

Influence of selected parenteral drugs on tightness and morphological parameters of enteroanastomosis in rats

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Summary

During the past few years many scientific investigations regarding the status of enteroanastomoses after surgical resections have been performed all over the world. General surgery wards perform intestinal resections on patients with neoplastic processes or with advanced nonspecific intestinal inflammation. Partial resections of the intestines are also performed on patients with necrotic changes caused by intestinal torsion or embolism of the branches of the mesenteric artery. These types of surgeries often require additional treatment which should cause a minimal strain for the patient in the postoperative period in order to obtain the highest percentage of recoveries. In proliferative diseases chemo- and radiotherapy are the most commonly used methods of treatment whereas general and local steroid therapy are used in inflammatory diseases. Additionally, immunomodulating agents are used with conventional therapy to improve the therapeutic effect.

In a few studies, the influence of drugs on the local status of the intestine and wound healing status after surgeries was examined. The influence of selected agents on the status of experimental enteroanastomoses performed on the large intestine of rats was evaluated based on physical and morphometric tests. The study was performed on 60 Wistar rats, all of whom were male, of a similar body weight (250-350 grams) and the same age (2 months old). The rats were operated on to create an experimental anastomosis of the ascending colon. Then we administered selected drugs to evaluate their effect on the status of the newly created intestinal anastomosis. Selected parameters were evaluated after 4, 7 and 14 days after the surgery. In our experimental cycle animals were divided into 4 groups with 15 individuals in each group. The first group (I) was given physiological saline intraperitoneally (control group). Animals in the second group (II) were given the immuno-modulating drug interferon (Intron A) (45.000 IU/kg of b.w./day) after the surgery. Animals in the third group (III) were given the steroid Dexaven (0.5 mg/day) after the surgery. Animals in the fourth group (IV) were given a cytostatic agent (5-fluorouracil) after the surgery (20 mg/kg/day). All of the drugs were administered in the same form as intra-peritoneal injections with the same amount of fluid.

The study was planned for 5 cycles. 12 animals participated in one experimental cycle: 3 animals each from groups I, II, III and IV were operated on. The drugs were given for 5 days starting from the second day after the surgery. The rats were dissected after the 4th, 7th and 14th days of the experimental cycle to evaluate the status of the enteroanastomosis and the skin, according to previously mentioned parameters. Macroscopic changes of the enteroanastomosis were examined. Swabs were taken from all individuals with features of inflammation around this location. Next, the tightness of the anastomosis was evaluated by examining the appearance of air in the area of the anastomosis. The most important and last part of the experiment was amorphometric analysis of the large intestine with the anastomosis. In preparations stained with hematoxylin and eosin the following parameters were evaluated: amount of active intestinal crypts/mm, amount of non-active intestinal crypts/mm,

total amount of intestinal crypts/mm, thickness of the mucous membrane, thickness of the submucous membrane, thickness of the muscular layer, fractal dimension of the mucosal membrane, fractal dimension of conjunctive tissue. Under the influence of steroid agents tightness disorders of the anastomosis were observed.

Evaluation of the influence of the used substances on the morphometry of the enteroanastomosis revealed evident changes in the later days of the trial. In the days following the dissection of the animals from the control group histological changes were also observed that can suggest that the surgical intervention in itself influenced the morphometric parameters and caused disorders in the activity of intestinal crypts, decreased the dimension of the submucous membrane and altered the regeneration of the mucous membrane of the large intestine. Generally, agents used in the study caused disorders in the healing of the anastomosis, which significantly decreased particular dimensions of analyzed parameters. The substances examined influenced the thickness of the submucous membrane by making it thinner. Steroid and immunotherapy changed the reconstruction of conjunctive tissue which significantly affected the status and the resistance of the anastomosis. Intraperitoneal administration of selected therapeutical substances, especially Dexaven and Intron A have influenced the complete healing process of the anastomosis by disturbing the regenerative processes.

Keywords: morphology, anastomosis, dexamethasone, intron, 5-fluorouracil

For several years research on the condition of enteroanastomoses after surgical resections have been conducted worldwide (1, 2, 5, 11, 16, 24, 25, 28). These procedures are one of the most frequently performed procedures in general surgery, due to neoplastic diseases and, in some cases, in advanced stages of inflammatory bowel disease. The indication for colorectal resection in the case of non-specific inflammations is intestinal perforation, massive haemorrhage, *megacolon toxicum* or the fulminant course of the disease. Partial resections are also performed in Crohn's disease and in cases of necrotic changes caused by torsion of the intestine or embolism of the mesenteric artery branch.

After most of the procedures it is necessary to introduce additional treatment which, with the minimum burden for the patient, would guarantee the best outcomes. Treatment with antineoplastic agents is very burdensome for the patient. The most commonly used drugs have been tested for systemic harmfulness; however, few studies discuss the influence of these drugs on the local state of the anastomosis and wound healing after surgery. Research on the state of the anastomosis has a significant impact on the further condition of the patient. The possibility of disengagement of the anastomotic fragment of the intestine carries the risk of repeated surgery. It results from an anastomotic leak which causes the appearance of intestinal contents in the peritoneal cavity and subsequently its infection. Postoperative wound infection requires antibiotic therapy. Infected wounds take longer to heal, the sutured part of the intestine separates and scar tissue overgrows. This entails the need for another surgical intervention, this time in the field of plastic and reconstructive surgery (19).

Thanks to the use of laboratory animals in the course of this research, it was possible to observe the effect of these drugs on individual stages of anastomotic healing in comparison with a control group. The conducted experiment investigated the effect of three medical substances (the steroid Dexamethasone; the cytostatic-

5-fluorouracil; and the immunomodulator, used in the adjuvant therapy of colorectal cancer, Interferon alpha) on the state of experimental anastomoses in the large intestine of Wistar rats. The experiments conducted so far have demonstrated the existence of a relationship between general steroid therapy and the effect of tissue healing. We know that these substances inhibit collagen synthesis, stabilize the lysosomal membranes of leukocytes, inhibit the migration and concentration of pro-inflammatory cells and the growth of new blood vessels (4, 8, 32, 33). In our experiment, it was assessed whether steroid therapy applied after the surgery had a significant impact on the state of the enteroanastomosis. This is important for those patients who, due to long-term inflammation of the intestines and pathological changes in the intestinal walls, had to have the most pathologically changed fragments removed. However, they will still need long-term treatment with steroids. The influence of the most commonly used cytostatic 5-fluorouracil on the speed and quality of healing was also investigated. Recent reports show a beneficial effect of interferons in adjuvant therapy after resection procedures for colorectal neoplasms (15, 21, 26, 29). In the experiment, these compounds were administered in the maximum doses allowing the survival of the animals, at the same time with appropriate calculations analogous to those used in humans. This will allow better application of the results towards use in humans.

Morphometric analysis enables an objective, quantitative evaluation of histological parameters. Although widely used in the study of skin and bone tissue, it has also found application in experiments on the gastrointestinal tract. This method is of great importance in research on the detection of early changes in gastrointestinal tissue, that is, in the diagnosis of neoplastic diseases. With the help of morphometric studies, it is possible to differentiate changes between normal mucosa, dysplasia, and early forms of invasive cancer. This method also allows for a precise assessment of changes in the intestine caused by chemo- and radiotherapy used in treatment of various forms of malignant

neoplasms. Thanks to the use of this method, we obtain additional data on the development of neoplasms (9, 13, 17, 23, 36).

The lack of satisfactory data regarding the influence of Dexamethasone, the cytostatic-5-fluorouracil and Interferon alpha on the tightness and on the morphometric parameters of enteroanastomoses convinced us to carry out the experiment.

Material and methods

The research was carried out on 60 two-month-old male Wistar rats, with a similar weight of 250-350 grams. An experimental anastomosis was made on the ascending colon in these individuals, the selected drugs were systematically administered and tests on the effect of these drugs on the condition of the newly formed anastomosis were performed. The selected parameters were assessed after 4, 7, 14 days post surgery. Rats came from a laboratory animal breeder, Teresa Górzowska. The study was approved by the Local Bioethics Committee for Animal Research. Before the surgery, animals were kept for 7 days in the animal quarters in order to adapt, reduce transport stress and, thus minimize the death of animals after the surgery. Rats were housed in appropriately marked cages, three animals each. During this period, they were fed standard food, had access to water and the cages were cleaned daily. The care of the animals was carried out by a lab technician. The animals for the planned experimental cycle were divided into four groups of 15 animals each. The first group (I), the control group, was given physiological saline intraperitoneally. Animals in the second group (II) were given the immuno-modulating drug interferon (Intron A, Merck Sharp & Dohme) (45.000 IU/kg of b.w./day). Animals in the third group (III) were given the steroid dexamethasone (Dexaven, Pharmaswiss) (0.5 mg/day). Animals in the fourth group (IV) were given a cytostatic agent (5-fluorouracil, Ebewe Pharma) (20 mg/kg/day). All of the drugs were administered post-surgically in the same form of intra-peritoneal injections with the same amount of fluid. The study was planned for five cycles. Twelve animals participated in one experimental cycle: three animals each from groups I, II, III and IV were operated on. Animals were anesthetized with Nembutal (5-ethyl-5 (1-methylbutyl) barbituric acid) administered by intraperitoneal injection at a dose of 50 mg/kg b.w., which provided anesthesia for about 1-2 hours. After the skin was incised, the colon was removed and clamps were established. The incision was made just behind the caecum for the purpose of proper identification of the anastomotic site. Fragments of the intestine were joined with non-absorbable sutures (Surgipro 5.0). Immediately after awakening, animals received only water with 5% glucose in the ratio of 1 : 1. They received solid food on the second day after the procedure. The drugs were started on the second day post-surgery, and were given for five days. The rats were euthanized, then dissected after the 4th, 7th and 14th days of the experimental cycle to evaluate the status of the enteroanastomosis and the skin according to previously mentioned parameters. A fragment of the large intestine with the experimental anastomosis was collected from each individual.

The next step was to check the tightness of the experimentally created anastomosis. A 3 cm long fragment of the intestine, which was fastened on both sides, was put into a vessel with water and immersed under the surface. An air-filled syringe with a ball-point needle was inserted at one end of the anastomosis and air was injected. The proper filling and volume expansion of the intestinal fragment was confirmed. The sample was then checked for the appearance of gas bubbles in the area of the seam.

The next stage of the experiment was to collect a narrow fragment of the large intestine containing a suture for histological examination and morphometric tests. A piece of tissue was fixed in 4% formalin. After fixation, intestinal sections were rinsed in running water, and then dehydrated in increasing concentrations of ethyl alcohol (50%, 60%, 70%, 80%, 90%, 99%). The intestinal sections were then placed in an anhydrous alcohol-xylene mixture (1 : 1) and then in pure xylene. Subsequently tissues were transferred to a xylene-paraffin mixture for paraffin infusion. The sections were then embedded in paraffin blocks. The blocks were trimmed and then sliced on a microtome (Microm HM 360) into 5 µm thick tissue sections. From each block, 20 large intestine transverse sections were obtained and cut at an interval of 20 µm after every five sections. The sections obtained in this way were placed on glass slides and glued to the glass surface with a small amount of a mixture of albumin and glycerol (1 : 1). The obtained colon tissue samples were stained using the H + E technique. Next, microscopic photos were taken using a Nikon Eclipse E800 light microscope (magnification 40 times) and a Nikon D70 digital camera. Five pictures were taken from each tissue section. The following parameters of the large intestine were analyzed: number of active intestinal crypts/mm, number of inactive intestinal crypts/mm, total number of intestinal crypts/mm, mucosa thickness, submucosa thickness, muscle layer thickness, fractal dimension of the mucosa (Box Counting Method), and fractal dimension of the connective tissue (UV microscope).

The obtained data was analyzed statistically. All data was reported as means, standard error of the mean, and confidence interval for the mean. Group differences were analyzed using one-way analysis of variance (ANOVA) and Duncan's post test as adjustments for multiple comparisons. In the absence of a normal distribution of the data or unequal variance, the analysis of differences between the groups was performed using the Mann-Whitney test. Differences between groups with $P < 0.05$ were considered statistically significant. The Statistica 6.0 program was used for statistical analysis.

Results and discussion

Out of 60 animals subjected to surgery, two individuals from the control group died immediately after the surgery. During the performed necropsy, no significant abnormalities were found in the macroscopic assessment of the abdominal cavity, no symptoms of peritonitis, no abscesses or postoperative adhesions were observed. It was therefore concluded that the death of the animals was not due to postoperative complications but, as in similar experiments, due to the action of the

drug used to anesthetize rats (Nembutal) or the stress that accompanied the surgical intervention.

As a result of the leak test of the anastomotic fragment of the large intestine, anastomotic coherence was noticed on 3 out of 5 of the animals from the Dexaven group dissected on the 7th day.

By analyzing the morphometry of the anastomotic fragment of the intestine for individual substances in the consecutive days of the experiment, the following conclusions were obtained. The number of active intestinal crypts was the highest on the 7th day of the experiment in the group of animals treated with Intron A. The number of inactive intestinal crypts was the highest on the 14th day of the experiment for the group treated with Intron A. The total number of crypts showed the greatest dimension in the 4th day of the Dexaven group. The mean size of the mucosa turned out to be the largest on the 4th day of the experiment for the 5-Fluorouracil group. The size of the submucosa reached the highest value on the 4th day in the control group. The muscular layer was thickest on the 14th day for the 5-Fluorouracil group and the highest value of fractal size of the mucosa was reached in the control group on the 14th day of the experiment (Tab. 1-3).

On the 4th day after the surgery, no statistically significant differences were found in the number of active and inactive intestinal crypts, the total number of crypts and the fractal dimension of the mucosa in any of the studied groups of individuals. The mucosa thickness showed significant differences in the group of animals treated with Intron-A and 5-Fluorouracil. There was an increase in its thickness as compared to the control group. The group of animals treated with Dexaven did not show any differences in this parameter compared to the control group. The submucosa clearly decreased in thickness after administration of Dexaven, Intron A and 5-Fluorouracil compared to the control group. However, no significant differences were observed between the groups. The muscular layer was the thickest in the 5-Fluorouracil group. However, in the remaining groups, the size of the muscular layer did not show any significant differences compared to the control group.

On the 7th day after the surgery, the histomorphometric tests showed no statistically significant differences between the groups, analyzing the number of active intestinal crypts, the total number of crypts, and the thickness of the mucosa and muscles. Only in the groups treated with Dexaven and 5-Fluorouracil increased in

Tab. 1. Results of morphometric tests of anastomosis on the 4th day of the experiment (mean values $\pm$ standard error); $p < 0.005$

4 th day of experiment	Control group	Intron A	Dexaven	5-Fluorouracyl
Number of active intestinal crypts/mm	3.88 $\pm$ 0.36	3.84 $\pm$ 0.56	4.36 $\pm$ 0.63	4.33 $\pm$ 0.59
Number of inactive intestinal crypts/mm	5.91 $\pm$ 0.29	5.16 $\pm$ 0.57	5.74 $\pm$ 0.43	4.88 $\pm$ 0.26
Total number of intestinal crypts/mm	9.79 $\pm$ 0.44	9.00 $\pm$ 0.32	10.10 $\pm$ 0.51	9.22 $\pm$ 0.55
Mucosa thickness, μ m	498.9 $\pm$ 23.05	604.8 $\pm$ 25.94	480.3 $\pm$ 41.91	828.10 $\pm$ 28.15
Submucosa thickness, μ m	120.2 $\pm$ 10.77	69.1 $\pm$ 5.51	67.2 $\pm$ 1.53	74.8 $\pm$ 3.36
Muscular layer thickness, μ m	487.1 $\pm$ 46.49	428.3 $\pm$ 42.46	465.9 $\pm$ 34.98	609.7 $\pm$ 36.85
Fractal dimension of the mucosa, [D]	1.5017 $\pm$ 0.0143	1.4980 $\pm$ 0.0199	1.4589 $\pm$ 0.0314	1.4404 $\pm$ 0.0232

Tab. 2. Results of morphometric tests of anastomosis on the 7th day of the experiment (mean values $\pm$ standard error); $p < 0.005$

7 th day of experiment	Control group	Intron A	Dexaven	5-Fluorouracyl
Number of active intestinal crypts/mm	4.43 $\pm$ 0.30	4.54 $\pm$ 0.74	3.46 $\pm$ 0.34	3.61 $\pm$ 0.41
Number of inactive intestinal crypts/mm	4.30 $\pm$ 0.29	5.02 $\pm$ 0.31	5.67 $\pm$ 0.17	6.09 $\pm$ 0.59
Total number of intestinal crypts/mm	8.73 $\pm$ 0.40	9.56 $\pm$ 0.47	9.12 $\pm$ 0.34	9.70 $\pm$ 0.47
Mucosa thickness, μ m	494.72 $\pm$ 17.64	574.05 $\pm$ 72.27	491.25 $\pm$ 37.17	511.13 $\pm$ 36.73
Submucosa thickness, μ m	80.94 $\pm$ 4.27	77.90 $\pm$ 5.54	78.74 $\pm$ 5.80	63.43 $\pm$ 4.85
Muscular layer thickness, μ m	437.25 $\pm$ 18.34	426.11 $\pm$ 23.55	444.30 $\pm$ 17.59	440.69 $\pm$ 20.91
Fractal dimension of the mucosa, [D]	1.3876 $\pm$ 0.0237	1.5263 $\pm$ 0.0094	1.5209 $\pm$ 0.0122	1.4162 $\pm$ 0.0246

Tab. 3. Results of morphometric tests of anastomosis on the 14th day of the experiment (mean values $\pm$ standard error); $p < 0.005$

14 th day of experiment	Control group	Intron A	Dexaven	5-Fluorouracyl
Number of active intestinal crypts/mm	2.48 $\pm$ 0.24	3.09 $\pm$ 0.56	4.23 $\pm$ 0.52	3.45 $\pm$ 0.39
Number of inactive intestinal crypts/mm	6.09 $\pm$ 0.13	6.37 $\pm$ 0.35	4.93 $\pm$ 0.34	5.28 $\pm$ 0.29
Total number of intestinal crypts/mm	8.57 $\pm$ 0.27	9.45 $\pm$ 0.25	9.16 $\pm$ 0.57	8.73 $\pm$ 0.20
Mucosa thickness, μ m	445.77 $\pm$ 33.99	482.08 $\pm$ 13.06	551.74 $\pm$ 22.91	524.64 $\pm$ 29.95
Submucosa thickness, μ m	92.00 $\pm$ 4.70	68.38 $\pm$ 2.86	70.27 $\pm$ 3.59	59.66 $\pm$ 3.37
Muscular layer thickness, μ m	538.95 $\pm$ 46.96	297.73 $\pm$ 12.02	423.29 $\pm$ 43.27	478.74 $\pm$ 29.36
Fractal dimension of the mucosa, [D]	1.5488 $\pm$ 0.0008	1.3782 $\pm$ 0.0226	1.3782 $\pm$ 0.0226	1.4190 $\pm$ 0.0259

the amount of inactive crypts compared to the control. In animals treated with 5-Fluorouracil, the submucosa decreased in thickness compared to the control and the group treated with Dexaven. In animals exposed to Intron A, no significant differences were found in the thickness of this layer. The fractal dimension of the mucosa was greater in the Intron A and Dexaven groups compared to the control group and animals treated with 5-Fluorouracil. On the 14th day after surgery, an increased number of active intestinal crypts was observed in the Dexaven group compared to the control group. None of the studied groups showed statistically significant differences in comparison with the control group in the total number of intestinal crypts on the 14th day after surgery. However, there were differences between the groups Intron A and 5-Fluorouracil. The thickness of the mucosa in the animals treated with Dexaven and 5-Fluorouracil compared to the control group showed an increase in size. Moreover, thinning of the submucosa was observed on that day in all groups as compared to the control group. The muscular layer showed a statistically significant reduction in size in the group of animals treated with Intron A and Dexaven compared to the control group. The fractal dimension of the mucosa was reduced in all groups tested compared to the control group.

When assessing the influence of the tested substances on the morphometric parameters of the large intestine during the experiment, a decrease in the number of active intestinal crypts was found in the control group on the 14th day after the surgery. The number of inactive crypts was the lowest on the 7th day after the operation. The total number of crypts in the control group did not differ in time from the surgery. The thickness of the mucosa and the muscles showed no significant differences, and differences were observed in the submucosa on the 7th day after the procedure, a decrease in thickness was noted. The fractal dimension of the mucosa in this group showed a decrease in value on day 7 and an increase in dimensions on day 14.

In the group treated with Intron A, no differences in time were observed in the following parameters: the number of active intestinal crypts, the total number of crypts, and the thickness of the submucosa. As for the number of inactive crypts, an increase in their number was observed on the 14th day after the surgery compared to the 7th day. The mucosa thickness in the Intron A group was lower on day 14 compared to day 4. Muscle thickness and mucosal fractal dimension were lowest on day 14 after anastomosis.

On the 14th day of the experiment, in the group of animals treated with Dexaven, the decrease of thickness of submucosa and muscular layers was observed.

In the group treated with 5-Fluorouracil, no time differences were observed in the number of active intestinal crypts, inactive intestinal crypts, total number of crypts and the fractal dimension of the mucosa. The size of the mucosa was lower on days 7 and 14 compared to day 4. The size of the mucosa was lower on day 14

compared with day 4, and the size of the muscular layer was reduced on days 7 and 14.

By examining the condition of the connective tissue of the large intestine under the influence of the administered drugs on individual days of the experiment, it can be concluded that: on the fourth day of the experiment, no statistically significant differences were observed in the behavior of the fractal dimension of the connective tissue of the large intestine under the influence of the administered drugs in the dissected animals. On the seventh day after surgery, the fractal dimension of the connective tissue of the colon in the group treated with Intron A was smaller compared to the control. No statistically significant differences were observed in the remaining groups of animals. On the 14th day of the experiment, the fractal dimension of the connective tissue of the large intestine in the group treated with Dexaven showed a significant reduction compared to the other groups of animals.

Research has already been carried out the use of non-specific immunomodulators such as interferons (alpha, gamma) in stand-alone therapy, or in combination with 5-Fluorouracil as an adjuvant therapy in the treatment of colorectal cancer (3, 9, 21, 22, 26). Wistar rats were used as experimental animals due to the ease of breeding, high resistance to stress related to surgical intervention, and above all because some of the metabolic processes in them are similar to those in humans. The experiment was extended to steroid drugs, which used in the treatment of intestinal diseases, seem to have a significant impact on the local state of the anastomosis after surgery and, consequently, on the further fate of patients.

With regard to histomorphometry as a method ideally describing the condition of the intestines, the first observation that comes to mind is that scientific literature treats the subject of morphometry in an experimental anastomosis quite briefly. The experience so far deals with morphometric, but only in the context of the intestines as a whole. The influence of diet or certain chemicals such as recombinant growth hormone or igf-1 on the histological state of the intestine (6, 7, 11, 12) has been widely analyzed. Only individual centers have conducted animal studies to check the changes in the histological image of the intestinal anastomoses (27, 31).

In our own study, based on the changes taking place in the following days of dissecting the animals in the control group, it was found that the surgical intervention itself changes the morphometric parameters of the anastomosed fragment. This was most evident in the thickness of the submucosa and the number of active and inactive intestinal crypts. These parameters, as we know, decreased in the following days of animal sectioning, compared to day 4. As for the fractal dimension of the mucosa, it also changed over time, indicating the remodeling of this layer.

Until now, studies have also been conducted to assess the effect of diet on intestinal morphometry. Scientists have already proved that natural food stimulates the

structure of the intestinal wall, which in turn affects the digestibility and absorption of food (6, 10, 14). Morphometric changes also occur in the reverse situation; i.e. limited access to food due to the use of a restrictive diet (20). The influence of the early introduction of food on anastomosis was also investigated. The impact of the surgery on the intestinal transport processes was assessed (18, 34, 35).

In our experiment, all individuals were fed the same standard food. The research was not intended to introduce an additional variable, that is diet. This is precisely due to the fact that chemical substances administered with food disturb or favorably modify the histological state of the intestines.

Based on the results of our own research, it was determined that the surgical intervention itself disturbed the morphometry of the large intestine. However, it was the chemical substances administered after the procedure that significantly modified the parameters of anastomosis. In the following days of dissecting the animals, following the effects of the complex fragment of individual substances over time, the most pronounced changes occurred in the group of animals treated with 5-Fluorouracil. There was a thinning of the mucosa, submucosa and the muscular layer of the large intestine. In animals administered Intron-A, the changes were similar. Only a decrease in the size of the submucosa was not observed. Additionally, there was a decrease in the fractal dimension of the mucosa and an increase in the number of inactive intestinal crypts. On the other hand, the lack of changes during the experiment in anastomosis treated with Dexaven turned out to be quite remarkable. The preparations on Day 14 were similar to those on Day 4.

Few studies refer to the description of changes in the structure of anastomosis under the influence of the preparations we have selected for our research. The centers that worked on it studied the influence of these substances on the selected parameters of the complex fragment. The changes were macroscopically assessed, physically examining the influence of intestinal pressure on the state of the anastomosis, and biochemical changes were analyzed. By examining the influence of early nutrition on the condition of the anastomosis after prior treatment with cytostatics and steroids, it was proved that early feeding has positive effects on healing of the anastomosis (10, 14, 30).

In the conducted experiment, 5-Fluorouracil was administered as a single drug, but in more than twice the typical dose. Comparing the activity of this cytostatic with the control group, it caused the most pronounced changes in the submucosa. On each of the analyzed days of the experiment, the thinning of this layer was visible in comparison with the control group. The authors of other publications analyzed the problem of combined administration of 5-Fluorouracil and interferon alpha in the treatment of advanced colorectal cancer. In their experiences, they often came to contradictory conclusions (21, 26). Studies on the effect of interferons have

shown that its administration, both local and systemic, impairs healing by reducing new collagen fibers. In our experiment, this drug undoubtedly affects the state of the intestinal anastomosis. This substance changed the dimensions of individual layers of the intestinal wall as compared to the control group. The most important changes were observed in the thickness of the submucosa (there was a reduction in size on days 4 and 14). Also, the fractal dimension of the mucosa was changed under the influence of intron A. After growth (compared to control) on day 7 there was a marked decrease on day 14. This may indicate that interferon alpha caused a disturbance in mucosal renewal after anastomosis surgery (Tab. 2). The influence on the fractal dimension of the connective tissue also influences the integrated fragment of the intestine. When analyzing the influence of the convalescence period on this parameter under the influence of individual substances, a statistically significant difference was found in the group exposed to interferon. The increase in this dimension on day 14, compared to day 4, may indicate that new tissue is stimulated to expand. The last element that we would like to mention in the discussion is the influence of the substances given on the resistance of the intestinal anastomosis, on the pressure in the intestine, which is strongly dependent on the influence of these parameters on the connective tissue of anastomosis. In the authors' own research, it was Dexaven that significantly lowered the endurance of anastomosis to intestinal pressure. Visible disturbances in the tightness of the anastomosis took place on the 7th day of animal selection. The fractal dimension parameter was used for the analysis of connective tissue.

There is no work in the literature analyzing connective tissue on the basis of the fractal dimension. This term is not only used for the intestines, but primarily for bone and skin tissue. It proves the degree of complexity and irregularity of a given structure. Combined with other morphometric data it is a very useful parameter. In the present experiment, no differences were observed in the fractal dimension at the beginning of the experiment, as compared to the control group. This may indicate the initial lack of effect of these drugs on the connective tissue, or that the surgical intervention fluid and the administered drugs were different and mutually canceling each other out. Analyzing the effect of individual substances on the connective tissue of the anastomosis during the experiment, statistically significant differences were noticed in the group treated with Intron A, demonstrating the influence of an immunomodulating drug. Until now, scientists have tried to study the effect of certain processes and substances on the morphometry of the left part of the intestine. However, there was no interest in studying the place of the anastomosis and the processes taking place in it. Using a similar research method, scientists showed changes in this unit under the influence of various factors. The main aspect was the resection of a large fragment of the small or large intestine and the effect of this procedure in combina-

tion with additional factors such as chemicals, diet or the type of surgery on the intestinal histomorphometry. Surgical intervention alone modified the histology of the large intestine. At the beginning of the experiment, changes in morphometric parameters under the influence of drug administration, as compared to the control group, were the least marked.

In conclusion, the tightness of the experimental anastomosis was disturbed under the influence of steroid therapy. All substances impaired anastomotic healing on different levels. The most pronounced effect of tested substances was the thinning of the submucosa and on 14th day of experiment it was 5-Fluorouracyl which caused the biggest damage. Intron A decreased thickness of muscular layer on 14th day of the experiment. Steroid therapy and immunotherapy changed the structure of connective tissue, also affecting the tightness of the anastomosis.

References

- Bading J. R., Yoo P. B., Fissekis J. D., Alauddin M. M., D'Argenio D. Z., Conti P. S.: Kinetic modeling of 5-fluorouracil anabolism in colorectal adenocarcinoma: a positron emission tomography study in rats. *Cancer Res.* 2003, 63 (13), 3667-3674.
- Baer U., Souchon R., Bauknecht K. J., Winter S.: Anastomosis healing in the irradiated colon. II. Influence of irradiation on new formation of connective tissue. An animal experiment study. *Strahlentherapie* 1983, 159 (8), 474-482.
- Berthet B., Di Costanzo J., Arnaud C., Ledoray V., Choux R., Assadourian R.: Treatment of caustic burns of the esophagus with interferon gamma. Comparison with epidermal growth factor. Experimental study in rats. *Gastroenterol. Clin. Biol.* 1994, 18 (8-9), 680-686.
- Bittencourt D. G., Barreto M. W., França W. M., Gonçalves A., Pereira L. A., Sbragia L.: Impact of corticosteroid on intestinal injury in a gastroschisis rat model: morphometric analysis. *J. Pediatr. Surg.* 2006, 41 (3), 547-553.
- Brodsky J. T., Cohen A. M.: Peritoneal seeding following potentially curative resection of colonic carcinoma: implications for adjuvant therapy. *Dis. Colon Rectum* 1991, 34 (8), 723-727.
- Bühler C., Hammon H., Rossi G. L., Blum J. W.: Small intestinal morphology in eight-day-old calves fed colostrum for different durations or only milk replacer and treated with long-R3-insulin-like growth factor I and growth hormone. *J. Anim. Sci.* 1998, 76 (3), 758-765.
- Fadrique B., Lopez J. M., Bermudez R., Gomez de Segura I. A., Vazquez I., De Miguel E.: Growth hormone plus high protein diet promotes adaptation after massive bowel resection in aged rats. *Exp. Gerontol.* 2001, 36 (10), 1727-1737.
- Furst M. B., Stromberg B. V., Blatchford G. J., Christensen M. A., Thorson A. G.: Colonic anastomoses: bursting strength after corticosteroid treatment. *Dis. Colon Rectum* 1994, 37 (1), 12-15.
- Gao C., Zhao J., Gregersen H.: Histomorphometry and strain distribution in pig duodenum with reference to zero-stress state. *Dig. Dis. Sci.* 2000, 45 (8), 1500-1508.
- García-Olmo D., Payá J., García-Olmo D. C.: Role of immediate postoperative feeding on colonic anastomoses resistance. An experimental study in a rat model. *Nutr. Hosp.* 1996, 11 (5), 279-285.
- Gómez de Segura I. E., Aguilera M. J., Codesal J., Codoceo R., De-Miguel E.: Comparative effects of growth hormone in large and small bowel resection in the rat. *J. Surg. Res.* 1996, 62 (1), 5-10.
- Griga T., Tromm A., Schmiegel W., Pfisterer O., Müller K., Brasch F.: Collagenous colitis: implications for the role of vascular endothelial growth factor in repair mechanisms. *Eur. J. Gastroenterol. Hepatol.* 2004, 16 (4), 397-402.
- Gulbinowicz M., Berdel B., Wójcik S., Dziewiątkowski J., Oikarinen S., Mutanen M., Kosma V., Mykkänen H., Moryś J.: Morphometric analysis of the small intestine in wild type mice C57BL/6L – a developmental study. *Folia Morphol.* 2004, 63 (4), 423-430.
- Hedemann M. S., Højsgaard S., Jensen B. B.: Small intestinal morphology and activity of intestinal peptidases in piglets around weaning. *J. Anim. Physiol. Anim. Nutr.* 2003, 87, 32-41.
- Ita M. I., Wang J. H., Jeffron C. C., Power D. G., Nolan Y., Toulouse A., Lim C. C. H., Fanning N., Redmond H. P.: Therapeutic Response Evaluation in Advanced Melanoma Patients Incorporating Plasma cfDNA, LDH, VEGF, PD-L1, and IFN- γ Measurements. *Anticancer Res.* 2022, 42 (2), 801-810.
- Kiseleva E. B., Ryabkov M. G., Sizov M. A., Bederina E. L., Komarova A. D., Moiseev A. A., Bagryantsev M. V., Vorobiev A. N., Gladkova N. D.: Effect of Surgical Technique on the Microstructure and Microcirculation of the Small Intestine Stump during Delayed Anastomosis: Multimodal OCT Data. *Sovrem Tekhnologii Med.* 2021, 13 (4), 36-45.
- Kisielinski K., Willis S., Prescher A., Klosterhalfen B., Schumpelick V.: A simple new method to calculate small intestine absorptive surface in the rat. *Clin. Exp. Med.* 2002, 2 (3), 131-135.
- Kiyama T., Onda M., Tokunaga A., Yoshiyuki T., Barbul A.: Effect of early postoperative feeding on the healing of colonic anastomoses in the presence of intra-abdominal sepsis in rats. *Dis. Colon Rectum* 2000, 43 (10 Suppl), 54-58.
- Lin H., Chang C. L., Huang E. Y., Changchien C. C.: Early-onset sigmoid colon perforation during concurrent chemoradiation in a patient with cervical cancer. *Int. J. Gynecol. Cancer* 2004, 14 (2), 381-383.
- Nuñez M. C., Bueno J. D., Ayudarte M. V., Almendros A., Ríos A., Suárez M. D., Gil A.: Dietary restriction induces biochemical and morphometric changes in the small intestine of nursing piglets. *J. Nutr.* 1996, 126 (4), 933-944.
- Wiesenfeld M., O'Connell M. J., Wieand H. S., Gonchoroff N. J., Donohue J. H., Fitzgibbons Jr R. J., Krook J. E., Mailliard J. A., Gerstner J. B., Pazdur R.: Controlled clinical trial of interferon-gamma as postoperative surgical adjuvant therapy for colon cancer. *J. Clin. Oncol.* 1995, 13 (9), 2324-2329.
- Palmeri S., Meli M., Danova M., Bernardo G., Leonardi V., Dastoli G., Rausa L., Russo A., Filippelli G., Palmieri G., Russo A., Della Vittoria Scarpati M., Lo Russo V., Di Lauro L., Colucci G., Bruni G., Piazzi M., Gebbia N., Spada S.: 5-Fluorouracil plus interferon alpha-2a compared to 5-fluorouracil alone in the treatment of advanced colon carcinoma: a multicentric randomized study. *J. Cancer Res. Clin. Oncol.* 1998, 124 (3-4), 191-198.
- Riordan S. M., McIver C. J., Wakefield D., Duncombe V. M., Thomas M. C., Bolin T. D.: Small intestinal mucosal immunity and morphometry in luminal overgrowth of indigenous gut flora. *Am. J. Gastroenterol.* 2001, 96 (2), 494-500.
- Savage F. J., Lacombe D. L., Boulos P. B., Hembry R. M.: Role of matrix metalloproteinases in healing of colonic anastomosis. *Dis. Colon. Rectum* 1997, 40 (8), 962-970.
- Scappaticci F. A., Fehrenbacher L., Cartwright T., Hainsworth J. D., Heim W., Berlin J., Kabbavar F., Novotny W., Sarkar S., Hurwitz H.: Surgical wound healing complications in metastatic colorectal cancer patients treated with bevacizumab. *J. Surg. Oncol.* 2005, 91 (3), 173-180.
- Shippinger W., Jagoditsch M., Sorré C., Gnant M., Steger G., Hausmaninger H., Mlineritsch B., Schaberl-Moser R., Mischinger H. J., Hofbauer F., Holzberger P., Mittlböck M., Jakesz R.: A prospective randomised trial to study the role of levamisole and interferon alfa in an adjuvant therapy with 5-FU for stage III colon cancer. *Br. J. Cancer* 2005, 92 (9), 1655-1662.
- Simmy T., Anup R., Prabhu R., Balasubramanian K. A.: Effect of surgical manipulation of the rat intestine on enterocyte populations. *Surgery* 2001, 130 (3), 479-488.
- Smith J. B., Zhao J. B., Dou Y.-L., Gregersen H.: Time-dependent viscoelastic properties along rat small intestine. *World J. Gastroenterol.* 2005, 11 (32), 4974-4978.
- Staib L., Link K. H., Beger H. G.: Toxicity and effects of adjuvant therapy in colon cancer: results of the German prospective, controlled randomized multicenter trial FOGT-1. *J. Gastrointest. Surg.* 2001, 5 (3), 275-281.
- Stark A., Nyska A., Madar Z.: Metabolic and morphometric changes in small and large intestine in rats fed high-fiber diets. *Toxicol. Pathol.* 1996, 24 (2), 166-171.
- Szende B., Marcsek Z., Kocsis Z., Tompa A.: Effect of simultaneous administration of Avemar and cytostatic drugs on viability of cell cultures, growth of experimental tumors, and survival tumor-bearing mice. *Cancer Biother. Radiopharm.* 2004, 19 (3), 343-349.
- Theogaraj E., John C. D., Christian H. C., Morris J. F., Smith S. F., Buckingham J. C.: Perinatal glucocorticoid treatment produces molecular, functional, and morphological changes in the anterior pituitary gland of the adult male rat. *Endocrinology* 2005, 146 (11), 4804-4813.
- Thiesen A., Tappenden K. A., McBurney M. I., Clandinin M. T., Keelan M., Thomson B. K. A., Drozdowski L. A., Wild G., Thomson A. B. R.: Dietary lipids alter the effect of steroids on the transport of fructose following intestinal resection in rats. *Dig. Dis. Sci.* 2008, 53 (8), 2126-2139.
- Wright N. A.: Epithelial stem cell repertoire in the gut: clues to the origin of cell lineages, proliferative units and cancer. *Int. J. Exp. Pathol.* 2000, 81 (2), 117-143.
- Tran L., Greenwood-Van Meerveld B.: Age-Associated Remodeling of the Intestinal Epithelial Barrier. *J. Gerontol. A Biol. Sci. Med. Sci.* 2013, 68 (9), 1045-1056.
- Zaitoun A. M., Cobden I., Mardini H.: Morphometric studies in rectal biopsy specimens from patients with ulcerative colitis: effect of oral 5 amino salicylic acid and rectal prednisolone treatment. *Gut.* 1991, 32 (2), 183-187.

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