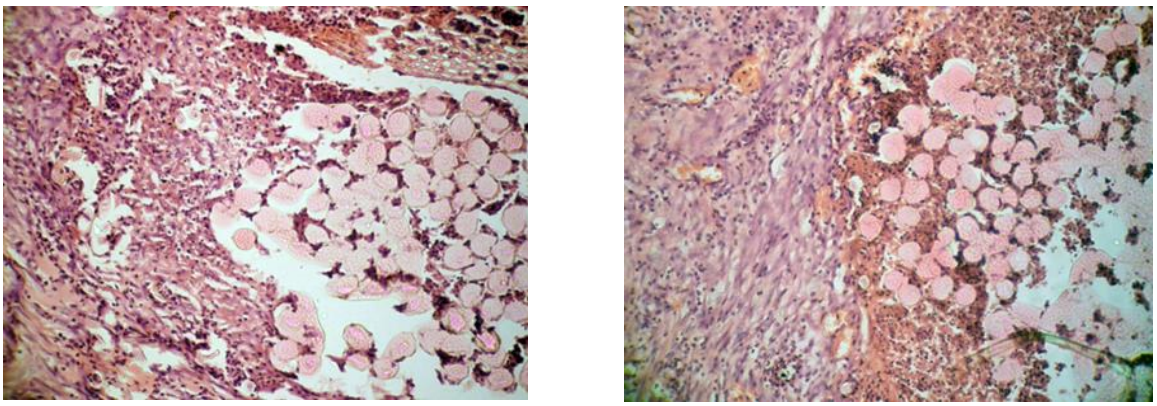
Fig. 5 Histological preparations of the rats' small bowel walls in 3 days after suturing, hematoxylin-eosin staining (objective 20X, eyepiece 10X, total optical magnification 200X): A – control group, B – research group

The value of R/BC in the control group (Fig. 6 A) significantly ($p<0.05$) decreased to 1.9±0.06 in 5 days after suturing. The value of R/BC in the research group (Fig. 6 B) decreased to 1.4 ±0.08. It was statistically significantly smaller than both from the control group ($p<0.01$) and from the indicators in 3rd day ($p<0.05$). At the same time, although the gaps between the ligatures decreased, it remained in the control group (Fig. 7 A), and significant amount of fibrin was found between the fibbers of the ligatures (Fig. 6 A). There were almost no gaps between the ligatures and the channel (Fig. 7 B) in the research group at this time, and the growth of granulation tissue was found between the fibbers of the ligatures.



A B

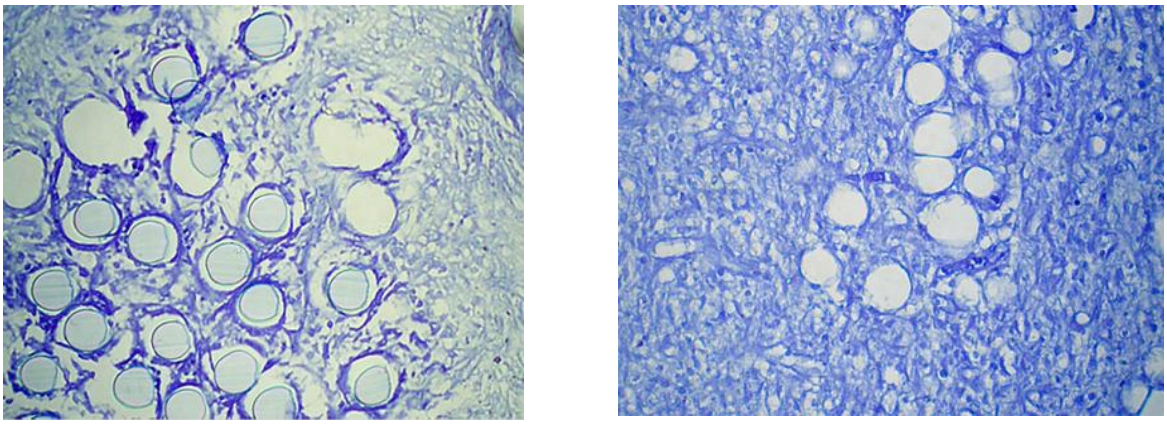
Fig. 6 Histological preparations of the rats' small bowel walls in 5 days after suturing, Mikel Calvo staining (objective 20X, eyepiece 10X, total optical magnification 200X): A – control group, B – research group



A B

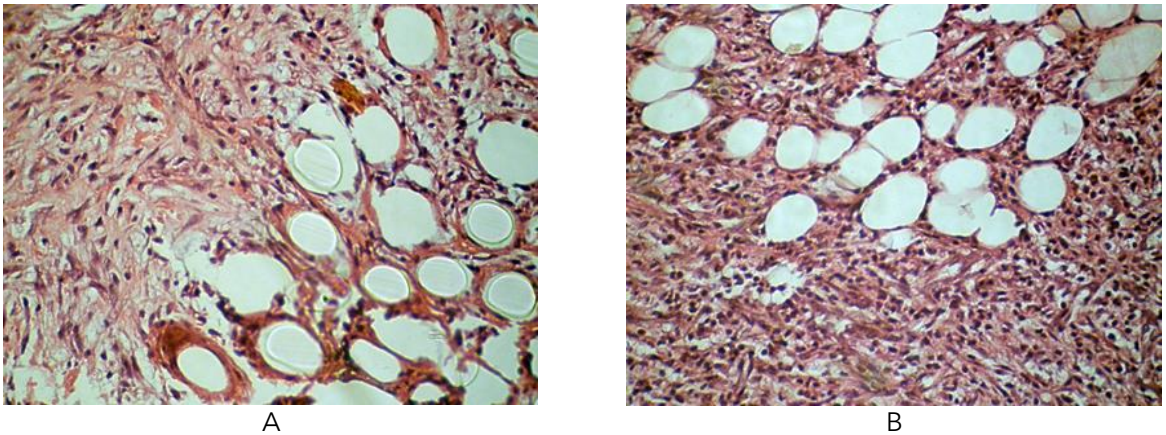
Fig. 7 Histological preparations of the rats' small bowel walls in 5 days after suturing, hematoxylin-eosin staining (objective 20X, eyepiece 10X, total optical magnification 200X): A – control group, B – research group

The value of R/BC in the control group in 7 days after suturing (Fig. 8 A) decreased to 1.7±0.13. It was smaller than in 5 days, but insignificant ($p>0.05$). At the same time the value of R/BC in the research group (Fig. 8 B) decreased to 1.21±0.07. It was statistically significantly smaller than the control group ($p<0.01$) and it was smaller than in 5 days, but insignificant ($p>0.05$). The germination of ligature fibbers by granulation tissue was found (Fig. 9) in both groups. But separate areas (Fig. 9 A) of loose adhesion of ligatures to the canal walls and the gap between the ligature fibbers were found in the control group.



A B

Fig. 8 Histological preparations of the rats' small bowel walls in 7 days after suturing, Mikel Calvo staining (objective 20X, eyepiece 10X, total optical magnification 200X): A – control group, B – research group



A B

Fig. 9 Histological preparations of the rats' small bowel walls in 7 days after suturing, hematoxylin-eosin staining (objective 20X, eyepiece 10X, total optical magnification 200X): A – control group, B – research group

Discussion

The observed increase in R/BC indicators within 12 hours following the injection of autofeces is believed to be a result of the consistent activation of oxidation reactions during acute IAI [8,48,53]. Reactive oxygen species are not just associated with various pathologies, but that they act as physiological redox signaling messenger with important regulatory functions [30]. The main sources of reactive oxygen species in IAI are, first of all, activated polymorphonuclear leukocytes and endothelial cells [8]. The lipid peroxidation products of polyunsaturated fatty

acids and cholesterol induce adaptive response and enhance defense capacity against subsequent oxidative insults [50]. That is, factors of oxidative reactions and oxidation products are important regulators of the inflammatory process [8,53]. However, according to the specifics of their action, mediators of oxidative reactions are so-called «damage mediators» [8]. Free radicals can damage cells and tissues [53]. The products of oxidative reactions are toxic [8,53]. The negative effect of the oxidative reactions middlemen is balanced by the factors of the antioxidant system [50]. But if antioxidant factors are not enough, the negative effect of oxidation factors may manifest [8,51].

The increase in the intensity of protein oxidation in 1 day after suturing the bowels is a consequence of the regular inflammatory process activation, due to a surgery injury. Predominance of R/BC indicators in the control group may be due to the greater expressiveness of inflammatory tissue changes in the area of the seams, as indicated by results of histological examination (significant infiltration of polymorphonuclear leukocytes, a greater degree of oedema). The experimental conditions in both groups were the same, except for the type of ligatures. Therefore, the lower activity of the inflammatory reaction and the lower activity of protein oxidation in the research group can be explained by the local effect of the antioxidant agent, which was the impregnated ligatures. The antioxidant agent apparently neutralised the local effect of oxidative factors, the number of which increased as a result of the inflammatory reaction, the activity of which increased as a result of surgical injury.

Some increase in the values of the R/BC indicators in the control group in 3 days after suturing testifies to high level of proteins oxidation. The decrease in the activity of protein oxidation in the research group, under the same other conditions of the experiment in both groups, can be explained by the local effect of the inhibitor of oxidation reactions used in the ligatures. The increased activity of protein oxidation can disrupt the regeneration [26]. This could possibly account for the findings observed in the histological examination of the control group – the gaps between the fibers of the ligatures and the channel formed by the ligatures. At the same time, a small number of such breaks and fibrin deposition between the fibers of the ligatures in the control group indicates better regeneration processes. In addition, more expressive signs of ongoing inflammation were found in the control group: stronger tissue oedema, a greater number of polymorphonuclear leukocytes in the tissues. This may be a consequence of the negative effect of oxidative reactions factors, which are damage mediators, that is, they are capable of damaging cells and tissues by themselves, if their action is not balanced by the antioxidant factors action.

The decrease in the activity of protein oxidation in the control group in 5 and 7 days after the suturing is a consequence of the inflammation activity reduction. The histological examination conducted during this period revealed a decrease in tissue edema and a reduction in the number of leukocytes present in the tissues. Instead, lymphocytes, fibroblasts, and plasma cells were found. This indicates the development of proliferation processes that complete the inflammatory process. But R/BC in the control group was significantly higher than in the research group. On the one hand, it could be a manifestation of high activity of oxidative processes that regulate inflammation. On the other hand, considering the reduction in the activity of the inflammatory response in both groups, the lower R/BC values in the research group should be considered a consequence of the local effect of the antioxidant that was used in the ligatures.

Faster normalisation of protein oxidation in the research group obviously contributed to the acceleration of regeneration. Between the fibers of the ligatures, the growth of granulation tissue was detected already after 5 days. At this time, there were still gaps between the ligatures in the control group, although fibrin deposits were already detected.

Germination of ligature fibers by granulation tissue was detected in both groups in 7 days after the suturing. This indicates a full-fledged regeneration process. At the same time, separate areas of the ligature's loose adhesion to the canal walls and the gaps between the ligature's fibers, that were found in the control group, indicate a comparatively lower activity and incompleteness of the regeneration processes.

It should be emphasized that suture leakage is most likely to occur within a period of 5-7 days after the bowels have been sutured, caused by the regeneration processes disruptions. One of the causes for suture leakage in IAI can be incomplete regeneration. The spaces between the fibers of the ligatures, the incompletely closed ligature channel ligature can be the path for invasion of microorganisms, which are able to support and intensify the inflammatory process in the bowel wall. This can cause activation of inflammation with negative results. One of the preconditions for preventing such processes is stimulation of regeneration.

So, the above makes it possible to conclude that the use of Kapron the ligatures impregnated with a 5% solution of ethylmethylhydroxypyridine succinate for suturing the small bowel in animals with models of IAI is associated with the accelerated decrease in the local activity of protein oxidation in the bowel tissues in the area of the ligatures and acceleration of regeneration processes in the areas of ligatures. This indicates that the creation of a suture material with antioxidant properties may be a promising direction for the prevention of suture leakage.

The presented way of influencing the regeneration of a sutured wound of the bowel wall and other similar known ways [18-24,26,27] are aimed at optimizing regeneration processes. The advantage of such ways, including ours, over ways based on the modification of bowel sutures [3,15,16,20] is that the regression of inflammation is

accelerated, and the formation of connective tissue is stimulated. The modification of the bowel sutures improves conditions for bowel wound healing but does not improve regeneration. The advantage of such ways over ways based on the strengthening of bowel sutures [17-19] is that there is an effect on the processes of regeneration, and not just strengthening of the bowel sutures line. The advantage of such ways over ways based on the use of stomas or intestinal tubes [10-14] also is the possibility of stimulating regeneration. The use of stomas or intestinal tubes improves the conditions for bowel wound healing but does not improve regeneration.

Various ways of stimulating regeneration (our and well-known ones) differ in ways of influencing regeneration mechanisms. It is impossible to determine the optimal way since different mechanisms are violated in different cases. Developing a sophisticated approach that enables concurrent action across multiple mechanisms would be advantageous. This can be achieved, for example, by creating a biodegradable ligature that will contain several necessary agents. Ways of regeneration stimulation should be combined with other ways of bowel suture leakage prevention for a comprehensive effect.

Limitations of the Study

The presented study has a number of limitations. This is a previous study. This study explored the principal possibility of using ligatures impregnated with an antioxidant agent. Experimental studies were performed using non-standardized impregnated ligatures. To confirm the data, a study using standardized impregnated ligatures is needed to accurately account for the amount of antioxidant agent. 5% ethylmethylhydroxypyridine succinate solution was used for impregnation of ligatures. It is necessary to conduct research with other antioxidant agents to detect the optimal antioxidant agent. Experimental studies with other parts of the bowels and with stomach are appropriate.

The small sample size (63 rats) was used in the study. It is necessary to conduct additional experiments on a larger number of animals to confirm the data. Female rats were used in this study. Therefore, male rats should be used in order to confirm the data in future studies.

The previous study used rats. To confirm the data, it is advisable to conduct experiments with other species of animals. Experimental data, as is generally accepted, must be verified in clinical research to definitively determine its validity. Clinical research can be conducted after the creation of industrial samples of ligatures with antioxidant properties. Research can be carried out only with the voluntary consent of patients after familiarizing themselves with all the risks.

But, considering the known physiological and pathological regularities common to all warm-blooded mammals, these limitations will not have a significant affect the generalizability of the results. It can be expected that the basic regularities will be confirmed, but some indicators may change slightly.

Prospects for Future Research

The presented research is part of the scientific work of the Department of Surgery № 1, Bukovinian State Medical University. One of the scientific work objectives is the complications prevention in abdominal surgery. The presented research is one of the scientific work series on ways to preventing bowel sutures leaking. The objective of the research series is to discover the most effective method for preventing the leakage of bowel sutures. It is necessary to cooperate with specialists to create samples of standardized ligatures. Research with a larger number of animals will be done in the future. Research will also be applied to patients.

Conclusions and Implications

1. The use of ligatures impregnated with a 5% ethylmethylhydroxypyridine succinate solution for suturing the small bowel in rats with intra-abdominal infection models is associated with the significant decrease in the activity of local proteins oxidation and the significant acceleration of local regeneration processes.

2. The creation of a suture material with antioxidant properties may be a promising direction for the prevention of suture leakage.

3. Further studies using ligatures with antioxidant properties for suturing the large bowel and stomach are needed to evaluate the effects.

4. Technological research and solutions are needed to create ligatures with antioxidant properties.

Declarations

Author Contributions

Conceptualization, F. Grynchuk; methodology, F. Grynchuk; validation, F. Grynchuk; formal analysis, F. Grynchuk and A. Shurma; investigation, A. Shurma; resources, A. Shurma; data curation, A. Shurma; writing-original draft preparation, A. Shurma; writing-review and editing, A. Shurma, F. Grynchuk; visualization, A. Shurma; supervision, F. Grynchuk; project administration, F. Grynchuk. All authors have read and agreed to the published version of the manuscript.

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- Data sharing not applicable: No new data were created or analyzed in this study. Data sharing is not applicable to this article.

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Institutional Review Board Statement

While performing the work, the norms of conducting research in the field of biology and medicine were observed: the Vancouver Conventions on Biomedical Research (1979, 1994), the Council of Europe Convention on the Protection of Vertebrate Animals Used in Experiments and for Other Scientific Purposes (1986).

Informed Consent Statement

Not Applicable.

Conflicts of Interest

The authors declare that there is no conflict of interests regarding the publication of this manuscript. In addition, the ethical issues, including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancies have been completely observed by the authors.

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