MORPHOLOGY AND PATHOMORPHOLOGY

Effect of Esophagoplasty on Structural Reorganization of Colon Transplant

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 153, No. 2, pp. 238-243, February, 2012
Original article submitted December 16, 2010

We studied ultrastructural reorganization of the colon transplant in delayed period after esophagoplasty. It was found that during functioning of the artificial esophagus, a complex of adaptive and pathologic processes occurs in the mucosa. Focal sclerosis of the mucosa and slight epithelial degeneration with hyperplasia and hypersecretion of goblet cells were found in biopsy specimens with stenosis of the anastomosis. In case of colonopathy of the transplant, more pronounced epithelial degeneration and proliferation accompanied by abundant polymorphocellular stromal infiltration were seen. Deformation of the transplant was characterized by progressive atrophic and sclerotic reorganization of the mucosa. Goblet cells with ultrastructural signs of hypersecretion predominated in the population of epithelial cells of the colon transplant; oligomucus and poorly differentiated cell and colonocytes with signs of alteration and degenerative changes in cytoplasmic organelles were also found.

Key Words: *artificial esophagus; colon transplant; morphology; ultrastructure*

Reconstructive technologies are now actively used for correction of benign pathologies of the esophagus. This requires comprehensive study of the long-term effects of esophagoplasty [2,5,7]. Single-stage esophagoplasty meets modern principles of reconstructive surgery of the esophagus. Stomach and colon are the major donor organs for creation of the artificial esophagus. Esophagocolonoplasty is often an operation of choice for corrosive strictures of the esophagus due to the fact that the stomach is considerably damaged and cannot be used as the plastic material [1,6,8,13].

Successful application of colon transplants for replacement of damaged esophagus is provided by pronounced trunk type of the donor organ blood flow, resistance of tissue components to hypoxia and aggressive factors of gastric secretion, and little influence of excluded colon prolonged segments on digestion. Many authors prefer transplant formation from the left portion of the colon arguing the choice by the features of its structure (sufficient length and small diameter) and maintaining the stable blood supply after mobilization of extended segments which enables various reconstructive operations, such as shunting, subtotal, or total esophagoplasty [6].

In the late postoperative period, there is a risk of development of pathological syndromes, which together constitute a heterogeneous group of the diseases of artificial esophagus [5]. The development of ideas about the morphological substrate of the pathology stimulated research in this direction, but systematic approach to the study of structural mechanisms underlying reorganization of the transplants in different

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types of esophagoplasty is still absent. The accumulated information in this area indicates that a wide range of pathological phenomena, from minimal inflammatory reaction to ulceration, fibrosis, and neoplasia, can occur in colon transplant [3,4,9,10,12]. The integrated structural analysis allows to clarify the nature of structural changes and establish pathomorphogenetic landmarks of the diseases of artificial esophagus.

The aim of the work was to study ultrastructural reorganization of the colon transplant during the late period after esophagoplasty carried out for benign diseases of the esophagus.

MATERIALS AND METHODS

We performed a comprehensive morphological study of artificial esophagus formed from the left half of the colon in 36 men and 21 women (age 18-73 years) undergoing esophagoplasty for corrosion strictures of the esophagus. During the late postoperative period, clinical and endoscopic monitoring of pathological conditions of artificial esophagus was carried out. The time of observation ranged from 1 month to 11 years; in 1 case, plastic repair was carried out about 26 years; in 3 cases, more than 40 years before this examination. During endoscopic revision of artificial esophagus, mucosa biopsy specimens were collected from altered sites of the transplant, esophagocolono- and colonogastroanastomoses.

For light microscopy, tissue specimens were fixed in 10% neutral formalin and subjected to routine histological processing. Paraffin sections were stained with hematoxylin and eosin in combination with Pearl's reaction, after van Gieson with poststaining of elastic fibers with Weigert resorcin-fuchsin, and by PAS method. Paraffin sections were examined under universal Leica DM 4000B microscope. Micrographs were obtained using Leica DFC 320 digital camera Leica QWin software.

Specimens for electron microscopy were fixed in 4% paraformaldehyde, post-fixed in 1% OsO_4, processed by standard methods, and embedded in epon—araldite mixture. Semithin sections were stained by the drop method with Schiff's reagent and 1% azur II. Ultrathin sections were contrasted with uranyl acetate and lead citrate and examined under a JEM1400 electron microscope (Jeol). Images were obtained with digital Veleta camera and iTEM software (Olympus).

RESULTS

Clinical and endoscopic study of artificial esophagus showed that after plastic surgery, stenosis of esophagocolonoanastomosis developed most often (26 cases, 45.6%); stenosis of distal anastomoses developed less frequently (5 cases, 8.8%); 16 patients showed signs of transplant colonopathy (28.0%), in 4 cases complicated with ulcer of colonogastroanastomosis. Deformation of the colon transplant was a severe complication of esophagoplasty, which more frequently occurred at the longest terms of functioning of the artificial esophagus (10 cases, 17.5%). In some patients, combined pathology of the artificial esophagus developed.

Light microscopic examination of biopsy specimens revealed that the mucosa of colon transplant maintained the typical microarchitectonics in the majority of cases. Adaptive epithelial remodeling was typical and manifested as the tendency to enlargement and more compact organization of crypts of Lieberkühn, and signs of hyperplasia and hypersecretion of goblet cells. In the epithelium, large secreting cells predominated. In some places they were placed randomly generating pseudomultilayer structure; the number of columnar colonocytes decreased.

In stenosis of esophagocolonoanastomosis, focal mucosal sclerosis was detected in biopsies. It was sometimes associated with the growth of young fibrous connective tissue, proliferation of blood capillaries and indirectly pointed to the ischemic component of the fibrosis development (Fig. 1, *a*). At the same time, in transplant mucosa, signs of mild degeneration of the colon epithelium formed by highly differentiated goblet cells with abundant PAS-positive cytoplasm, were primarily recorded (Fig. 1, *b*).

In transplant colonopathy, more significant diffuse degenerative changes of the epithelium were observed. Increased desquamation of cells was locally recorded; foci of interstitial edema and capillary plethora and abundant polymorphocellular stromal infiltration were seen (Fig. 1, *c*). Height of colonocytes in the epithelial layer varied. The cytoplasm was vacuolated; nuclei were displaced to the apical pole and located at different levels. Elongation of epithelial cambial zone of and increased number of mitoses were noticeable. In the cell population, the number of young colonocytes increased. In the upper segments of the crypts, incompletely differentiated secreting cells were identified indicating the imbalance between proliferation and cell differentiation. In two cases, signs of weak colon epithelial dysplasia were revealed.

In case of deformation of artificial esophagus, mucosa of colon transplant was thinner, dystrophic and atrophic epithelial changes were recorded, crypts of Lieberkühn were shorter and their number reduced (Fig. 1, *d*). An important feature of the biopsies was stromal sclerotic reorganization; that was manifested by focal interstitial condensation, overgrowth of thin bundles of collagen fibers, and fibrosis of muscular plate and submucosal layer. In these cases, collagen growth in mucosa may be due to the fact that the transplant was suvjected to long-lasting functional loads in

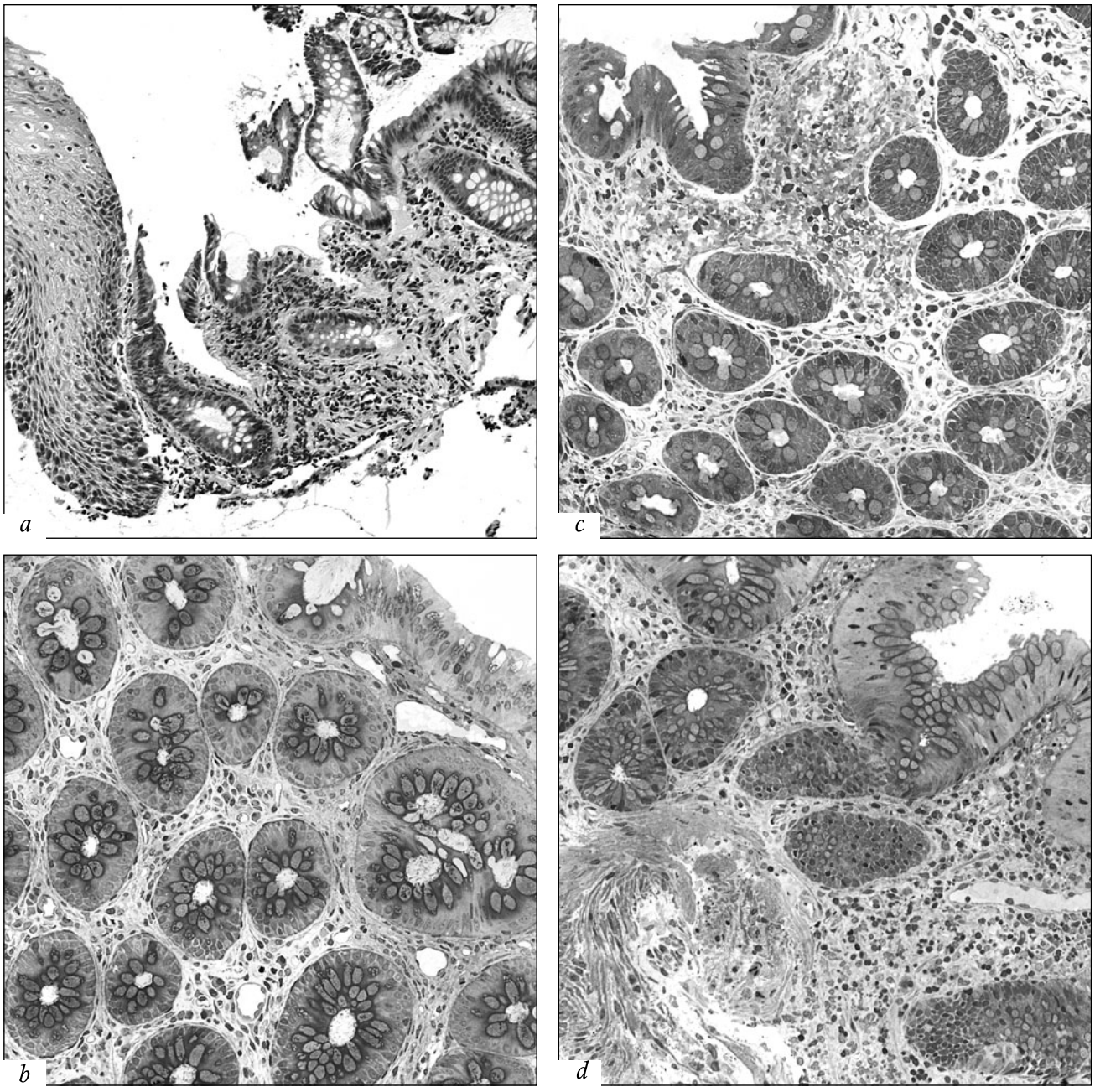

Fig. 1. Pathomorphological changes in the mucosa of colon transplant during the late period after esophagoplasty. Staining with hematoxylin and eosin (*a*); semithin sections, azure II staining (*b,c,d*). *a)* degeneration of stratified squamous and colon epithelium, focal stromal sclerosis in the zone of esophagocolonoanastomosis, ×140; *b)* large crypts of Lieberkühn, hyperplasia and hypersecretion of epithelial goblet cells, ×280; *c)* degeneration of crypt epithelial cells, hyperemia and lymphoplasmacytic infiltration of the stroma, ×280); *d)* atrophy of crypts of Lieberkühn, fibrosis of the mucosal muscle plate, ×280.

the anti-physiological conditions. On the other hand, there is a risk of progression of involution process in the wall of the artificial esophagus associated with aggravation of tissue ischemia, which is particularly manifested in elderly patients.

Electron microscopy of biopsies of colon transplant revealed ultrastructural heterogeneity of mucosal epithelium. Goblet cells with signs of high secretory function formed the basis of the cell population. Their cytoplasm was overfilled with abundant mucus globules merging into conglomerates; flattened nucleus were pushed aside to the cell basal pole (Fig. 2, *a*). In the cytoplasmic matrix free of secretory globules, fragments of proliferated lamellar complex, isolated tubules of granular cytoplasmic reticulum, single mitochondria with short cristae could be seen.

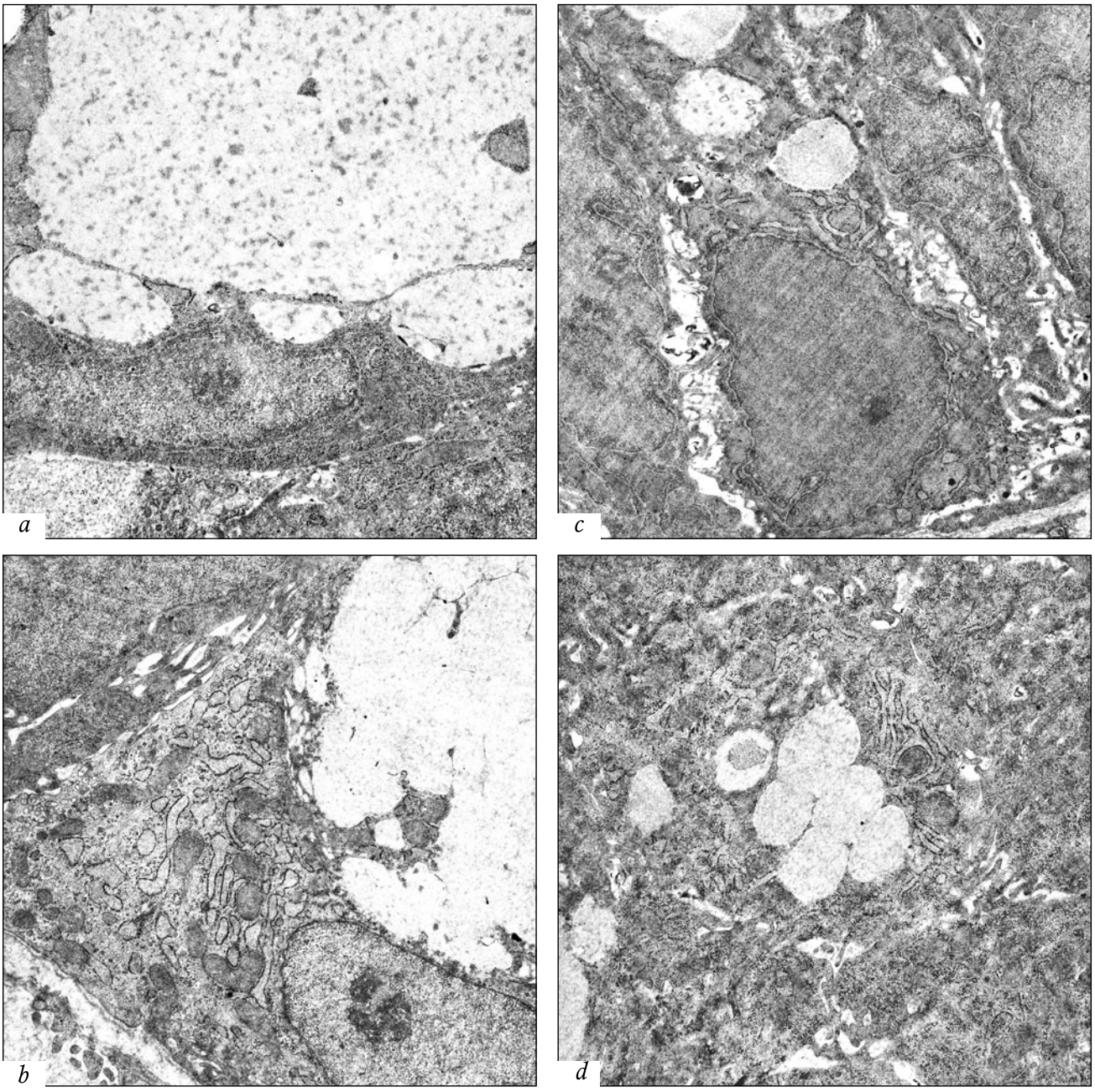

Fig. 2. Ultrastructural changes in the mucosal epithelium of colon transplant during the late period after esophagoplasty. *a)* fragment of goblet cell, abundance of secretory granules in the cytoplasm, ×20,000; *b)* fragment of epithelium: goblet cells is filled with granules of secretion, in the cytoplasm of undifferentiated colonocyte mitochondria are surrounded by cisterns of granular endoplasmic reticulum, ×15,000; *c)* colonocytes with signs of degeneration, increased intercellular spaces, ×8000; *d)* fragment of epithelium, colonocytes with low content of secretory granules in the cytoplasm, ×20,000.

In transplant colonopathy, the number of oligomucus cells increased in functional zones of the crypts of Lieberkühn. Their intracellular organization was characterized by single electron-transparent mucus globules, large elements of the lamellar complex, well-developed endoplasmic reticulum cisterns with numerous ribosomes. Sometimes the undifferentiated colonocytes were visualized close to mature goblet cells. The latter had large nuclei with looped nucleoli. Cytoplasmic matrix contained a large number of mitochondria surrounded by numerous cisternae of granular cytoplasmic reticulum. Numerous free ribosomes and polysomes, small clusters of small vacuoles were seen (Fig. 2, *b*).

In epithelium, colonocytes with signs of alteration were identified; they were characterized by polymorphism of the mucus globules with optically dense inclusions, increased invaginations of nuclear membrane

and expansion of the perinuclear space, swelling of mitochondria, vacuolization of endoplasmic reticulum. Intercellular contacts mediated by interdigitations were locally destructed so that the lateral plasma membrane processes bulged freely into the expanded intercellular spaces; osmiophilic lamellae were occasionally visualized there (Fig. 2, *c*). In areas of mucosal atrophy of the transplant, colonocytes became cubic; heterochromatin-rich nuclei were localized pericentrally, cytoplasmic matrix was denser with poorly visible biosynthetic organelles; the number of mucus globules decreased significantly (Fig. 2, *d*).

Thus, during the late period after esophagoplasty, colon transplant undergoes certain restructuring. These changes arise and develop primarily due to the reconstructive intervention directly associated with the formation and movement of the transplant, and the new conditions of functioning, which leads to the mobilization of protective mechanisms of the mucosa [4,11]. Pathological processes in the artificial esophagus can be caused by ischemia, prolonged contact of mucosa with aggressive components of the gastro-intestinal refluxate, decrease in the effective clearance and deformation of colon transplant, *etc*. [3,7,10,14,15]. The effect of these circumstances, separately and in the complex, causes the development of epithelial degenerative changes, destabilization of cell renewal, formation of vascular and cell reactions of the mucosa, and subsequently leads to focal destructive changes and accelerated sclerotic reorganization of the transplant.

Based on the results of the study we can conclude that pathological state of the artificial esophagus formed from the colon is accompanied by various structural modifications of the mucosa. Focal sclerosis, signs of hypersecretion and low degeneration of the epithelium are characteristic of the stenosis of esophagocolonoanastomosis. Degenerative changes in epithelial cells in combination with abundant polymorphocellular stromal infiltration are more expressed during transplant colonopathy. Dystrophic and atrophic changes in the epithelial compartment and sclerotic reorganization of the mucosa are progressing during the deformation of artificial esophagus. Ultrastructural modifications of colon epithelium depending on the leading pathological syndrome reflect intensified secretion of goblet cells, alteration and degeneration of cytoplasmic organelles, and appearance of immature colonocytes in the cell population. Overall pathological phenomena recorded in the mucosal biopsies of the colon transplant are interpreted in the context of ideas about structural markers of the disease of artificial esophagus.

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