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MORPHOMETRIC ANALYSIS OF THE STRUCTURAL COMPONENTS OF THE DUODENAL WALL IN SIMULATED PANCREATITIS

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The paper presents the data of the morphometric study of the duodenal wall in cryogenic pancreatitis. It has been established that the degree of morphometric changes correlated with the duration of the simulated pathological process. An increase in the thickness of the mucous membrane, the height and thickness of the villi, the thickness of the lamina propria of the mucous membrane and the submucous layer has been noted. It was associated with edema and the inflammatory process in the investigated organ. There was also a tendency to increase in the width and depth of crypts, the height of columnar epitheliocytes in the middle part of the villi, which indicated pronounced hypertrophic changes.

Key words: alimentary system, villi, crypt, duodenum, cryogenic pancreatitis, morphometry.

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МОРФОМЕТРИЧНИЙ АНАЛІЗ СТРУКТУРНИХ КОМПОНЕНТІВ СТІНКИ ДВАНАДЦЯТИПАЛОЇ КИШКИ ЗА УМОВ ЗМОДЕЛЬОВАНОГО ПАНКРЕАТИТУ

У роботі представлені дані морфометричного дослідження стінки дванадцятипалої кишки за умов кріогенному панкреатиті. Встановлено, що ступінь мофрометричних змін взаємозалежав від тривалості змодельованого патологічного процесу. Виявлено зростання товщини слизової оболонки, висоти і товщини ворсинок, товщини власної пластинки слизової оболонки і підслизової основи, що пов'язане із набряком та запальний процес в досліджуваному органі. Також відзначалася тенденція до зростання ширини і глибини крипт, висоти стовпчастих епітеліоцитів в середній частині ворсинок, що вказує на виражені гіпертрофічні зміни.

Ключові слова: травна система, ворсинка, крипта, дванадцятипала кишка, кріогенний панкреатит, морфометрія.

The relevance of problems related to the current state of gastroenterology can be explained by the significant rise in the prevalence of diseases of the organs of the pancreatohepatobiliary system [10, 12]. Unfortunately, the abovementioned pathologies are characterized by a severe and progressive course, leading to early disability and death of working-age patients. In the etiology and pathogenesis of damage to the organs of the digestive tract, the primary place is given to the consumption of poor-quality and contaminated food and water, the chemicalization of all areas of human life, the complex ecological situation in general, the uncontrolled use of medications that cause stress, metabolic and microcirculatory disorders [4, 5]. The anatomical and physiological synergy of the organs of the digestive system determines the development of systemic lesions [1]. In this regard, pancreatic disorders are no exception, in which other digestive system organs are involved in the pathological process [2].

Currently, no clear idea exists of the prime cause and the distant consequences of combined pathologies of the organs of the pancreatohepatobiliary area. At the same time, it should be noted that the duodenum is an important component of the unified hepatobiliary system and is the subject of detailed and comprehensive studies by morphologists, endocrinologists, gastroenterologists, and immunologists to date.

The purpose of the study was to establish the dynamics of morphometric changes in the structural components of the duodenal wall in cryogenic pancreatitis.

Material and Methods. The experimental study was conducted on 40 laboratory white rats, which were assigned into two groups. Group I (n=10) involved intact animals; Group II (n=30) involved animals with experimental pancreatitis. Experimental damage to the pancreas in the animals was modeled by local freezing of both its surfaces with chloroethyl. Control animals underwent an identical laparotomy without freezing the pancreas.

Animal housing and experiments on them have been carried out in compliance with the rules of the Strasbourg "European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes" and "General Ethical Principles of Animal Experiments" (Kyiv, 2013).

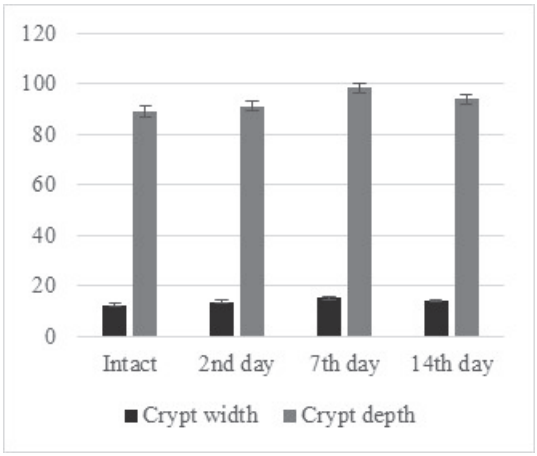
Animals with experimentally simulated cryogenic pancreatitis were sacrificed on days 2, 7 and 14. For histological studies, the duodenal material was collected, and pieces of the organ were cut out from its middle part. The material was fixed in a 10 % neutral formalin solution with three changes of the fixative, dehydrated in alcohols of increasing concentration and embedded in paraffin blocks. Sections with a thickness of 5–6 μm were stained with hematoxylin and eosin [1].

A system of visual image analysis using the Vision CCD Camera and the SEO ImageLAB Bio software was used for morphometric histological studies of histological preparations. Morphometrically, in histological preparations, the thickness of the duodenal mucous membrane, submucous layer, muscle and serous membranes, the height and width of the villi, the depth and width of the lumen of the crypts, the thickness of the lamina propria and muscle lamina of the mucous membrane, the height of columnar epitheliocytes with a brush border were measured. Quantitative parameters were processed statistically using Microsoft Excel software.

Results of the study and their discussion. The findings of the morphometric studies have established that on day 2 of the experimental cryogenic pancreatitis, the structural changes of the duodenal wall were characterized by a slight thickening of its mucous membrane from 687.4±13.0 to 701.1±24.1 μm. The height of the villi increased and accounted for 596.1±17.2 μm, while in the control group this parameter was 585.4±10.3 μm (Fig. 1A), which was statistically insignificant. In this experiment period, the villi's thickness increased significantly by 1.3 times and accounted for 56.31±1.42 μm ($p<0.001$) in the experimental group of animals. The height of epitheliocytes in the middle part of the villi increased significantly ($p < 0.05$). It accounted for 18.22±0.41 μm, which was by 9.0 % greater compared to the similar parameter of the group of intact animals.

The crypts of the duodenal mucosa became larger, and their depth was 91.26±3.53 μm, which was not statistically significantly different from the similar parameter in the group of intact animals. The width of the crypts on day 2 of the experiment increased statistically significantly and accounted for 13.72±0.47 μm, which was by 9.8 % greater ($p<0.05$) compared to the animals of the intact group (Fig. 1B).

A



B

Fig. 1A, B – Morphometric parameters of changes in the villi and crypts of the duodenum in experimental animals at different observation periods.

At the bottom of the crypts, cells in different phases of mitosis were noted less often compared to the control group, which was clearly visible from the characteristic pattern of chromatin. The mitotic index of the columnar epitheliocytes during this period of the experiment accounted for 1.908±0.053 %, that was by 1.7 times lower ($p<0.05$) compared to the similar parameter in the group of intact animals (Fig. 2).

The thickness of the submucous layer of the duodenum on day 2 of the experiment increased statistically significantly and accounted for 278.0±9.2 μm, that was by 1.2 times greater ($p<0.001$) compared to the value of the intact group. These changes were caused by hypertrophy of the duodenal glands and vascular wall edema (Fig. 3).

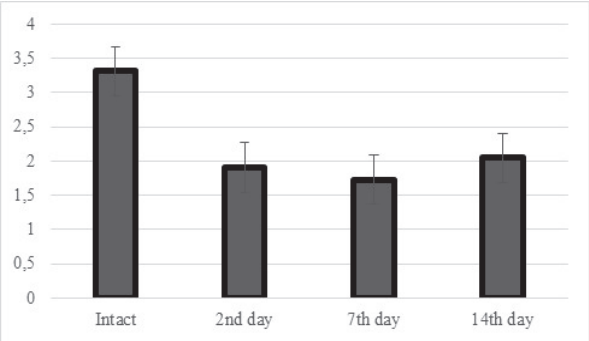


Fig. 2. The dynamics of changes in the mitotic index of duodenal crypts in the animals with experimental cryogenic pancreatitis at different periods of observation.

Fig. 3. Dynamics of morphometric parameters of the duodenum in experimental cryogenic pancreatitis.

The connective tissue of the lamina propria was swollen and cleared, its thickness was 11.33±0.35 μm ($p<0.05$), which was by 9.2 % greater compared to the animals of the intact group. At this time period of the experiment, the abovementioned changes were statistically significant. The muscle lamina of the mucous membrane was 7.13±0.21 μm, and the muscular coat was 31.17±1.15 μm; statistically, they did not differ from similar parameters of animals of the intact group, accounting for 7.02±0.26 μm and 31.37±1.12 μm, respectively. The serous membrane of the duodenum retained its characteristic two-layer structure and had a thickness of 4.39±0.11 μm, remaining practically unchanged (the parameter of the comparison group was 4.25±0.15 μm).

The thickness of the duodenal mucosa increased statistically significantly during the abovementioned period of observation and accounted for 732.1±19.2 μm ($p<0.05$), which was by 6.5 % greater compared to the similar parameter in the group of intact animals. At the same time, the height of its villi was 617.3±12.6 μm ($p<0.05$), which was by 5.5 % greater compared to the similar parameter in the group of intact animals. The specified changes in this term of the experiment were statistically significant.

On day 7 of the experimental study, the thickness of the villi increased statistically significantly to 63.84±1.65 μm ($p<0.001$), which was by 1.4 times greater than the similar parameter in the group of healthy rats. Compared with the previous period of observation, the height of epitheliocytes decreased slightly and accounted for 17.97±0.35 μm. During this period of observation, the depth of the crypts increased statistically significantly to 98.31±2.24 μm ($p<0.001$), which was by1.1 times greater than in the group of intact animals. It should be noted that the crypts became by 1.2 times wider and their width was 15.11±0.36 μm ($p<0.001$) (Fig. 4). This difference was statistically significant.

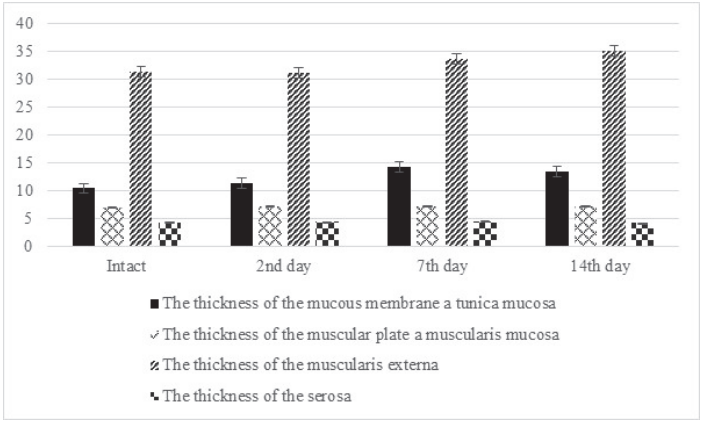


Fig. 4. Dynamics of morphometric parameters of the duodenum in experimental pancreatitis.

At the bottom of the crypts, the number of proliferating epitheliocytes decreased, the mitotic index accounted for 1.727±0.065 % ($p<0.05$) that was by 1.9 times lower compared to the studied parameter in the group of intact white rats.

Similar to the previous observation period, the lamina propria of the mucous membrane and its submucous layer was tending to thick. Thus, the thickness of the lamina propria of the mucous membrane of the duodenal wall was 14.22±0.44 μm ($p<0.001$) that was by 1.3 times significantly greater than the similar parameter in the group of intact animals. On day 7 of the observation, the thickness of the submucous layer was statistically significantly greater and accounted for 306.7±10.2 μm ($p<0.001$), which was by 33.2 % greater compared to the group of intact animals.

The thickness of the muscle lamina of the mucous membrane was 7.24±0.14 μm, and the muscular coat enlarged to 33.57±1.41 μm; however, the above values did not statistically significantly differ from those in the comparison group of animals. The thickness of the serous membrane of the duodenum was 4.43±0.12 μm, and was not significantly different from the control value of 4.25±0.15 μm.

The thickness of the duodenal mucosa during the specified period of the observation was 690.3±24.3 μm, and practically did not differ from the similar parameter in the group of comparison animals. The height of the villi was almost normal, accounting for 579.0±14.4 μm. The difference between the above values was statistically insignificant.

On day 14 after experimental cryogenic pancreatitis, the thickness of the villi decreased slightly and accounted for 57.23±1.72 μm ($p<0.001$), though it was statistically significantly greater compared to the intact value. The height of epitheliocytes accounted for 17.12±0.48 μm, that was by 2.4 % greater compared to the similar parameter in the group of intact animals.

The depth of the crypts during the specified period of the observation was 93.95±3.37 μm, with no significant difference compared to the similar parameter in the comparison group. The width of the crypts decreased and accounted for 14.14±0.48 μm ($p<0.001$), that was by 1.1 times statistically significantly greater compared to the similar parameter in the group of intact animals. They were less tortuous than on day 2 and 7 of the experiment. During the specified period of the observation, the number of dividing epitheliocytes increased at the bottom of the crypts. The mitotic index during this period of the experiment accounted for 2.045±0.054 %, that was by 1.6 times lower compared to the studied parameter in the group of intact white rats.

The thickness of the lamina propria of the mucous membrane of the duodenum on day 14 of the experiment was statistically significantly greater compared to the intact animals and was 13.42±0.37 μm ($p<0.001$), that was by 1.3 times greater compared to the similar parameter in the group of intact white rats. The thickness of the submucous layer during the specified period of the observation decreased compared to the animals on day 7 day of the observation, but statistically significantly exceeded the similar parameter by 1.0 times and accounted for 261.4±4.78 μm ($p<0.001$) compared to the intact group of animals. As in the previous observation periods, the serous membrane remained without significant changes, and its thickness was 4.17±0.16 μm.

The findings of the morphometric study of the duodenal wall in experimental cryogenic pancreatitis are, to a certain extent, consistent with the available data in the literature [5]. We established that the degree of damage to the structural components of the duodenal wall was depended on the duration of the simulated pathological process in the pancreas, which are consistent with the findings [3]. The increase in the thickness of the mucous membrane, the lamina propria of the mucous membrane and the submucous layer, the height and thickness of the villi can be explained by the edema that accompanies the inflammatory process in the studied organ, which corresponds to the data of several researchers [9].

Hypertrophic changes that occur in the duodenal wall in cryogenic pancreatitis lead to an increase in the width and depth of the crypts, the height of the columnar epitheliocytes in the middle part of the villi, which also are in concordance with the literature data [11]. The experiment's findings [7] indicate the regenerative mechanisms triggering in the duodenum conditions of concomitant damage to the pancreas. According to our research, at the bottom of the crypts, we noted a significant suppression of the mitotic activity of epitheliocytes on day 2 of the observation, and only on day 14 of the experiment this parameter was tending to increase.

Conclusion

Thus, in cryogenic damage to the pancreas, the development of destructive changes in the duodenal wall is characterized by remodeling of all wall membranes and to the greatest extent by thickening of the mucous membrane and submucous layer. The peak values of the morphometric parameters of the structural components of the duodenum were recorded on day 7 of the experiment, which can be explained by the edema of the stroma of the villi and the intercellular substance, hemostasis in the elements of the hemomicrocirculatory bed, and inflammatory leukocyte infiltration.

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