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Efficacy of Intraoperative Lavage with Ozonized Physiological Solution in Case of Experimental Peritonitis

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Abstract

The efficacy of sanitization of the abdominal cavity with ozonized physiological solution under conditions of simulating experimental peritonitis has been examined. By the results of histological examination of the small and large intestinal walls reduced inflammatory process and destruction of the muscular layer in the form of focal moderately pronounced inflammatory infiltration were found. Condition of APUD system was estimated by the amount of APUD (amine precursor uptake and decarboxylation)-cells, found practically in all the glands. An average number of APUD-cells (142+20,4) was close to the control one (326+16,8), which is indicative of an increased functional activity of apudocytes under conditions of introduction of ozonized physiological solution.

Keywords: purulent peritonitis, APUD-system, ozonized physiological solution.

1. Introduction

In spite of the progress and improvement of surgical methods of treatment and introducing of new antibacterial means into surgical practical work, lethal outcome in case of diffuse purulent peritonitis remains high.

One of the most dangerous complications in the post-operative period in case of peritonitis of appendicular genesis is acute commissural intestinal obstruction constituting 4,5 % of all surgical diseases of the abdominal cavity. It is the most severe sign of commissural peritoneal disease and occupies a leading place among other kinds of intestinal obstruction (Upalakalin et al., 2006; Adegbola et al., 2005).

APUD-system (Amine Precursors Uptake and Decarboxylation system) – is the system of cells having general embryonic origin able to produce and accumulate biogenic amines and (or) peptide hormones. Considering the fact that practically half of APUD-cells are located in the gastrointestinal tract, the role of the system in secretory regulation becomes understandable, but its influence in the development intra-abdominal commissures and course of post-operative period is not studied practically (Амирасланов и др., 2010, Курик и др., 2013; Осадчук и др., 1996).

Recently an increased interest to alternative non-medical methods of treatment has been noticed which is connected with a number of factors: a high frequency of allergic reactions to medical agents; a great number of contraindications and side effects in case of administration of potent medical preparations (Штикер, 2000).

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Objective: to study in the experiment histological changes of the intestinal wall and condition of APUD-system in case of peritonitis and efficacy of sanitization of the abdominal cavity with ozonized physiological solution.

2. Materials and methods

The experimental studies were conducted on 30 rats with the body weight of 270 ± 26 mg. Peritonitis was simulated by means of intra-abdominal administration of 10% autofeces mixture. 48 hours later laparotomy was performed, the abdominal cavity was cleaned with antiseptic solutions (group of comparison), washing with ozonized physiological solution (main group). The operative wound was sutured layer-by-layer.

Surgery was performed under conditions of vivarium at the Higher Educational Establishment of Ukraine “Bukovinian State Medical University” according to “General Ethic Principles of Experiments on Animals” (Kyiv, 2011), in accordance with “European Convention on Protection of Vertebrate Animals Used for Experiments and Other Scientific Purposes” (Strasburg, 1985). Blood from the posterior vena cava and the walls of the small and large intestines was taken on the 5th and 10th days after laparotomy had been performed under general i/v anaesthesia (calypso solution 12,5 mg/kg).

Peritonitis was simulated by means of intra-abdominal administration of 10 % autofeces mixture.

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Euthanasia of rats was conducted 10 days after the experiment. Portions of the ileum and caecum were taken for morphological examination.

The fragments of the intestinal wall were fixed in 10 % neutral formalin solution. After common preparation of specimens they were saturated with paraffin. Then microscopic sections 3-5 microns thick were made on the rotation microtome. The sections obtained were stained with hematoxylin-eosin and impregnated with silver by Grimelius and Mason-Gamperl staining.

The number of endocrine cells was calculated in the light microscope Olympus – CX 41 by means of detection of an average amount of APUD-cells in 10 visual fields of every section magnified x 200, x125.

The results were statistically processed by the common methods using Student t-criterion. Differences were considered to be statistically reliable with reliability level 0,05 and higher.

3. Results and discussion

The intestinal wall in the portion of the ileocecal angle in the control group of rats was found to be of usual histological structure. The mucous membrane was in the condition of normal physiological functioning with secreting glands; the glands are with clear enterocytes in the small intestine and colonocytes in the large intestine, as well as a considerable amount of cup-shaped cells secreting mucus (Fig. 1).

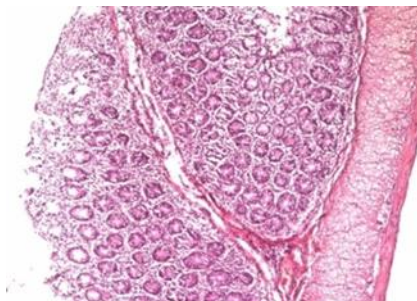


Fig. 1. Portion of the ileocecal angle. Staining with hematoxylin-eosin; magnified: x 125

In the submucous membrane the vessels with moderate blood filling, single lymphocytes, macrophages were found. The muscular membrane was with clear structure of fibers. Nerve plexuses were detected in the muscular layer.

In the control group of rats endocrine cells (APUD-cells), which granules were stained with silver by Grimelius and Mason-Gamperl staining, were found in great amounts in the glands of the mucous membrane. The granules were located in cells both in the basal and apical part of the cells; the granules were of different shape and occupy a considerable part of the cell which is indicative of normal secretory activity of the endocrine cells.

An average amount of APUD-cells in the mucous membrane of the ileocecal angle in the control group of rats was $326 \pm 16,8$ (Fig. 2).

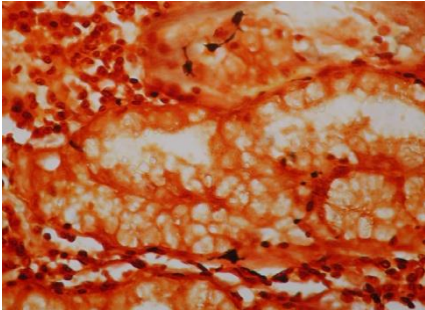


Fig. 2. Mucous membrane in the portion of the ileocecal angle. Impregnated with silver by Grimelius method; magnified: x 200

In the comparison group the portions of Bauhin's valve (ileocecal valve) are found in the sub-mucous layer of the intestinal wall, the walls of the small intestine were inflamed, granulomas were mostly of lymphocytes, macrophages that can be estimated as a sign of chronic inflammatory process; the mucous membrane in this case was with dystrophic changes of the villi epithelium (Ajisaka et al., 2003). In the sub-mucous and muscular membranes of the small and large intestines adjacent to the portion of Bauhin's valve diffuse moderate infiltration with lymphocytes, granulocytes, macrophages, plasmatic cells were found (Fig. 3).

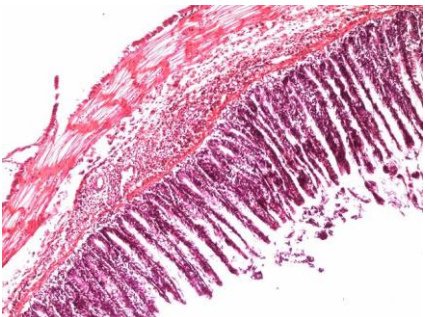


Fig. 3. The portion of Bauhin's valve. Stained with hematoxylin-eosin; magnified: x 200

Moderate infiltration with lymphocytes and macrophages are detected in the intestinal wall in the portion of Bauhin's valve in the sub-mucous layer; hyperemia, stasis in the blood vessels of the sub-mucous membrane. Thickened serous membrane, it focal fibrosis were found as well as its moderate infiltration with lymphocytes and macrophages. In the wall of the large intestine moderate dystrophic changes in the epithelium of the mucous membrane, focal fibrosis of the serous and muscular layers was detected (Fig. 4).

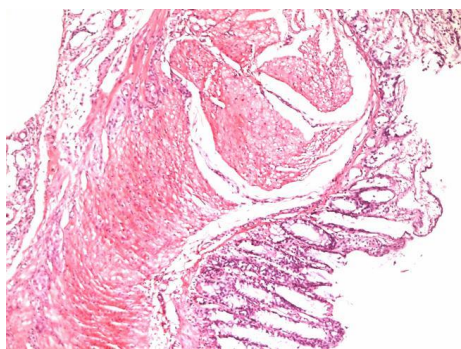


Fig. 4. The portion of Bauhin's valve. Stained with hematoxylin-eosin; magnified: x 125

Inflammatory infiltration was found in the wall with focal superficial ulceration of the mucous membrane. It might be caused by trophic disorders of the intestinal wall at the expense of partial involvement of the mesentery with vessels into commissural process, due to which necrosis, inflammation and ulceration in the mucous membrane occurred, further inflammation expanded to the muscular membrane. In four cases in the group of comparison massive lymphoid-cellular infiltration of the sub-mucous membrane with formation of lymphoid follicles was found which was the reaction of the immune system to pathological process, in this case – on the development of commissures with disorders of the intestinal function (Fig. 5).

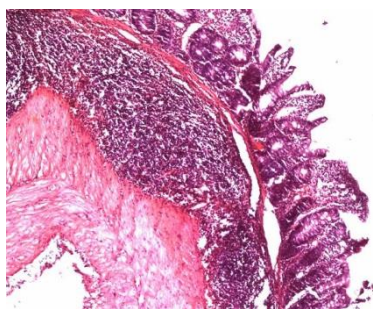


Fig. 5. The portion of Bauhin's valve. Stained with hematoxylin-eosin; magnified: x 125

Examination of APUD-system detected reduced amount of cells, APUD-cells were found only in separate glands, a small amount of argyrophilic and argentaffin granules (Fig. 6) which is indicative of decreased functional activity of APUD-cells. An average amount of APUD-cells in the mucous membrane of the intestine was $96 \pm 18,3$.

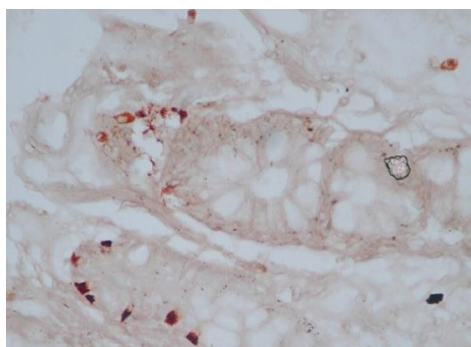


Fig. 6. The mucous membrane of the portion of the ileocecal angle. Impregnation with silver by Mason-Gamperl method, magnified: x 200

In the main group changes in the intestinal wall were less pronounced than in the previous group where ozonized physiological solution NaCl 0,9 % was not used. Thickening of the serous layer in the walls of the small and large intestines of the rats from this group was detected,

although at the same time, changes in the muscular and mucous membranes were not found practically, which was indicative of the fact that these portions of the intestine were not involved in commissural process so much as it was in the group of comparison.

In some cases in the portion of the small intestine hyperchromic glands were available, which is indicative of certain changes of their functional activity, that is, decreased production of mucus at the expense of decreased amount of cup-shaped cells looking light with foamy cytoplasm (Fig. 7).

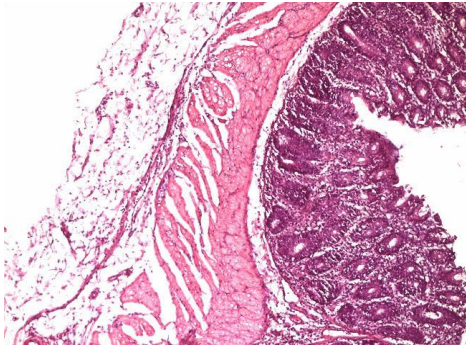


Fig. 7. The portion of the small intestine. Staining with hematoxylin-eosin; magnification: x 200

In some cases dystrophic changes were found in mucous villi. In one case from this group pronounced stasis was found in the lumen of blood vessels on the border of the sub-mucous and muscular membranes which is a sign of certain circulatory disorders. In single cases considerable diffuse lymphoid-cellular infiltration was found in the form of lymphoid follicles of big sizes in the sub-mucous layer of the intestinal wall.

In some cases dystrophic changes in the mucous villi were detected. In one case from this group pronounced stasis was found in the lumen of blood vessels on the border of the sub-mucous and muscular membranes which is a sign of certain circulatory disorders. In single cases considerable diffuse lymphoid-cellular infiltration was found in the form of lymphoid follicles of big sizes in the sub-mucous layer of the intestinal wall (Ando et al., 2006).

Thickened serous membrane with moderate infiltrations with lymphocytes, histocytes was found in the intestinal wall, although the muscular and mucous membranes were not practically changed. In the mucous membrane of the large intestine a focal desquamation of the villi epithelium was detected; the majority of glands was with integral histological structure, in the normal functional condition, with availability of a considerable amount of cup-shaped cells in the glands together with colonocytes (Fig. 8).

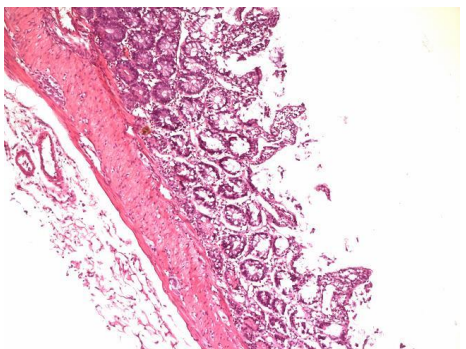


Fig. 8. The portion of the large intestine. Staining with hematoxylin-eosin; magnification: x 200

Moderate fibrosis of the sub-mucous membrane, moderate focal infiltration with lymphocytes and macrophages of the serous membrane was found in the wall of the small intestine. The glands of the mucous membrane were hyperchromic, enterocytes prevailed in them; the number of cup-shaped cells was reduced, although the glands were practically in normal functional condition.

In the portion of the ileocecal angle in some cases the glands were hyperchromic with moderately pronounced dystrophic changes in the epithelium, in some cases dystrophic changes were more focally pronounced. At the same time, focal pronounced lymphocyte infiltration of the mucous and sub-mucous membranes with formation of lymphoid follicles was detected (Fig. 9).

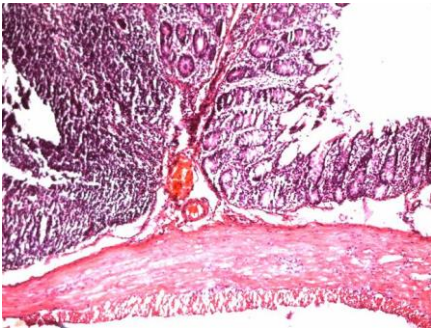


Fig. 9. The portion of the ileocecal angle. Staining with hematoxylin-eosin, magnification: x 125.

In case of staining of the intestinal wall by Grimelius and Mason-Gamperl method a bigger amount of APUD-cells was found in the glands as compared with the group where in simulating commissural disease ozonized physiological solution was not used. The cells were found almost in all the glands (Fig. 10); the number of granules and their sizes were bigger, the granules were located both in the basal and apical parts of cells. An average amount of APUD-cells in this group was $142 \pm 20,4$.

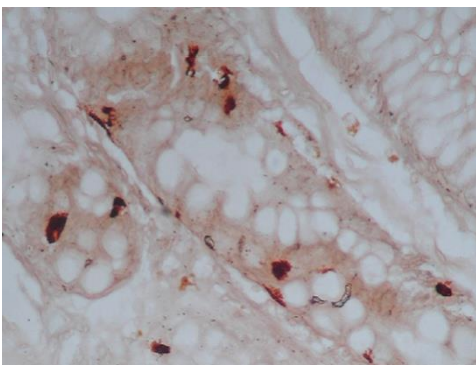


Fig. 10. The mucous membrane of the portion of the ileocecal angle. Impregnation with silver by Mason-Gamperl method, magnification: x 200.

4. Conclusion

1. Histological examination of the walls of the small and large intestines in case of experimental peritonitis resulted in detection of a positive effect with intra-operative administration of ozonized physiological solution NaCl 0,9 % at the expense of decreased inflammatory process, decreased destruction of the muscular membrane in the form of focal moderately pronounced inflammatory infiltration.

2. Examination of APUD-system in case of intra-operative administration of the ozonized physiological solution NaCl 0,9 % found APUD-cells practically in all the glands, the number of granules and their sizes were larger, the granules were located both in the basal and apical parts of cells. The average number of APUD-cells ($142 \pm 20,4$) was close to the control ($326 \pm 16,8$), which is indicative of an increased functional activity of APUD-cells.

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