HISTOMORPHOLOGIC CHANGES OF ESOPHAGEAL MUCOSA IN EXPERIMENTAL THIRD DEGREE STRICTURE

HISTOMORFOLOGICZNE ZMIANY BŁONY ŚLUZOWEJ PRZEŁYKU W EKSPERYMENTALNYM MODELU ZWĘŻENIA III STOPNIA

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ABSTRACT

Introduction: Nowadays the level of early and late complications after the operations for esophageal corrosive strictures such as esophago-organ anastomotic leak, development of infections, pneumonia, pleural empyema, mediastinitis, peritonitis, postoperative corrosive stricture development etc. remains rather high. Besides, postoperative mortality rate is high as well – 3.5-30 %. For that reason, an experimental model of esophageal stricture was suggested and ultrastructural mucosal changes in the stricture itself were studied to elaborate the unified pathogenic approach in treatment of esophageal stricture and improvement of its results.

The aim of our work was to study the dynamics of ultrastructural changes both in normal esophageal walls and in third degree esophageal stricture

Materials and Methods: The experiment was carried out on white male rats weighting 250-300 grams, to whom the third degree esophageal stricture model was created. After layer-by-layer incision of anterior abdominal wall abdominal portion of the esophagus was completely ligated (10 rats). In the control group (6 rats) anterior abdominal wall was opened with its subsequent layered closure. The animals were withdrawn from the experiment on the third day by ketamine overdose, and the samples were taken for ultrastructural study.

Results: Electron microscopic study of submicroscopic organization of basal, prickle, superficial epithelial cells in stratified non-squamous epithelium, smooth myocytes of muscle plate and contractile elements in esophageal muscular layer was carried out. Nuclear membrane, membranes of mitochondria, endoplasmic reticulum and cytoplasmic Golgi complex were found to be subjected to focal lysis. The third degree esophageal stricture caused destructive lesions in ultrastructural architectonics of stratified non-squamous epithelium cells, smooth myocytes of muscle plate and contractile elements in esophageal muscular layer of rats.

Conclusion: Thus, catabolic processes leading to organelle disintegration develop in esophageal cells of rats with third degree stricture.

KEY WORDS: ultrastructure of esophageal cells, esophageal stricture, catabolic processes.

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INTRODUCTION

Recent introduction of various modern methods in diagnostics and treatment of constrictive esophageal diseases [1, 2, 3] neither improved the outcomes nor decreased the complication level. There remains a rather high level of both early and late complications such as esophago-organ anastomotic leak, development of infections, pneumonia, pleural empyema, mediastinitis, peritonitis, postoperative corrosive stricture development etc. Besides, postoperative mortality rate is high as well – 3.5-30% [4, 5, 6]. For that reason, we suggested the experimental model of esophageal stricture and studied ultrastructural mucosal changes in the stricture itself to elaborate the unified pathogenic approach in treatment of esophageal strictures and improvement of therapy outcomes [7].

THE AIM

The aim of our work was to study the dynamics of ultrastructural changes both in normal esophageal walls and in third degree esophageal stricture.

MATERIALS AND METHODS

Experimental studies were carried out on white male rats weighting 250-300 grams. The experiments were done in accordance with general principals of animal experiments adopted by I National Bioethical Congress (Kiev, 2001) and conformed to European Convention for the protection of vertebrate animals used for experimental and other scientific purposes (Strasbourg, 1986).

In all animals of the studied group the model of third degree esophageal stricture was created. In our experiments the model was created in the following way: after layer-by-layer incision of anterior abdominal wall abdominal portion of the esophagus was completely ligated. In the control group (6 rats) anterior abdominal wall was opened with its subsequent layered closure. The animals were withdrawn from the experiment on the third day by ketamine overdose, and the samples were taken for ultrastructural study.

The excised tissues of esophageal mucosa taken from rats with modelled third degree stricture served the material for electron-microscopic analysis. Esophageal mucosal

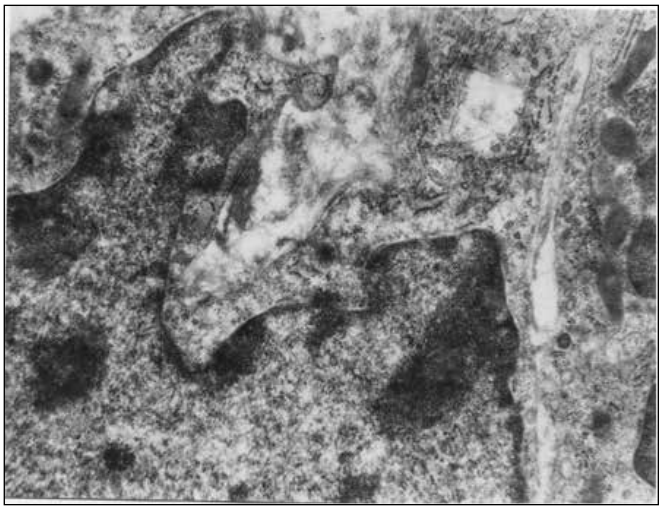

Fig.1. Ultrastructure of basal epithelial cells of stratified esophageal non-squamous epithelium in rats with modelled third degree stricture. Nuclear membrane invaginations (arrow) and loosening foci, x 38000.

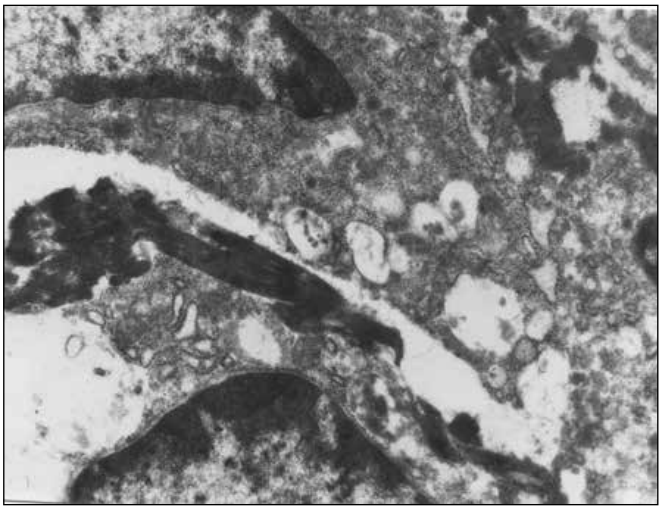

Fig.2. Ultrastructure of basal epithelial cells of stratified esophageal non-squamous epithelium in rats with modelled third degree stricture. Lysis of mitochondrial cristae (arrow), x 36000.

tissues taken from intact experimental animals were used to evaluate the quality of histologic processing. Immediately after sampling the material was immersed into 2.5% buffered gluter-formaldehyde solution at 4°C for pre-fixation. After washing in buffer solution the material was transferred into 1% buffered solution of osmium tetroxide for 3-4 hours for final fixation. Dehydration was carried out in ascended alcohol series and acetone. The tissue was embedded in epoxide resin mixture (epon-araldite) according to conventional methods. Polymerization of blocks was done in thermostat for two days at 60°C. Ultrathin sections were made on ultramicrotom device UMTD-3 from the blocks obtained. They were placed on electrolytic grids, and after lead citrate contrasting they were studied under electron microscope EMV-100 BR in increasing voltage 75 kW.

RESULTS AND DISCUSSION

Electron microscopic study of esophageal cells of intact rats confirmed the satisfactory histologic tissue fixation. Submicroscopic organization of organelles of esophageal mucosa cells corresponded to modern concepts. No destruction of intracellular membrane structures were found in the preparations.

Electron microscopic study of basal epithelial cells of stratified esophageal non-squamous epithelium with third degree stricture revealed predominance of destructive lesions in intracellular membranes and organelles of these cells. Nuclear membrane was loosened with multiple lysis foci. Nuclear matrix was strongly cleared, predominantly in the central part of the nucleus. Nuclear membrane had shallow and deep invaginations (Fig.1).

Nucleoli of the cells were virtually absent. The major portion of nuclear chromatin was in condensed state, its blocks were concentrated along the nuclear membrane. Perinuclear spaces were irregularly enlarged. In perinuclear region of cytoplasm there were mitochondria with cleared matrix and disorganized cristae. Focal lysis of external membranes and mitochondrial cristae was often observed. At the same time there were mitochondria with totally destructed cristae in the cells. Those mitochondria were presented as vacuoles filled with the conglomerate of structureless substance with various degree of electron density (Fig.2).

Cisternae of granular endoplasmic reticulum were enlarged, appeared as vacuoles filled with predominantly electronic-transparent substance. No ribosomes were virtually seen on the membranes of granular endoplasmic reticulum. There was a very few number of free ribosomes and polyribosomes in cytoplasm. Basal epithelial cells with fragmented membranes of granular endoplasmic reticulum occurred in the preparations.

Cytoplasmic Golgi complex was found to be subjected to reduction with randomly oriented smooth membranes surrounded by a small number of large electron-transparent vesicles. In cytoplasm secondary lysosomes and small-size lipid inclusions were detected in the region of cytoplasmic Golgi complex (Fig.3).

Cytoplasmic membrane was thickened and subjected to deformation, had loosened structure and high degree of electron density. Occasionally multiple foci of its lysis and loosening were seen.

Prickle epithelial cells of esophageal mucosa contained clusters of glycogen osmiophilic granules. In peripheral portions of cytoplasmic epithelial cells of prickle layer randomly oriented and occasionally disorganized tonofibrils were located. Nuclear chromatin was predominantly condensed, its blocks were collected into a dense osmiophilic ring located along nuclear membrane margins. Irregular vacuole-like enlargement of perinuclear spaces was seen. Loosening of nuclear membrane was followed by the appearance of multiple lysis foci. In cytoplasm of epithelial cells a small number of mitochondria with electronic transparent matrix were located. Mitochondria had isolated short cristae. There were destruction foci in the great majority of external mitochondrial

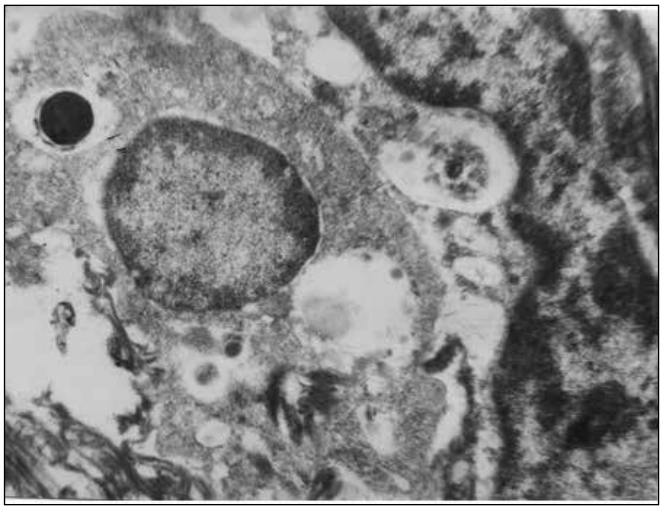

Fig.3. Ultrastructure of basal epithelial cells of stratified esophageal non-squamous epithelium in rats with modelled third degree stricture. Secondary lysosomes in cytoplasm (arrow), x 40000.

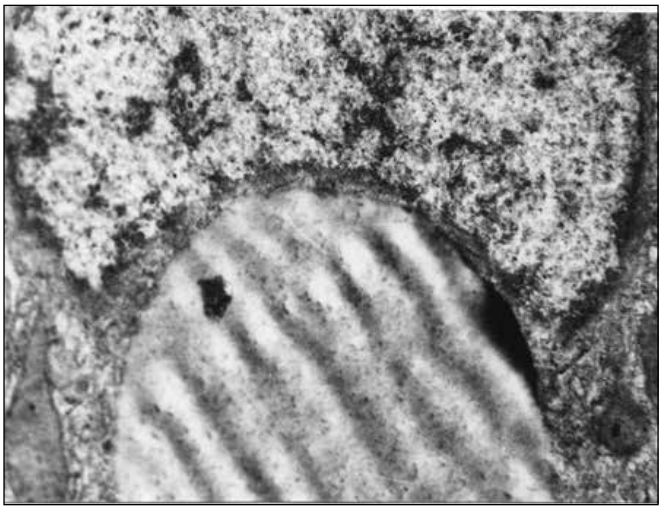

Fig.4. Ultrastructure of prickle epithelial cells of stratified esophageal non-squamous epithelium in rats with modelled third degree stricture. Large inclusions of lipids in cytoplasm (arrow), x 41000.

membranes and cristae. Totally destructed mitochondria were seen as well. Enlargement of cisterns of granular endoplasmic reticulum was accompanied by reduction in the number of membrane-bound ribosomes. Prickle epithelial cells with fragmented membranes of granular endoplasmic reticulum were also observed. Free polyribosomes and ribosomes were virtually absent in cytoplasm. Cytoplasmic Golgi apparatus was reduced and presented by isolated smooth membranes with large electron transparent vacuoles being located next to them. In the region of cytoplasmic Golgi apparatus secondary lysosomes and large lipid inclusions were detected (Fig.4).

Intercellular spaces were considerably enlarged, contained a small number of cytoplasmic processes and were filled with electron-transparent substance. Cytoplasmic membrane was considerably loosened and had a great number of destruction foci. Isolated microvilli were totally destructed.

Superficial epithelial cells of multilayer esophageal epithelium were flattened. Nuclear membrane of superficial epithelial cells was totally destructed. A small number of destructive and degenerative organelles were detected in their cytoplasm. Organelles and membrane complexes had destruction foci in most of the cells. A small number of desmosomes was seen on cytoplasmic membrane. Cytoplasmic membrane was thickened, its well-defined contoured structure was damaged.

Ultrastructural organization of smooth myocytes of muscle plate of esophageal mucosa in rats with modelled third degree stricture underwent destructive and dystrophic changes. Nuclei of smooth myocytes were elongated, nuclear chromatin was decondensed with regular distribution of its granules on the surface of nucleus section. Occasionally blocks of condensed chromatin, randomly scattered in the matrix, were observed. Perinuclear spaces were uniformly enlarged. Lysis of nuclear membrane areas was often found. Generally nuclear membrane was considerably loosened. Organelle clusters in cytoplasm of smooth myocytes were located in perinuclear region. There were few mitochondria in various degrees of swelling containing electron-transparent matrix. Mitochondria had isolated disorganized cristae. Occasionally destructive foci of outer membranes and cristae were seen in mitochondria. Cisterns of endoplasmic reticulum were enlarged and filled with microfiber substance. In some smooth membranous myocytes of endoplasmic reticulum the membranes were subjected to fragmentation. Cytoplasmic membrane was considerably loosened and thickened, next to it a small number of caveolas were found in cytoplasm. In cytoplasm of smooth myocytes there were randomly oriented clusters of myofilaments.

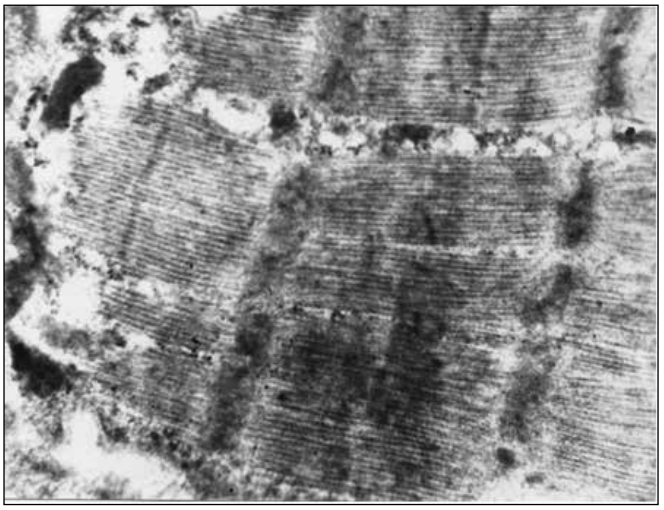

Fig.5. Ultrastructure of esophageal striated muscle tissues in rats with modelled third degree stricture. Clearing of sarcoplasm and lysis of mitochondrial membrane (arrow), x 39000.

Striated muscle tissue of esophageal muscular coat in the region of third degree stricture had submicroscopic organization intrinsic to the prevalence of destructive over dystrophic processes. Nuclei of striated muscle fibers preserved typical dysrophic elongated form and contained, for the most part, uniformly distributed granules of decondensed chromatin. Nuclear matrix was cleared. Nuclear membrane was loos-

ened containing considerable number of destruction foci. Perinuclear spaces were moderately and uniformly enlarged. Mitochondria were localized between myofibrils in the form of oval irregular inclusions bounded by elementary membrane. Mitochondrial matrix had rough fibrous structure and very high electron density. There were foci of lysis on external membranes and mitochondrial cristae. Totally destructed mitochondria were frequently observed (Fig.5).

Parallel arrangement of myofibril bands and cross striation was preserved in sarcoplasm. There were areas of myosymplast with branched and thinned contractile elements. Sarcoplasm was cleared, clusters of glycogen granules were found between myofibrils. Tubules of sarcoplasmic rough were enlarged and presented as vesicles filled with electron-transparent substance. Sarcoplasmic membrane lost its definite contoured structure and assumed high degree of osmiophilia. Frequently lysis areas were determined in it.

Nuclei of blood capillary endotheliocytes of esophageal mucosa in the region of third degree stricture were of irregular form. Nuclear membrane formed multiple shallow and deep invaginations. Nuclear matrix had increased electron density. Condensed nuclear chromatin was concentrated along nuclear membrane. In the central part of nuclear matrix electron transparency zone was formed with isolated granules of condensed chromatin and blocks of decondensed chromatin, randomly scattered in the matrix. Ribosomes were also localized in that area. Hyaloplasm of blood capillary endotheliocytes was swollen, had low electronic density and contained a large number of organelles, ribosomes and polyribosomes. In cytoplasm of several endotheliocytes there were secondary lysosomes and lipid inclusions. Mitochondria of endotheliocytes were small, had oval form and electron-transparent matrix. They were considerably swollen, their cristae were subjected to lysis and fragmentation. Mitochondrial matrix was cleared, occasionally myelin-like structures were found. Granular endoplasmic reticulum was subjected to vacuolization. There were endotheliocytes containing fragmented membranes of granular endoplasmic reticulum. Cytoplasmic Golgi complex was reduced, its smooth membranes being disorganized and mostly destructed. In cytoplasm of endotheliocyte processes a small number of micropinocytotic vesicles were detected. Cytoplasmic membrane of endotheliocytes contacting with blood had multiple lysis areas. In capillary lumen, dendrite consisting of degenerative membrane fragments, organelles and amorphous substance, presumably of lipoprotein origin, was found in addition to cellular blood elements.

Electron microscopic study of submicroscopic organization of basal, prickle, superficial epitheliocytes of stratified esophageal non-squamous epithelium, smooth myocytes of muscle plate and contractile elements of esophageal muscular layer in rats with modelled third degree stricture revealed the prevalence of destructive lesions. Nuclear membrane, membranes of mitochondria, endoplasmic reticulum and cytoplasmic Golgi complex were subjected to focal lysis. Bioenergy of all esophageal cells suffered because of those lesions. Reparative, metabolic and synthetic intracellular processes were deactivated, structurally confirmed by the decrease of ribosome and polyribosome number in cytoplasm as well as loosening, focal or total lysis of granular endoplasmic reticulum membranes. Ultrastructural changes of smooth myocyte organelles and striated muscle fibers in the region of esophageal stricture indicated the decrease of contractile ability of these cells. Submicroscopic lesions of epitheliocytes of esophageal capillary bed suggested the development of intracellular edema. Absence of micropinocytotic vesicles in cytoplasm of endotheliocyte processes implied nearly complete interruption of transcellular transport of nutrients, water and electrolytes. Cellular ultrastructure of the esophagus with third degree stricture suggested the increase of catabolic intracellular processes, structurally confirmed by the presence of secondary lysosomes and lipid inclusions in cytoplasm.

CONCLUSIONS

1. Modelled third degree stricture causes destructive lesions in ultrastructural architectonics of stratified esophageal non-squamous epithelium, smooth myocytes of muscle plate and contractile elements of esophageal muscular layer in rats.
2. Cellular ultrastructure of the esophagus with third degree stricture suggests the increase of catabolic intracellular processes.

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