

HISTOLOGICAL CHANGES IN THE DUODENAL WALL IN EXPERIMENTAL PANCREATITIS

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The morphological state of the duodenum in simulated cryogenic pancreatitis has been studied during the experiment on white rats. It has been established that on day 2 of the experiment the reactive changes occurred in the in the duodenal wall that were characterized by the dilatation of lumens and plethora of the vessels, edema of the connective tissue of the mucous and submucous membranes, thickening and deformation of the villi. On day 7 of the experiment histological changes in structural components of the duodenum became more drastic, manifested by the damage and desquamation of the columnar epithelial cells with border, edema of the stroma, leukocytic infiltration, plethora and destruction of the walls of the vessels of microvasculature, hypertrophy of the acini of the duodenal glands. On day 14 of the experiment, the lesions in duodenum wall were less pronounced in contrast to the previous time period. The plethora of the vessels, edema of connective tissue, leukocytic infiltration and damage of the cells of epithelial lamina was decreasing. The detected histological changes in the duodenum in the experimental pancreatitis are crucial in the search for effective corrective factors that will lead to normalization of its structural components.

Key words: histological changes, duodenum, experimental pancreatitis.

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The publications report that pancreatitis can cause a range of structural and functional changes in the duodenum. In this part of the digestive tube the alimentary tracts of the stomach, liver and pancreas are "intersected". Therefore, destabilization of one of these organs can lead to disorder of morphological status of the other ones [6, 11]. It has been established that disorders of the pancreas cause the lesions in the duodenum [2, 4], and the severity and nature of morphological abnormalities are correlated with duration of the disease [3, 9]. Deformed villi of the duodenal mucosa and desquamation of the epithelial cells occur in patients with acute pancreatitis. The lumens of blood capillaries in the intestinal villi are dilated and plethoric. The amount of goblet cells increase both in crypts and on the villi. The connective tissue of the lamina propria and submucous layer is edematous with increased number of lymphocytes, histocytes, sporadic eosinophil cells and tissue basophils. These changes were associated with impaired hemodynamics in the vessels of the microvasculature [1, 11, 18]. It has been found that cryogenic damage to the pancreas leads to the development of the significant changes in the small intestine mucosa [3]. It has been confirmed that the epithelium of the mucous membrane, which provides its primary function, contributes to the compensatory processes aimed at recovering the morphological state of the small intestine [8]. Therefore, the microscopic and ultramicroscopic studies of the structural changes in the duodenal wall in pancreas lesions are theoretically and practically relevant to date.

The purpose of the paper was to determine changes in the rat duodenal wall in dynamics after experimental cryogenic pancreatitis at the microscopic and ultramicroscopic levels.

Materials and Methods. Histological studies of the reorganization of the duodenal wall in the animals with pancreatitis have been carried out on 20 laboratory male white rats with mean body weight of 200 ± 5 g. The animals were kept in the standard conditions of the vivarium at the SHEI "I. Horbachevsky Ternopil State Medical University", Ukraine. Rats were inspected daily for the overall health, body weight and lethality. The latter accounted for not greater than 5% in the group of animals with experimental pathology. The experiments were performed in compliance with the "scientific and practical recommendations on the housing of laboratory animals and experiments on them" [5] and the European Convention on the protection of laboratory animals [10]. No violations of ethical standards in the conduct of scientific research have been revealed by the commission on bioethics. The laboratory animals were assigned into two groups: Group 1 (n=5) involved the intact white rats; Group 2 (n=15) involved animals with induced cryogenic pancreatitis of different duration. Experimental lesions of the white rats' pancreas were induced by the local freezing of both surfaces of the pancreas with chloroethane during 10 s according to S.O. Shalimov. The animals underwent medial laparotomy under sodium thiopental anesthesia to assess the pancreas. The animals were taken out of the experiment on day 2, 7 and 14. For histological studies the slices of the duodenum were fixed in 10% neutral formalin solution. The subsequent processing of the material with the follow up embedment into

paraffin blocks was conducted using the conventional methods [2]. The sections, obtained on the sliding microtome, were stained with hematoxylin-eosine [7]. Histological specimens have been studied using the light microscope SEO SCAN and the images were made with Vision CCD Camera with image output system of histological preparations. The material for electron microscopic study of the duodenum was taken using the conventional technique. The duodenal specimens were fixed in the Millonig buffered 2,5% glutaraldehyde solution (pH 7,2-7,4). Postfixation was made by Millonig buffered 1 % Osmium(IV) oxide solution during 60 min with subsequent dehydration in spirits and acetone and embedment into the mixture of epoxy resins and araldite. The ultrathin sections, made on the LKB-3 (Sweden) ultramicrotome, were contrasted by 1 % uranyl acetate solution and lead citrate according to Reynolds and analyzed on the PEM-125K electron microscopy.

Results and Discussion. Microscopic studies of the duodenal wall on day 2 after modeling of cryogenic pancreatitis have shown in the mucous membrane and submucous layer reactive changes in blood vessels, manifested by dilatation and plethora of its lumens. Aggregation of the blood corpuscles was noted in blood capillaries and venules. In the mucous membrane the villi were thickened and deformed; edema of the lamina propria was found and lumens of the crypts were moderately dilated. Infiltration of loose fibrous connective tissue of the mucous membrane and submucous layer by lymphocytes and plasma cells was detected (Fig.1).

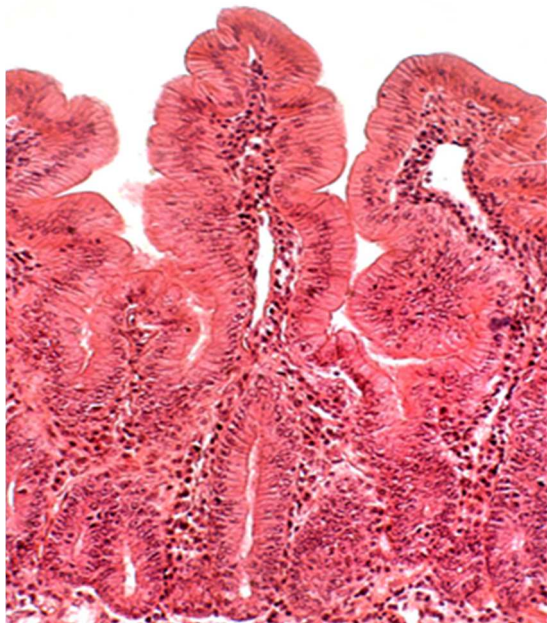


Fig. 1. Microscopic changes of the duodenal mucosa on day 2 of the experiment. Thickening and deformation of the villi of the duodenal mucosa, edema of the connective tissue of the lamina propria, enlargement of the lymphoid cells. H&E stain. Magnification $\times 200$.

Changes in the cells of the epithelial lamina of the mucous membrane were detected in the early periods of the simulated acute pancreatitis. In the columnar epithelial cells with border the nuclei shifted from the basal membrane to the center of the cell; brush border lost its clearness at their apical pole. Desquamation of the part of the epithelial cells was noted at the top of the villi.

The significant widening of the cytoplasm of the goblet cells that produce mucus was detected both in the villi and crypts. Their oval bodies were clearly defined due to the presence of numerous clear secretory granules, and oblate basophilic nuclei were located in the basal portion of the cells. The enlargement of the area of the acini of the duodenal glands in the submucous layer was observed and the cytoplasm was filled with secretory granules in the glandulocytes. At this period of the experiment the electron microscopic studies of the duodenum showed vascular lesions of the microvasculature in the lamina propria of the duodenal mucosa. Dilatation of lumens and plethoric capillaries was noted. Inhomogeneous thickening of cytoplasmic areas of the endothelial cells, destruction of organelles and decrease in number of pinocytic vesicles was detected.

The basal membrane was inhomogeneous, thickened in some segments and lost clearness. Edema of connective tissue and clearing of the amorphous component of the intercellular substance was noted. The nuclei with inhomogeneous karyolemma with the presence of heterochromatin in the karyoplasm were found in the middle part of the intestinal villi in the majority of the columnar epithelial cells; the karyoplasm had considerable osmiophilic areas, especially near the karyolemma. The significant sign of the impaired parietal digestion and absorption is the altered structure of the microvilli. Their height decreased, part of them were fragmented and some of them were destroyed (Fig. 2). In such epithelial cells organelles were destructively changed. In the basal poles of the cells the length of individual tubules of the granular endoplasmic reticulum decreased; not much ribosomes were found on its surface, and its membranes were poorly circumscribed. Edema, dilatation and vacuolization of the components of the Golgi complex were detected. Part of the mitochondria was hypertrophic with focally cleared matrix and reduced cristae. Goblet cells were noted among the epithelial cells. The apical part of their cytoplasm was filled with large secretory granules, and osmiophilic nuclei were pyknotic, locating in the basal pole. On the submucous layer of the acini of the duodenal glands the glandulocytes with considerable amount of secreta prevailed, corresponding to the stage of its aggregation. On day 7 of the experiment the changes in structural components of the duodenum became more drastic. Thickened villi, damaged surface epithelium and prominent leukocytic infiltration were detected in the mucous membrane.

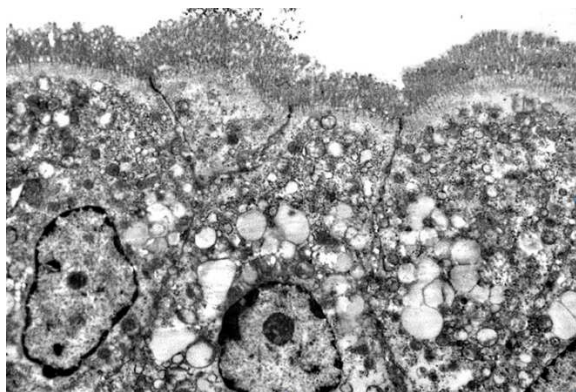


Fig. 2. Submicroscopic changes of the columnar epithelial cells of the rat duodenal mucosa on day 2 after simulation of the cryogenic pancreatitis. Destruction of microvilli (1), altered nuclei (2), vacuolate structures in the cytoplasm (3). Magnification $\times 8\ 000$.

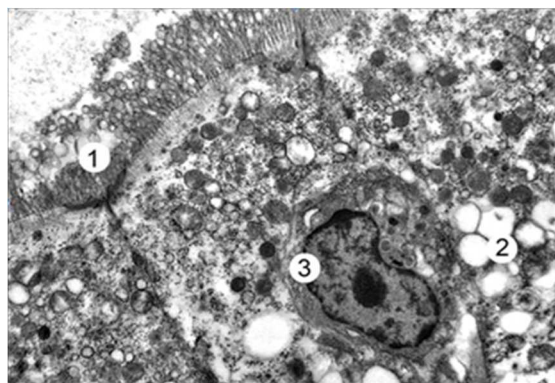


Fig. 3. Ultrastructural changes in the epithelial cells of the duodenal villus on day 7 after simulation of the cryogenic pancreatitis. Damaged microvilli (1), vacuoles (2) in the cytoplasm, interepithelial lymphocyte (3). Magnification $\times 7\ 000$.

The signs of hemostasis in the vessels of the microvasculature and perivascular edema were noted in the connective tissue of the mucous membrane. Against the background of the cleared amorphous substance numerous lymphocytes and plasma cells were well defined. In the stromal connective tissue of the submucous layer the plethoric vessels, especially veins and venules, as well as duodenal glands with the signs of hypertrophy of the acini were also found. Small basophilic nuclei of the exocrine cells were located in the basal portion of the cell and their apical areas were overfilled with secreta. Day 7 of the study of ultrastructure of the components of the duodenal wall showed the preserved dilatation and plethora of the vessels of microvasculature and destruction of the wall of blood capillaries. The loose connective tissue of the lamina propria was electron-clear, edematic with damaged fibrillar components. The increase in damaged epithelial cells was found in the epithelial lamina of the villi. The marked fragmentation and desquamation of the microvilli and reduce in their size was detected. The nuclei of the columnar epithelial cells were enlarged, their karyolemma was deformed and heterochromatin prevailed in the karyoplasm. The destruction of organelles was accompanied by their vacuolization, therefore, the oblong structures were found in the cytoplasm, especially in the paranuclear zone. In the extended interepithelial spaces lymphocytes were often found (Fig. 3). Goblet cells were noted among the epithelial cells, the apical part of which was filled with numerous secretory granules. On the duodenal submucous layer of the acini of the duodenal glands the glandulocytes with considerable amount of secreta were observed. The greater part of the cytoplasm of the cells was filled with large secretory granules. The increase in lymphocytes and plasma cells was frequently detected in the connective tissue.

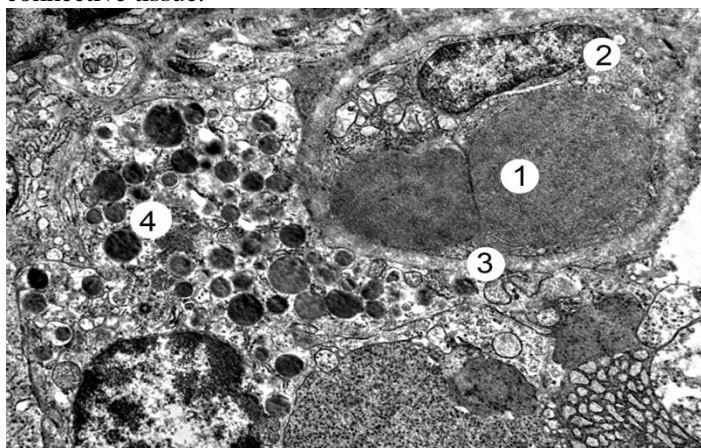


Fig. 4. Ultrastructural state of the lamina propria of the duodenal mucosa on day 14 after simulation of the cryogenic pancreatitis. The lumen of the blood capillary with red blood cells (1), endothelial cell (2), homogenous basal membrane (3), tissue basophil (4). Magnification $\times 8\ 000$.

On day 14 of the experiment the changes, similar to the previous period, were preserved in the duodenum, though they were less manifested. Edema of the villous stroma was reducing. However, the plethora of blood vessels and damage to their wall were not so significant. Leukocytic infiltration of the stroma and epithelial lamina was moderate. In the submucous layer the hypertrophy of the acini of the duodenal glands has been noted, though the amount of secreta in the glandulocytes was not as significant as on day 7 of the experiment. At this period of the experiment the electron microscopic studies established the reduction in the plethora of the vessels of microvasculature, and dilatation of the

lumens of the blood capillaries was insignificant. Cytoplasmic areas of the endotheliocytes were moderately altered, most nuclei were elongated, though the basal membrane remained unevenly thickened, and in certain areas was not clearly circumscribed. Contact of tissue basophils with the basal membrane was noted, as well as their partial degranulation, which contributed to the reduction of the edema of loose connective tissue of the duodenal lamina propria (Fig. 4). In the villous epithelial lamina heterogeneous changes of the columnar epithelium were detected; some cells were significantly damaged, while in others their architecture has been

preserved. Submicroscopically it was manifested by the better state of the micrivilli on the apical surface of the cells. In the cytoplasm the destruction of organelles was less evident; hypertrophy of the mitochondria, cisterns of the Golgi complex and canaliculi of the endoplasmic reticulum was observed. The nuclei were of the regular structure, oblong, with predominance of euchromatin in the karyoplasms, though large nucleoli were also noted. On the duodenal submucous layer of the acini of the duodenal glands the glandulocytes with considerable amount of secretory granules were observed, and less amount of other secretory granules were found in the cytoplasm, indicating about relative normalization of secretion. The detected changes in the structural organization of the duodenal mucosa and submucous layer are consistent with data of the scientific research [1, 3, 11].

Conclusions

1. Restructuring of the duodenal wall in dynamics in the experimental pancreatitis is specific. In the early period (day 2) of the experiment the reactive changes, manifested by dilatation and plethora of the vessels of the mucous membrane and submucous layer, edema of the stroma, thickening and deformation of the villi, initial structural disorder of the columnar epithelial cells and changes in the glandular components of the organ (goblet cells and duodenal glands) have been histologically established.
2. In the experimental cryogenic pancreatitis the most pronounced destructive changes in the mucous membrane and submucous layer occur on day 7 of the observation. Major disorders of the vascular system of the organ are combined with the destruction of all components of the duodenal mucosa and submucous layer. The phased pattern of secretion of the exocrine cells of the duodenal glands' acini undergoes significant changes. Along with impairment of the wall of blood capillaries the intensification of leukocytic infiltration of the epithelial lamina and connective tissue of the duodenal wall occurs.
3. In the late period (day 14) of the experiment after the simulated pancreatitis the signs of the reparative regeneration were microscopically and submicroscopically established in the duodenal mucosa and submucous layer. The plethora of the vessels and stromal leukocytic infiltration decreased, the structure of the columnar epithelial cells was enhanced and the state of the goblet cells and acini of the duodenal glands normalized.

Perspectives of further research. *The findings of the experimental studies of the duodenum in the experimental cryogenic pancreatitis have established a considerable disturbing effect of this factor; therefore, it is necessary to continue the study with the use of corrective factors.*

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Реферати

ГИСТОЛОГИЧНІ ЗМІНИ В СТІНЦІ ДВНАДЦЯТИПАЛОЇ КИШКИ ЗА УМОВ ЕКСПЕРИМЕНТАЛЬНОГО ПАНКРЕАТИТУ
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В експерименті на білих щурах проведені дослідження морфологічного стану дванадцятипалої кишки в умовах експериментального криогенного панкреатиту. Встановлено, що на 2 добу досліду в стінці органу наступають реактивні зміни, які характеризуються

ГИСТОЛОГИЧЕСКИЕ ИЗМЕНЕНИЯ В СТЕНКЕ ДВЕНАДЦАТИПЕРСТНОЙ КИШКИ В УСЛОВИЯХ ЭКСПЕРИМЕНТАЛЬНОГО ПАНКРЕАТИТА
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В эксперименте на белых крысах проведены исследования морфологического состояния двенадцатиперстной кишки в условиях экспериментального криогенного панкреатита. Установлено, что на 2 сутки опыта в стенке органа наступают реактивные изменения, характеризующиеся расширением просветов

розширенням просвітів та кровонаповненням судин, набряком сполучної тканини слизової та підслизової оболонок, потовщенням та деформацією ворсинок. На 7 добу експерименту гістологічні зміни в структурних компонентах дванадцятипалої кишки нарастають, що проявляється пошкодженням та десквамацією стовпчастих епітеліоцитів з облямівою, набряком строми, лейкоцитарною інфільтрацією, кровонаповненням та деструкцією стінки судин мікроциркуляторного русла, гіпертрофією кінцевих секреторних відділів дуоденальних залоз. На 14 добу експерименту деструктивні зміни структур стінки дванадцятипалої кишки менш виражені, ніж у попередній термін. Зменшується кровонаповнення судин, набряк сполучної тканини та лейкоцитарна інфільтрація, пошкодження клітин епітеліальної пластинки. Встановлені гістологічні зміни дванадцятипалої кишки за умов експериментального панкреатиту необхідні для пошуку ефективних коригуючих чинників, що призведуть до нормалізації її структурних компонентів.

Ключові слова: гістологічні зміни, дванадцятипала кишка, експериментальний панкреатит.

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и кровенаполнением сосудов, отеком соединительной ткани слизистой и подслизистой оболочек, утолщением и деформацией ворсинок. На 7 сутки эксперимента гистологические изменения в структурных компонентах двенадцатиперстной кишки нарастают, что проявляется повреждением и десквамацией столбчатых эпителиоцитов с каемкой, отеком строми, лейкоцитарной инфильтрацией, кровенаполнением и деструкцией стенки сосудов микроциркуляторного русла, гипертрофией конечных секреторных отделов дуоденальных желез. На 14 сутки эксперимента деструктивные изменения структуры стенки двенадцатиперстной кишки менее выражены, чем в предыдущий срок. Уменьшается кровенаполнение сосудов, отек соединительной ткани и лейкоцитарная инфильтрация, повреждения клеток эпителиальной пластинки. Установленные гистологические изменения двенадцатиперстной кишки в условиях экспериментального панкреатита необходимые для поиска эффективных корректирующих факторов, которые приведут к нормализации структурных компонентов этого отдела пищеварительной системы.

Ключевые слова: гистологические изменения, двенадцатиперстная кишка, экспериментальный панкреатит.

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INDICATORS CELL CYCLE AND DNA FRAGMENTATION IN CELLS OF SMALL INTESTINE MUCOSA 14, 21 AND 30 DAYS AFTER SKIN BURNS ON THE BACKGROUND OF PRELIMINARY INFUSION OF SOLUTION LACTOPROTEIN WITH SORBITOL OR HAES-LX 5%

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In analyzing the data of cell cycle and DNA fragmentation of the cells of the mucous membranes of the small intestine of rats in the late stages after the thermal burn of skin 2-3 degrees, in the area of 21-23% of the body surface, on the background of the previous use of infusion solutions, it was found that "lactoprotein with sorbitol" or HAES-LX-5% have a positive effect on cell cycle performance: after 14 days, the S-phase data and the index of proliferation were increased compared with those in the burn group + 0.9% NaCl solution in the same period; after 21 days, the S-phase data and the index of proliferation of these two groups were significantly higher than those in the burns + 0.9% NaCl solution, and at the same time, the values of the SUB-G0G1 interval in both groups were lower than those in the group where was used 0.9% NaCl solution on the background of burn. After 30 days in the burn + HAES-LX-5% group, all cell cycle indices have no significant or trend-specific differences compared to those in the non-burning group, and with "lactoprotein with sorbitol", the G0G1 and the proliferation phases have been significantly lower than indicators in a group without skin burns.

Key words: cell cycle, DNA cytometry, small intestine, rats, thermal burn skin, "lactoprotein with sorbitol", HAES-LX 5%.

The small intestine is the target organ in a burn disease, which is caused by violations of its functioning, with increasing damage on the background of toxemia and microbiocenosis [6]. The peculiarity of these violations is their long-term negative impact. It has been established [12] that dysbiosis is observed 21 days after thermal damage, especially against the background of antibiotic therapy, which is the standard method of treating severe burns.

Particularly important data are violations in the light of the hypothesis that the small intestine is the motor of the development of syndrome of polyorgan dysfunction with burn disease [4]. In particular, in this hypothesis, there is a significant role of the violations of enterocyte apoptosis [8] as a trigger mechanism for activation of multiple organ failure syndrome, along with cytokine stimulation, dysregulation of intercellular interaction, activation of microbial flora and other factors. It is dangerous to have abdominal hypertension syndrome [1], which can develop at the background of elevated intraperitoneal pressure, requiring accurate calculation of the volume of infusion, modification of the volume of infusion by monitoring diuresis, but completely eliminate this complication is impossible without the use of active infusion solutions, preferably with hyperosmolar effect [5].