



O.M. Babay

Efficacy of Clinical Application of Phosphatidylcholine Liposomes: Comparative Ultrastructural Analysis of Gingival Epithelium at Different Stages of Treatment of Patients with Generalized Periodontitis

Department of Therapeutic Dentistry
Kharkiv National Medical University, Kharkiv, Ukraine

Keywords:

generalized
periodontitis;
ultrastructural
morphology;
treatment;
efficacy

Abstract.

On the basis of comparative ultrastructural analysis of the gingival mucosa (GM) before and during the stages of clinical application of phosphatidylcholine liposome there was revealed that the basal layer of the epithelium was relatively expanded and represented by cells that preserved the structure typical for normal mucous membrane. It indicated an increase in reparative potential of this layer filled with cambial cells to restore epithelial integrity. In the stratum spinosum cells were more distinctly joined to each other with desmosomes that provided the integrity of the connections between cells and maintenance of barrier properties of the epithelium; in the stratum granulosum complete recovery of cells was not observed, however, the structure of most keratinocytes resembled the normal one. The efficacy of using advanced therapeutic dental complex (TDC) (deposition of egg phosphatidylcholine liposomes (EPCL) by means of infiltrative injections and periodontal bandage) was also indicated by an increase in functional activity of gingival epithelial cells: the presence of large functional nucleoli in the nuclei of euchromatin which predominates over heterochromatin reflecting the activation of the RNA and protein biosynthesis on the background of the expansion of the nuclear pores as morphological equivalent of the intensification of the nuclear-cytoplasmic transport process.



Copyright © Author(s), 2016

Problem statement and analysis of the recent research

Despite the large number of methods that allow us to determine clinical efficacy of dental treatment the evaluation of the morphological component of this efficacy is still relevant today. Therefore, the analysis of status of the periodontal tissues is of particular interest to researchers and plays an important role in choosing therapeutic tactics. The structural and functional status of the periodontal tissues, the gingival mucosa (GM) in particular, being interconnected with hemocirculatory disorders which can result in tissue hypoxia and energy deficiency serves as a universal nonspecific criterion for this pathology and determines the direction of treatment. The use of liposomal forms of essential phospholipids was proven to be advisable in pathogenetic correction of these changes [4, 7]. The well-known egg phosphatidylcholine liposomes (EPCL) having high bioenergetic potential and antihypoxic action are representatives of this group. Their use improves not only tissue oxygenation due to an increase in the rate of oxygen diffusion from the lungs into the blood and from the blood into the tissues but microcirculation and rheological properties of blood [5, 10]. There is convincing evidence that the use of EPCL in targeted correction of these disorders is effective. We have introduced the inclusion of preparations of this group into therapeutic dental complexes (TDC) in patients with generalized periodontitis (GP) [2, 9, 11].

The objective of the research was to carry out a comparative analysis of ultrastructural changes in the gingival mucosa of patients with generalized periodontitis during the stages of complex treatment using egg phosphatidylcholine liposomes.

Materials and methods

The gingival mucosal tissues of 40 persons (10 healthy individuals and 30 patients with stages I-II GP in the exacerbation phase) were studied morphologically. The gums were cut away during tooth extraction according to medical indications. It allowed us to collect primary morphological material for conducting microscopic examination. Only patients who gave informed consent were recruited into the study. Bioptic specimens were taken during the various stages of clinical monitoring: before treatment (CM-I: 10 persons), 30 days after treatment (CM-III: 10 persons), 6 months after treatment (CM-IV: 10 persons). Treatment was performed using special therapeutic dental complex (TDC) that consisted in the deposition of EPCL by means of infiltrative injections and periodontal bandage [9] in addition to therapeutic interventions according to clinical protocol [1, 11].

For electron microscopic study of the GM the tissue fragments (1.00 mm³ in size) were prefixed in 5.0% paraformaldehyde - glutaraldehyde Karnovsky fixative followed by postfixation in 1.0% osmium tetroxide. The dehydration process was accomplished by passing the tissue fragments through a series of increasing alcohol concentrations and acetone; then, the material was embedded in an Araldite-Epon mixture (mixture of the ethoxyline resins) [6]. Semi-thin and ultrathin sections (1 mc and 50-70 nm, respectively) were prepared using the ultramicrotome UMTP-3M. Semi-thin sections were stained with a 1.0% solution of methylene blue and a 1.0% solution of borax (1:1); then, they were stained with methylene basic fuchsin – according to Aparicio [3]. Contrast enhancement of ultrathin sections was performed in saturated solution of uranyl acetate followed by Reynolds' lead citrate [11]; then, they were examined by means of transmission electron microscope. The prints from the negatives of electronogram were made using digital camera Canon EOS-300 D.

Results and discussion

The analysis of semi-thin sections of the GM in patients with GP revealed significant epithelial thinning; papillae of the lamina propria of the GM were represented by loose connective tissue and contained a large number of blood capillaries located close to the surface of the epithelium (it could lead to increased gum bleeding). In the areas of epithelial thinning there was

an intercellular edema; the destruction and death of cells of the superficial, granular, spinous and basal layers were observed.

The results of electron microscopic study indicated that in the granular, spinous and basal layers of the gingival epithelium closely adjacent cells were observed between which there were no desmosomal attachments as the destruction of desmosomes led to the detachment of cells and formation of endoepidermal cavities filled with transudate due to its effusion from the vessels of the lamina propria of the GM. There were observed both primary loss of cohesion between keratinocytes (acantholysis) and secondary one - intercellular edema or spongiosis that is formed due to the penetration of serous fluid in the intercellular spaces of the stratum spinosum.

The number of cytoplasmic spinous processes in epithelial cells reduced, and spinous processes were short; there was observed a focal lysis of the cytoplasmic membrane of epithelial cells with the presence of epithelial cells having significantly blade-like, occasionally fragmented cell nuclei. The basement membrane was found between the epithelium and the lamina propria of the GM being intermittent. There were multiple complex basement membrane contours.

In isolated epithelial cells of the basal layer containing the area of the destruction of cell membrane a focal destruction and degranulation of granular endoplasmic reticulum were observed. In some patients in keratinocytes of the granular, spinous and basal layers there was found a large amount of cells with pronounced destructive changes. Such cells contained intensely blade-like nucleus with various elongated bands; the areas of lysis of the nuclear envelope and fragmentation of the nucleus were observed. In the cytoplasm of keratinocytes the fragmentation and focal lysis of keratin tonofibrils, swelling and destruction of tubules, and in some cases, foci of destruction and vacuolization of the cytoplasm were detected. In some keratinocytes vacuoles occupied almost the entire cytoplasm and the areas of destruction were additionally seen in the cytoplasmic membrane. In addition, the strength of cell-cell junctions between keratinocytes reduced, desmosomes were not clearly visible, and in some desmosomes there were no junctions due to asymmetry of desmosomal structures.

The analysis of semi-thin sections of the GM in patients with GP after treatment revealed that there was a significant increase in cell density in the basal and spinous layers of the epithelium. The basal layer expanded compared to the GM: it is known to play an important role in the regeneration process as it contains cambial elements of the epithelium. This layer of the epithelium was represented by cubic or prismatic cells having the characteristics typical for normal basal cells. In the cytoplasm of basal cells there were low-density keratin filaments, as a result of which the cytoplasm was brightly coloured.

Electron microscopic study showed that basal cells contained oval nuclei with 2-3 nucleoli containing a small amount of heterochromatin localized around the nuclear envelope and distributed throughout the entire nucleus in the form of small blocks; euchromatin occupied almost the entire nucleus. The cytoplasm of these cells showed low electron density due to the presence of single keratohyalin cords in the cytoplasm. Cells were located at insignificant distances between each other and joined to each other with desmosomes of lamellar structure. Desmosomes were localized in various cytoplasmic processes – so-called spinous process providing both trophic and close connections among these cells. Only a few cells of this layer contained electron-dense nuclei with no differentiation between euchromatin and heterochromatin.

Electron microscopic analysis of the stratum spinosum of the GM revealed that in contrast to the basal layer it contained large epithelial cells being somewhat irregular in shape that is typical under normal conditions. A significant number of epithelial cells containing large euchromatic nucleus and nucleoli of various electron density was observed among the keratinocyte population. The nucleus of most cells contained one large or 2-3 moderate nucleoli. The presence of the nucleoli in cells indicated the activation of the RNA biosynthesis which plays an important role in protein biosynthesis. As in the basal layer, cells of the stratum spinosum were joined to each other with

desmosomes of lamellar structure indicating partial restoration of the structural integrity of this layer. Numerous osmiophilic tonofibrils were found in the cytoplasm of epithelial cells and desmosomal attachments were observed between cells. Keratin tonofibrils of epithelial cells penetrated cytoplasmic processes of cells and, therefore, came in contact with desmosomes.

The gingival epithelium in the transitional zone between the granular and spinous layers was predominantly narrow, contained small cells in the cytoplasm of which keratin filaments were oriented parallel to the granular layer of the epithelium. The nuclei of keratinocytes contained small nucleoli. There were cells with two and more nucleoli. Prevailing euchromatin was observed in the nuclei indicating their functional activity. The increase in the activity of the nuclear-cytoplasmic transport was also indicated by the presence of expanded nuclear pore complexes. In both spinous layer and the transitional zone between the spinous and granular layers there were desmosomes which provided the junctions between cells as well as biochemical and biophysical relationships between them. In the granular layer there were detected single keratinocytes with signs of destructive changes as indicated by zigzag-shaped nuclear membrane as well as by the cytoplasm containing multiple vacuoles and foci of homogenization.

Conclusions

1. The analysis of semi-thin sections of the gingival mucosa in patients with GP before and after treatment using improved methods [9] revealed that the basal layer of the epithelium was relatively expanded and represented by cells that preserved the structure typical for normal mucous membrane. It indicated an increase in reparative potential of this layer filled with cambial cells to restore epithelial integrity.

2. After treatment using improved methods [9] in the stratum spinosum cells were more distinctly joined to each other with desmosomes that provided the integrity of the connections between cells and maintenance of barrier properties of the epithelium. In the stratum granulosum complete recovery of cells was not observed, however, the structure of most keratinocytes resembled the normal one.

3. The efficacy of using advanced TDC (deposition of EPCL by means of infiltrative injections and periodontal bandage [9]) was also indicated by an increase in functional activity of gingival epithelial cells: the presence of large functional nucleoli in the nuclei of euchromatin which predominates over heterochromatin reflecting the activation of the RNA and protein biosynthesis on the background of the expansion of the nuclear pores as morphological equivalent of the intensification of the nuclear-cytoplasmic transport process.

References

1. Antonov SV. Dentistry: regulatory legal acts and documents. Tsentr medychnoho prava. Kyiv. 2009;696.
2. Babay OM, Krasnopol'skyi YuM. Efficacy of using "Lipin" in complex treatment of generalized periodontitis. Stomatoloh. 2003;5:32-38.
3. Grigoryan AS, Grudyanov AI. Key elements of the pathogenesis of periodontal diseases with consideration to cytomorphometric method of the research. Stomatologiya. 2001;1:5-9.
4. Kaplun AP, Shon Le Bang, Krasnopol'skyi YuM. Liposomes and other nanoparticles as a means of drug delivery. VMKh. 1999;3:3-13.
5. Kylymnychenko OI. Usage of lipin in complex treatment of hypoxia of different etiology [dissertation]. Donetsk National Medical University. Dnipropetrovsk. 1998;17.
6. Kunin AA, Ippolitov YuA, Lepekhina LI, Bykov EG. Clinical histochemistry of the barrier function of gingival mucosa in periodontitis. Stomatologiya. 2001;1:13-16.
7. Kushnirenko S. Properties of liposomes and their use in clinical medicine. Liky Ukrainy. 2003;1:18-20.

8. Leontyev VK. Healthy teeth and quality of life. *Stomatologiya dlya vsekh*. 1999;2(3):30-34.
9. Babay OM, Krasnopolskyi YuM, Babay AM, inventors; Babay OM, Krasnopolskyi YuM, Babay AM, assignee. Method of treatment of inflammatory and inflammatory-destructive processes in paradontium. Ukraine's patent UA 55454. 2003 Apr. 15.
10. Perova HI. Experimental and clinical substantiation of using the lecithin-antioxidant complex in treatment of generalized periodontitis [dissertation]. Odesa National Medical University. Odesa. 2002;21.
11. Babay OM, Merkulova UV, Deieva TV. Comparative clinical and morphological analysis of the periodontitis of different genesis. *Eksperymentalna ta klinichna medytsyna*. 2015;2(67):60-63.