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PROINFLAMMATORY AND ANTIAPOPTOTIC MARKERS OF THE STAGES OF ACUTE ENTERAL INSUFFICIENCY

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ABSTRACT — The diagnostic significance of the CD3 and Bcl-2 markers for determining the stage of acute intestinal failure was investigated. The authors used their own model. Loperamide at a dose of 0.09 mg/kg/day was used as a drug inducing intestinal failure. The study was carried out on 36 male Wistar rats weighing 250±30 g, comparable in age. The study groups included 12 animals. The length of the villi, the width of the villi, the depth of the crypts, and the width of the crypts were examined morphometrically. An UltraVision Quanto Detection System HRP Polymer (ThermoFisher, USA) was used as a detection system. In group I, there were pronounced inflammation in the form of lymphoplasmacytic infiltration, shortening and thinning of intestinal villi. The number of CD3+ cells, which were located mainly in the center of the upper part of the villi, increased significantly. Bcl-2 expression decreased. In group II, shortening and thinning of intestinal villi progressed. Paneth's cells disappeared. There was a significant decrease in CD3 + and almost complete absence of Bcl-2+ cells ($p \leq 0.05$). Thus, in this work, the dynamics of the expression of pro-inflammatory and anti-apoptotic markers and their relationship with a complex of morphological changes in the wall of the small intestine of rats during the modeling of acute enteric failure have been established.

KEYWORDS — enteral insufficiency syndrome, markers of apoptosis, modeling of intestinal obstruction, apoptosis of enterocytes, CD3, Bcl-2.

INTRODUCTION

The most common cause of acute enteral failure (AEF) in adults is acute intestinal obstruction (mechanical or paralytic ileus). The incidence of acute intestinal obstruction in Russia is about 5 cases per 100 thousand people. Mortality in this pathology remains high and, according to different authors, ranges from 5.1 to 8.4%. According to American authors, intestinal obstruction accounts for approximately 15% of all visits to the emergency department for acute abdominal pain [1, 2]. Many studies have been published on AEF, however, its pathogenesis remains a mystery. The etiology of AEF is multifactorial. Mechanisms involved in AEF include inhibitory sympathetic effects; release of hormones, neurotransmitters and other mediators; inflammatory response; and the effects of analgesics. Endogenous opiates that are released after surgery, according to some authors, may cause AEF. One of the most common causes of AEF is peritonitis. In this case, a superadded infection gradually occurs. Bacterial lipopolysaccharide causes AEF, initiating an inflammatory response in the layers of intestinal smooth muscles. The most dangerous, among others, in the pathogenesis of AEF, is the inhibition of the barrier function of enterocytes, which leads to bacterial translocation into the portal tract and generalization of the infection. With the use of immunohistochemical methods, the possibilities of understanding the pathogenesis of AEF have significantly expanded. A significant role in the development of inflammation belongs to various phenotypes of T-cells CD3, a multifunctional protein marker of T-lymphocytes, responsible for signal transmission after antigens recognition by the T-cell receptor. CD3 regulates the proliferation of T-lymphocytes, and the release of cytokines. CD3 is often considered as a quantitative marker of immune damage to the intestinal wall. In addition to this, a change in the expression of the antiapoptotic protein Bcl-2 makes it possible to assess the state of enterocytes [3, 4].

Purpose of the study is to study the diagnostic significance of markers CD3 and Bcl-2 for determining the stage of AEF under experimental conditions.

MATERIALS AND METHODS

The experimental part of the work was carried out in certified laboratories. The experiment was carried out in compliance with Directive 2010/63 / EU. There is a conclusion of the Ethics Committee (Minutes No. 3 dated 31.10.2011). As a model, we used a technique developed by us for the experimental reconstruction of AEF [5]. The study was carried out on 36 male Wistar rats weighing 250 ± 30 g, comparable in age. The animals were orally administered Loperamide (LSR-004065/09) 2 times a day at a dose of 0.09 mg/day

per kilogram of body weight with an interval between ingestions of 12 hours, for 5 days. Animals under anesthesia were withdrawn from the experiment after 72 and 120 hours with the formation of compensated (group I n = 12) and decompensated (group II n = 12) AEF. The results were compared with a control group of intact animals (n = 12). Small intestine samples were taken for morphometric and immunohistochemical studies. Paraffin blocks were prepared according to the standard procedure. Rabbit antibodies to Bcl-2, clone SP66 (Roche, Switzerland) were diluted 1: 200 in an antibody diluent, rabbit antibodies to CD3 (Prime-BioMed, Russia) were diluted 1: 200. An UltraVision Quanto Detection System HRP Polymer (ThermoFisher, USA) was used as a detection system. The obtained samples were examined using an Olympus BX53 microscope (Olympus, Japan) with a set of objectives UPlanFL N 4x/0.13, UPlanFL N 10x/0.30, and UPlanFL N 40x/0.75. Photos were taken with an Infinity 2 camera (Lumenera, Canada) at 40x and 100x magnifications. The following parameters were studied: length of the villi (VL), width of the villi (VW), depth of the crypts (CD), width of the crypts (CW). Optical density and expression area were determined for each marker. The relative area of expression (%) was calculated as the ratio of the area occupied by immunopositive cells to the total area of cells in the field of view and expressed as a percentage. The optical density of expression was measured in arbitrary units. To assess the differences between the groups of subjects, a parametric unpaired t-test was used. The level of 0.05 was chosen as the critical threshold of significance. To establish a correlation, the Pearson pair correlation coefficient was calculated for nominal variables. To describe the data, the mean value (m) was used, indicating the standard deviation (sd). Statistical processing of the data obtained during the experiment was carried out using the statistical software packages Microsoft Excel 2010 and STATISTIKA 6.0.

RESULTS AND DISCUSSION

In the control group, a thick mucosa was morphologically visualized, practically not disturbed from the side of the lumen. Wide and long villi. VL averaged 365.3 ± 4.3 μm, the width of the villi, VW, was 65.3 ± 0.9 μm, the CD — 76.3 ± 0.6 μm, the width of the crypts, CW, was 31.3 ± 0.4 μm. In the control group, cells, different from the background, stained with Bcl-2 were noted.

In the morphological study in group I, the histological picture was characterized by pronounced inflammation in the stroma of the villi, there was a pronounced lymphoplasmacytic infiltration without admixture of neutrophils with a small number of eosinophils and Paneth cells. Desquamation of the epithelium took place, however, no areas of exposure of the deep mucosa were observed. No cell lysis was observed. With morphometry, VL averaged 205.3 ± 6.3 μm, VW — 55.3 ± 1.3 μm, CD — 63.3 ± 0.9 μm, CW — 26.3 ± 0.5 μm ($p \leq 0.05$). The average number of boluses on the 2nd day was 1.1, and then there was no defecation. Immunohistochemical changes in the first group: there was a large number of CD3-positive (T-cells), which are located mainly in the upper part of the villi (in the center); in addition, Bcl-2+ cells are also found in the same zones. In the center of the intestinal villi, an inflammatory infiltrate with a predominance of CD3+ T lymphocytes is observed; T cells are also localized in the upper part of the villi (Fig. 1).

In the group II, the picture was characterized by thinning of the mucous membrane of the small intestine, lysis of cells in the upper part of the villi, while the base of the villi was enlarged due to the abundance of lymphoid cells. Paneth's cells were missing. Morphometric analysis revealed significant changes in the sizes of villi and crypts — VL 145.5 ± 5.2 μm, VW — 42.3 ± 0.8 μm, CD — 53.3 ± 0.9 μm, CW — 22.2 ± 0 .5 μm ($p \leq 0.05$). Defecation was absent at all observation periods. A decrease in the number of T-cells (CD3) was noted for due to the lysis of the villi, only their lower third was preserved, where the cells are located. There was practically no Bcl-2 expression in group II (Fig. 2).

Thus, the dynamics of the expression of markers and their relationship with the complex of morphological changes in the wall of the small intestine of rats have been established during the modeling of AEF. In the group I, pronounced inflammation in the form of lymphoplasmacytic infiltration was noted. In the epithelium of the villi, there are single dystrophically altered cells that look like large vacuoles. In single fields of view, desquamation of the epithelium of enterocytes in the apical part is noted. The number of CD3+ cells, which were located mainly in the center of the upper part of the villi, increased significantly. The expression of Bcl-2 decreased slightly in comparison with the control group ($p \geq 0.05$). In the group II, shortening and thinning of intestinal villi progressed. The thickness of the intestinal wall decreased. Paneth's cells disappeared. Massive desquamation of epithelial cells was noted both in the area of the upper parts and lateral walls with exposure of the connective tissue base. There was a significant decrease in CD3+ and almost complete absence of Bcl-2+ cells ($p \geq 0.05$).

CONCLUSION

Based on the studies carried out, it can be concluded that CD3 and Bcl-2 markers are diagnostically

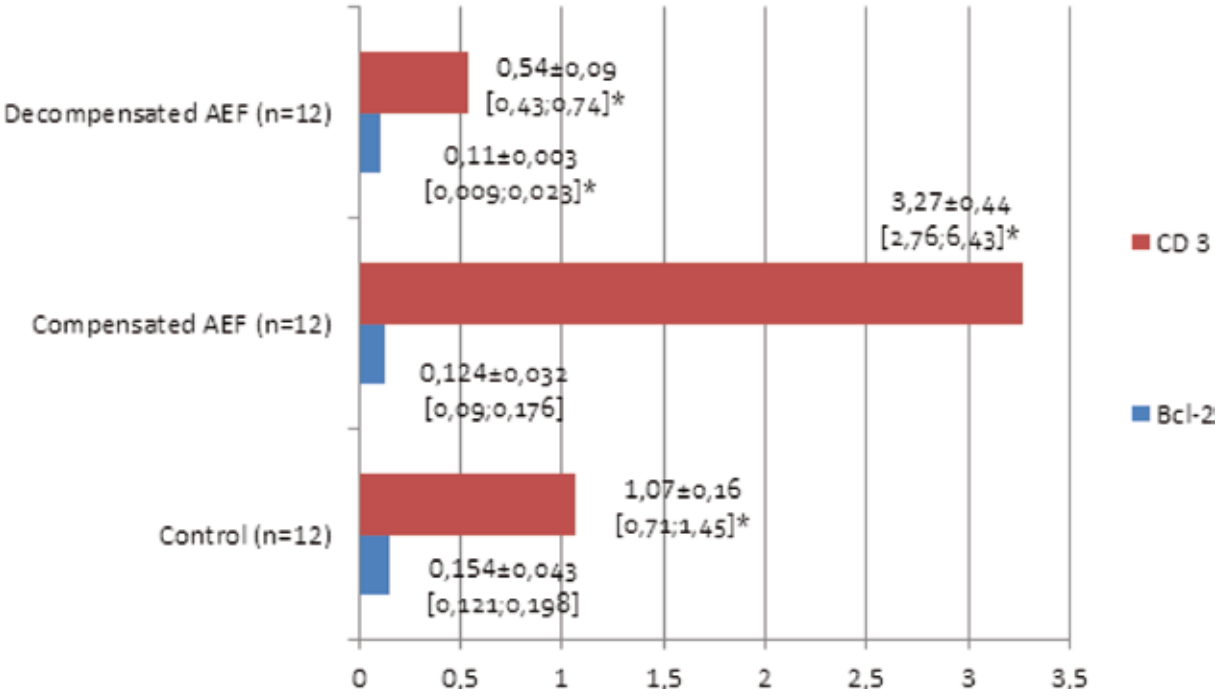

Fig.1. *Optical density of enterocytes immunopositive to Bcl-2 and CD3 in the study groups (s.u.; Me [min; max]; M ± sd)*
** — reliability of changes in values at p≤0.05 in relation to the previous study*

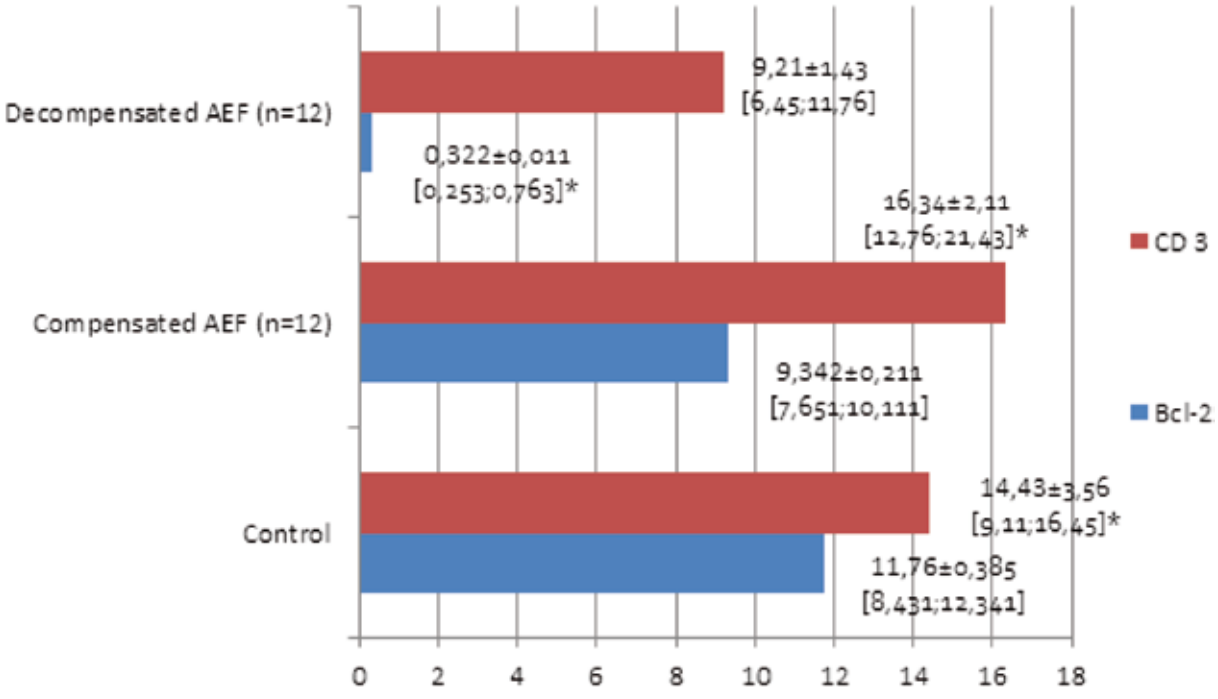

Fig. 2. *Optical area of expression of enterocytes immunopositive to Bcl-2 and CD3 in the study groups (%; Me [min; max]; M ± sd)*
** — reliability of changes in values at p≤0.05 in relation to the previous study*

significant for determining compensated and decompensated stages of AEF. The model used can be applied in experimental surgery to develop and test new treatment methods.

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