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Дослідження впливу тканинних факторів росту та електромагнітного випромінювання світлового діапазону на тонкокишкові анастомози в експерименті

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Investigation of impact of the tissue growth factor and electromagnet irradiation of a light diapason on the small bowel anastomoses in experiment

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Реферат

Мета. Розробка методу герметизації швів тонкокишкового анастомоза в умовах перитоніту.

Матеріали і методи. Експеримент виконано на 20 білих щурах–самцях лінії Вістар масою тіла близько 250 г. Догляд за тваринами, зміст та методи експериментальної роботи відповідали принципам Європейської конвенції щодо захисту хребетних тварин, що використовуються для дослідних та інших наукових цілей (Страсбург, 1986).

Результати. Проведеним експериментальним дослідженням показано можливості застосування фізичних (хвилі світла певної довжини) та біологічних (тканинні фактори росту) методів у профілактиці і лікуванні гострих запальних реакцій та стимуляції репаративних процесів.

Висновки. Перспективним напрямом подальшого дослідження методів профілактики неспроможності тонкокишкових анастомозів є застосування електромагнітного впливу низької інтенсивності світлового спектра в комбінації з аплікацією тканинних факторів росту.

Ключові слова: тонкокишковий анастомоз; неспроможність анастомоза; фототерапія; тканинні фактори росту; експеримент.

Abstract

Objective. Elaboration of method for hermetization of sutures of the small bowel anastomosis in conditions of peritonitis.

Materials and methods. The experiment was performed on 20 white male rats of a Wistar line owing a body mass approximately 250 g. The protocol for the laboratory animals care, the content and methods of experimental work were corresponded to principles of European Convention for defense of a vertebrate animals, which are used for research and scientific purposes (Strasbourg, 1986).

Results. The experimental investigation conduction have shown the possibilities of application of physical (the light waves of a certain length) and biological (the tissue growth factors) methods in prophylaxis and treatment of an acute inflammatory reactions and for stimulation of reparative processes.

Conclusion. The trend for further investigation of prophylactic measures for the small bowel anastomoses insufficiency, using the electro–magnet impact of the low–intense of the light spectrum in combination with application of the tissue growth factors, demonstrate a perspective.

Keywords: the small intestine anastomosis; insufficiency of anastomosis; phototherapy; the tissue factors of growth; experiment.

Introduction

Restoration of the continuity of the gastrointestinal tract in abdominal surgery is the most critical stage of any intervention, especially in an emergency situation, and remains an urgent problem [1, 2]. The healing of the anastomosis depends on the type of suture, the method of its application, immersion in the tissue and the condition of the intestinal wall. The body's protective reaction to the suture material, like a foreign body, is aimed at rejection of ligatures into the lumen of the hollow organ, which is inevitably accompanied by the formation of erosion, or they are organized along the fistula line by connective tissue [3, 4]. The healing process lasts a long time, is an inflammatory reaction and determines the direct outcome of the formed fistula, and in the long–term period its functional state. In general, the healing process of intestinal anastomosis fits into the concept of "healing by secondary intention" [5, 6]. The choice of the method of imposing intestinal anastomosis in peritonitis remains an urgent problem, since the frequency of suture failure remains high, which was the reason for conducting an experimental study [7, 8].

Materials and methods

Experiment design. After laparotomy, the jejunum was crossed by 3/4 of the lumen, after which the abdominal cavi-

Fig. 1.
Formation of small intestinal anastomosis in the experiment.

Fig. 2.
Drawing on the area of seams components of tissue growth factors (0.5–1 ml), followed by covering the seam line with a synthetic coating.

ty was sutured with occasional sutures. After 12 hours, relaparotomy, sanation of the abdominal cavity and suturing of the small intestine were performed with a single prolene continuous suture. All experimental animals after relaparotomy were observed a large amount of intestinal discharge in all parts of the abdominal cavity, swelling of the parietal peritoneum with vascular injection. The effusion was evacuated and the abdominal cavity was sanitized with antiseptic solutions [9, 10].

All animals are divided into three groups. The control group included 5 rats, which performed a simulating operation (laparotomy/relaparotomy) and evaluated metabolic parameters.

The comparison group consisted of 5 rats, who simulated EIA, studied the number of insolvency of jejunal sutures and mortality, as well as metabolic parameters (*Fig. 1*).

The study group (SG) consisted of 10 rats, which were modeled with AEP, 5 animals in two series.

SG A included animals that were sutured by the jejunum suture line after applying the components of tissue growth factors to the suture zone (0.5–1 ml) were covered with a synthetic wound coating PCL (polycaprolacton) (*Fig. 2*).

SG B included animals that, after applying the components of tissue growth factors (0.5–1 ml) to the zone of sutures and then covering the suture line with a synthetic coating, conducted electromagnetic irradiation of the zone of intestinal sutures with a wavelength of ʌ 660 nm (which corresponds to red light) (*Fig. 3*). The wavelength of the electromagnetic irradiation and the nature of the coating were chosen by us on the basis of literature data and the results of our own preliminary experimental studies [11 – 14].

The surviving animals were removed from the experiment on the 5th day of the experiment by decapitation.

As a source of electromagnetic irradiation, a quartz–polymer fiber with a diameter of 400 μm was used, with a radiation wavelength of λ = 660 nm, and a radiation power of 50 mW.

The study used the methods of light microscopy, enzyme immunoassay, spectrophotometry. The parameters of the barrier function of oxygen–dependent and oxygen–dependent phagocytosis, the level of serum cytotoxicity, the concentration of circulating immune complexes and interleukins were studied.

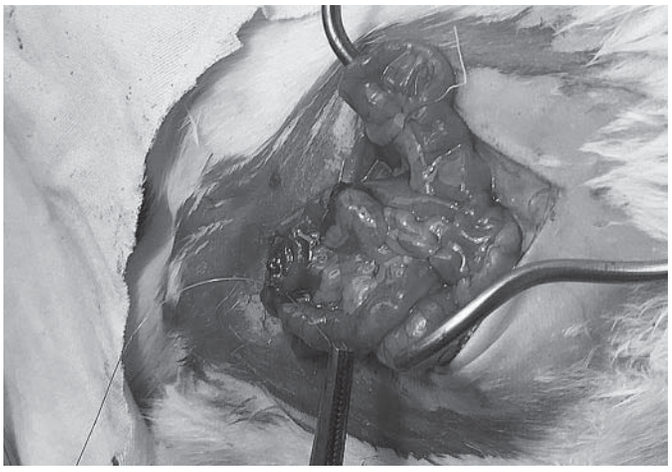

Fig. 3.
Irradiation of the anastomosis zone with a wavelength of λ 660 nm after rehabilitation of the abdominal cavity.

The content, care and methods of experimental work with which corresponded to the observance of the International Principles of the European Convention on the Protection of Vertebrate Animals (Strasbourg, 1986) [15].

Statistical data processing was carried out on a MacBook Pro personal computer and a set of statistical programs from the MS Office package.

Results

In the course of experimental studies, it was found that in 100% of the rats of the comparative group, an insomnia of anastomotic sutures developed in the observation period of 4–7 days, which led to the death of animals within 6–15 days. (mortality in the group – 100%).

In SG A, there were no deaths in the early postoperative period. However, in 3 (60.0.%) Experimental animals, they refused to eat food and water for 4–5 days, were adynamic, and there-

Table 1. Phagocytic activity of granulocyte neutrophils in experimental animals

Indicators of phagocytic activity of neutrophils	Control group (n=5)	Comparison group (n=5)	SG A (n=5)	SG B (n=5)
Phagocytic index, %	81,71 ± 2,3	40 ± 5,1	50 ± 3,2	56 ± 4,2
Phagocytic number	3,62 ± 0,5	2,08 ± 0,9	2,18 ± 0,7	2,23 ± 0,6
Phagocytosis Completion Index	1,44 ± 0,3	1,09 ± 0,4	0,82 ± 0,2	1,49 ± 0,5

Note. * - significance of difference with control p ≤ 0.05

Table 2. **Metabolic potential of neutrophilic granulocytes in experimental animals**

HCT test indicators	Control group (n=5)	Comparison group (n=5)	SG A (n=5)	SG B (n=5)
SP,%	7,0 ± 2,5	44,0 ± 2,3	29,0 ± 1,2	22,0 ± 1,8
ST,%	65,0 ± 5,6	79,0 ± 5,9	59,0 ± 3,6	67,0 ± 2,6
SCK SP, y.e.	0,80 ± 0,2	0,93 ± 0,3	0,34 ± 0,5	0,24 ± 0,3
SCK ST, y.e.	0,73 ± 0,1	1,55 ± 0,4	0,88 ± 0,3	0,97 ± 0,21
IS	5,0 ± 0,4	1,80 ± 0,3	2,0 ± 0,23	3,0 ± 0,3

Note. * - significance of difference with control p ≤ 0.05

Table 3. **The concentration of circulating immune complexes in experimental animals**

Indicators of phagocytic activity of neutrophils	Control group (n=5)	Comparison group (n=5)	SG A (n=5)	SG B (n=5)
Circulating immune complexes, U.E.	35,0 ± 0,05	0,210 ± 0,08	104,0 ± 0,9	102,0 ± 0,06

Table 4. **Expression of T and B lymphocyte receptors in experimental animals**

Subpopulation of lymphocytes	Control group (n=5)	Comparison group (n=5)	SG A (n=5)	SG B (n=5)
CD2+, %	NA	3,0 ± 1,1	5,0 ± 1,3	4,0 ± 1,6
CD3+, %	NA	7,0 ± 1,2	10,0 ± 1,3	8,0 ± 2,6
CD4+, %	NA	2,0 ± 1,8	6,0 ± 1,6	5,0 ± 3,0
CD8+, %	28,0 ± 2,6	5,0 ± 2,3	4,0 ± 2,4	3,0 ± 4,2
IRI, %	NA	1,5 ± 1,6	1,5 ± 0,9	1,7 ± 0,8

fore were derived from the experiment. At the autopsy, there were signs of early adhesive intestinal obstruction (dilatation of the small intestine leading sections with the formation of loose conglomerate of intestinal loops in the intestinal sutures. No signs of intestinal sutures were found to be insignificant. One observation on the 8th day showed a clinical deterioration of the laboratory animal, it died on the 12th day of the experiment: At the opening, signs of partial insolvency of intestinal sutures with the development of diffuse peritonitis were noted.

In SG In the early postoperative period was favorable. In one observation (20.0%) on the 10th day of the experiment, signs of intestinal obstruction were clinically detected, and therefore the animal was derived from the experiment. In the abdominal cavity in the region of the intestinal sutures, a single flat–sided fusion was determined, which was the cause of intestinal obstruction.

Phagocytic activity of granulocyte neutrophils in groups of experimental animals is presented in *Table 1*.

As shown in the *Table 1* data, in experimental peritonitis, inhibition of the phagocytic activity of neutrophils is noted, and against the background of a combination of growth factors and light exposure, an increase in these indicators is noted.

Table 2 presents data reflecting the metabolic potential of neutrophilic granulocytes in experimental animals in the studied groups.

The data presented indicate a sharp inhibition of the metabolic potential of neutrophilic granulocytes and a tendency to stabilize against the background of combined physical and biological effects.

Against the background of experimental peritonitis, a multiple increase in the level of CEC is observed, which after combined exposure decreased almost twofold (*Table 3*).

In the course of the studies, it was found that against the background of peritonitis in animals, the cellular immunity is inhibited, and against the background of the combined physical and biological effects, their relative stabilization occurs (*Table 4*).

In the *Table 5* presents the results of determining proliferative activity in the reaction of blast transformation of lymphocytes.

As shown in the *Table 5* data, with experimental peritonitis there is a sharp inhibition of proliferative activity of lymphocytes with an increase in the RBTL stimulation index. In animals of subgroup 2A, an increase (– PHA), a decrease (+

Table 5. **Proliferative activity in the reaction of blast transformation of lymphocytes**

Experimental groups animals	Without mitogen stimulation (-PHA),%	In the presence of mitogen (+PHA), %	Stimulation index
Control group (n=5)	13,5 ± 2,5	23,5 ± 4,5	1,06 ± 1,3
Comparison group (n=5)	7,0 ± 0,9	18,0 ± 1,3	1,57 ± 0,5
SG A (n=5)	9,0 ± 1,2	17,0 ± 1,1	0,89 ± 0,3
SG B (n=5)	11,5 ± 2,5	21,5 ± 3,5	1,01 ± 1,3

Table 6. **Serum IL-1, IL-6 and IL-10 levels in rats with acute experimental peritonitis**

Experimental groups animals	IL-1	IL-6	IL-10
Control group (n=5)	271±16	43,3±2,57	49,44±2,55
Comparison group (n=5)	211±13	56,6±3,88	38,6±1,88
SG A (n=5)	264±11	49,6±2,12	47,61±3,14
SG B (n=5)	259±16	47,61±3,18	45,14±2,12

PHA) and a stimulation index were noted. In animals of subgroup 2, the studied indices approached the control values.

A study of inflammatory mediators (IL–1, 6, and 10) in the blood serum of rats with EIAs of subgroups 2A and 2B showed an increase in the concentrations of IL–1 (by 20.1% and 18.5%, respectively) and IL–10 IL–1 (by 17.7% and 14.3%, respectively), as well as a decrease in the level of IL–6 (by 14.8% and 19.7%, respectively) in blood serum compared with animals of the comparison group, which may indicate the start of anti–inflammatory immune reactions (*Table 6*).

Discussion

The combined use of phototherapy and growth factors applied on a synthetic coating similar to the characteristics of the human dermis seems to be an effective way to seal the small intestinal anastomosis. Indicators of phagocytic activity of neutrophils (phagocytic index, phagocytic number and index of completeness of phagocytosis) against the background of combined exposure tend to normalize after exposure to light in the wave range λ = 630 nm. Depending on the activity of oxidative enzymes of neutrophils, which are formed under the influence of superoxide anion and hydroperoxides, which are formed in the NADPH–oxidase reaction, the reduction reaction of the nitro blue tetrazolium dye in a spontaneous NBT test and stimulated with zymosan was investigated. A high level of induction of enzymes with zymosan corresponded to normal immunoreactivity, and an increase in spontaneous oxidative activity led to a depletion of the oxidative reserve of phagocytic neutrophils. After the combined treatment, an increase in the stimulation index (IS) of phagocytosis was observed 3 times. A decrease in serum cytotoxicity was also observed on average from 60% to 40%. They revealed a decrease in the concentration of circulating immune complexes by a factor of 2 after exposure to light, probably due to the activation of their elimination.

In our opinion, the main mechanisms for implementing the effects of low–intensity electromagnetic radiation in the light range are associated with a modification of the immune status of the body in response to radiation, which is manifested in the mobilization of mature CD4 and CD8 T–lymphocytes, increasing IL–1 and IL–10 concentrations and reducing IL–6 levels and C–reactive protein in serum. Thus, through the inclusion of mediated signaling systems, a directed (anti–inflammatory) response of the organism to a combination of effective parameters of electromagnetic radiation can be carried out. The obtained results can serve as a basis for using low–intensity electromagnetic radiation of extremely high frequency in the complex for the prevention and treatment of various inflammatory diseases in order to increase the effectiveness of therapeutic measures.

A promising direction for further research is the use of a combination of sealing a suture line with the application of components of tissue growth factors (0.5–1 ml) in combination with a low–intensity electromagnetic effect of the light spectrum.

Thus, the use of physical and biological methods in the treatment of peritonitis caused by perforations of hollow organs, can more effectively maintain the stage of inflammation and stimulate repair processes, as they are a pronounced regulator of the stages of the inflammatory process and activate repair processes. The results obtained can serve as a basis for the use of low–intensity light exposure in the complex for the prevention and treatment of various inflammatory diseases in order to increase the effectiveness of therapeutic measures.

Findings

The results of experimental studies may indicate that in conditions of a thin anastomosis, accompanied by a high level of development, and attempts to seal the anastomosis with different development options in the early and after the periodic period, the occurrence of adhesions cannot be caused by pathology. A promising direction for further research is the use of a combination of sealing lines with the application of components of tissue growth factors (0.5–1 ml) in combination with a low–intensity electromagnetic effect of light radiation.

Підтвердження

Фінансування. Приватні кошти.

Інформація про внесок кожного учасника. Тимченко М. Е. – ідея, робота з експериментальними тваринами; Іванова Ю. В. – ідея, виконання маніпуляцій,

структурування; Клімова О. М. – збір, обробка та аналіз зразків; Щур О. І. – робота з джерелами і матеріалом, впорядкування.

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Згода на публікацію. Всі автори прочитали і схвалили остаточний варіант рукопису. Всі автори дали згоду на публікацію цього рукопису.

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