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STRUCTURAL CHANGES OF THE GINGIVAL EPITHELIUM IN RATS WITH SPONTANEOUS ARTERIAL HYPERTENSION UNDER ITS PHARMACOTHERAPY

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Summary. *The article provides data on the influence of Bisoprolol, Thiotriazolin and Quercetin on structural changes of gingival epithelium of linear rats with spontaneous arterial hypertension (SHR). For histopathological changes in the gingiva of rats with hypertension is characterized epithelial apoptosis. In this situation, the therapeutic usage of Thiotriazolin discovered the properties as inhibitor of apoptosis.*

Key words*: arterial hypertension, Bisoprolol, Thiotriazolin, Quercetin, gingival epithelium*

Introduction. Despite of the extensive experience of clinical investigations, significant advances in the study of pathogenesis mechanisms and, therefore, a large number of hypertension medicamental correction the problem of arterial hypertension have a verycurrent interest [3] In connection to these very actual is the investigations of research perspectives usage of metabolic drugs in combination therapy of hypertension [1, 2]. It is assumed that the very convenient and adequate experimental model for the investigation of antihypertensive drugs influence is spontaneous hypertension (SAH) in rats SHR line, which best suits arterial hypertension in humans [7]. In previous investigations we have found structural changes in connective tissue [8] and nerves [9] of gingiva of rats with SAHafter administration of beta-blocker Bisoprolol with metabolic drugs (Quercetin and Thiotriazolin). The purpose of this investigation was to study the changes in gingival epithelium of rats with SAH under correction this conditions by Bisoprolol, Tiotriazolin and Quercetin.

Materials and methods. Studies were carried out on 60 young mature (age 100 days) male rats of the SHR (Spontaneously Hypertensive Rats) strain and also on a group offive normotensive rats of the WKR (normotensive Wistar-Kyoto Rats) strain of the same age. Animals with SAH were divided into six groups (10 rats in each). The first group was the control; rats of the second, third, and fourth groups were injectedwith Bisoprolol, Thiotriazolin, and Quercetin, respectively. Animals of the fifth group were injected with Bisoprolol combined with quercetin, while rats of the sixth group were injected with Bisoprolol combined with Thiotriazolin.

Bisoprolol is a highly effective cardioselective beta-adrenoblocker [3], whose hypotensive effect after single injection lasts for 24 h. In this case, the physiological circadian rhythm of arterial pressure is preserved. Quercetin (3,5,7,3',4'-pentahydroxyfla vone) is a flavonoid compound of vegetable nature with a wide spectrum of positive pharmacological (in particular, antioxidant) effects [1]. Thiotriazolin (morpholine-3-methyl-1,2,3-triazoline-5 diacetate) is a synthetic metabolic preparation possessing antioxidant and anti-ischemic effects [2].

All the above-listed pharmacological agents were added to food (Thiotriazolin and Quercetin in doses of 25 mg/kg, and Bisoprolol in a dose of 20 mg/kg). The experiment lasted 90 days. In all rats, prior to the experiment and on the 90 day, we measured the arterial pressure (mm Hg) in the tail artery using the corresponding device. Intergroup differences between the studied indices were estimated using the Student's test; such differences were considered significant at $P < 0.05$.

Housing of and manipulations with experimental animals were performed according to the existing norms of the "General Ethical Principles of Experiments on Animals" approved by the First National Congress on Bioethics (Kyiv, 2001), as well as to the European Convention for the

Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (Strasbourg, 1985).

Sampling of the material (bioptic samples of gingival tissues) for further electron microscopic studies was performed under ether anesthesia. The obtained samples were treated using the conventional technique. Studies were carried out in the Department of Electronic Microscopy (scientific head, Prof. L. O. Stechenko) at the Institute for Problems of Pathology of the Bogomolets National Medical University of the Ministry of Public Health of Ukraine (Kyiv, Ukraine). Ultrathin slices were examined under an electronic microscope with magnifications from 2,000Ч to 30,000Ч on the screen and photographed.

Results and discussion. There was found that before the experiment the normotensive rats blood pressureranged from 100-110 mmHg virtually unchanged after 90 days. In SHR rats the blood pressure was equal to 160-170 mmHg.Bisoprolol reduced blood pressure up to 120-130 mmHg, using Thiotriazolin caused a moderate reduction of blood pressure (on 5-10 mm Hg), and Quercetin had no influenced on the high blood pressure.

The epithelium basal layer of the free and attached gingiva, gingival sulcus and the gingival margin normotensive rats is not essentially different in structure and consists of basal epithelial cells, preferably columnar, arranged in a row. Basal surface of these cells has a stable, congruent adjacent to the basement membrane configuration (Fig. 1). On the part of the cytoplasm of basal epithelial cells in the cell membrane that is in contact with the basement membrane, placing hemidesmosomes. Basal epithelial cells differ from the above placed cells layers by of smaller and fewer desmosomes. Cytoplasmic matrix of these cells has a high electron density; it contains numerous mitochondria (with high electron density matrix), the elements of granular endoplasmic reticulum, ribosomes and polysomes, lysosomes and included changing in the shape electron density particlesof unexplained functional nature. The nucleus are round or oval in shape. Nuclear chromatin is concentrated on the periphery karioplasm, in which located round shape compact nucleoli. The little quantity of tonofilaments concentrated around the nucleus and in the form of bundles terminates in the region of desmosomes and hemidesmosomes.

The epithelium basement membrane looks as continuous uniform layer of electron density material, in which at high magnification of electron microscope may distinguish globular (in the form of high electron density granules) and fibrillar (as interwoven fibrils of less electron density) components. From the outside to the epithelium basement membrane adjacent collagen fibers, fibroblasts and blood capillaries of the gingival mucous lamina propria.

The gingival epithelial prickle layer of normotensive rat formed by polygonal shape epithelial cells. Their bounds are separated from each other by narrow spaces crossed by thin spikes that look like spine. Neighboring epithelial prickle cells connected by numerous desmosomes (Fig. 2). At high magnification in electron microscope can see that desmosomes which are the most symmetrical and strictly parallel area of packed neighboring plasmalemmae (desmosomes plates). Plates separated by an interval in which the well-marked central and two lateral fine grain structure lamellae, separated from each other and from plasmolemmaby lighter intervals. Laterallamellae there are a continuation of the outer leaflet of the cell membrane. By desmosomes plate from the cytoplasm side adjacent tonofilamentes that are connected to it by transverse filaments fibrils.

Epithelial prickle cells of normotensive rats have a large spherical nucleus, which can be elektron light (due to the predominance of euchromatin) or have moderate electron density (due to the predominance of heterochromatin). The prickle cells cytoplasm characterized by the of fine developed fibrillar apparatus, which present by tonofibrilsand tonofilaments. Tonofibrils form varying length bundles. The epithelial prickle cells, which are located closer to the granular layer, contained small electron density granules surrounded by a membrane (keratynosomes). The contents of these granules is released into the intercellular space.

The gingival epithelial granularcells of normotensive rats differ by elektron light cytoplasm filled with keratohyaline granules and tonofibrils bundles,and granules, similar prickle cells granules. The keratohyaline granules not surrounded by a membrane and grouped into different configurations bulks. Keratohyaline masses associated with tonofibrils bundles and formed tonofibrils-keratohyaline complexes (Fig. 3), which is often associated with ribosomes.

Tonofibrils-keratohyaline complexes (formed due to the keratohyaline pervades tonofibrslls) are a direct morphological precursor of keratin fibrils of the stratum corneums quames.

The epithelium keratinous layer of the free gingiva of normotensive rats formed flat keratinous squames (Fig. 4), which do not contain nucleus and organelles and filled with myelinlike single cells, vacuoles with of elektron transparent or elektrondensity contents (remnants of degraded mitochondria) located in osmiofil matrix (Fig. 5). Cytoplasmic matrix has a different density due to uneven distribution of keratin fibrils, location of some small keratohialine granules and amorphous loose clusters of keratohyaline masses. Plasmolemma of all keratinous squamesthickened because of congruent adjoining tangents plasmolemmas neighboring squames) / The characteristic feature of the stratum corneum is ordered stratification. One of the main features of this stratification is the gradient of the electron density of cytoplasmic matrix of successive bands of keratinous squames –the matrix density increases in the direction of the granular layer.

For gingival epithelium of rats with SAH (1, 3, 4, 5 groups) which were not treated by Thiotriazolin, characterized by the reduction (up to a total loss) in the number of desmosomes and hemidesmosomes in the basal layer, in which shifted some epithelial prickle cells (both with preserved ultrastructure and apoptotic changed cells). Loss of desmosomes and hemidesmosomes by epithelial cells accompanied with degradation of underlying extracellular matrix, which is represented by the remnants of the basement membrane in the form of irregular masses of lamellae like and tonofibrils material which is concentrated near the basal

(often spine form) areas adjacent epithelial cells. Overall, these changes lead to disruption of rows and orientation of epithelial prickle cells and basal layers cells (Fig. 6) and output enable apoptotic epithelial cells in the loose connective tissue of the lamina propria of the mucosa, where apoptotic cells are subject to phagocytosis.

In gingival epithelium of 1, 3, 5 rats groups, and especially 4 groups the processes of shrinkage and compaction of cytoplasm ofprickle epithelial cellsoccurred. Due to this intercellular spaces expanded (Fig. 7), as in the case with spongiosis (intercellular edema of the prickle cell layer).In different prickle cells cytoplasmic matrix has different degree of electron density, but in some cases, in certain groups of cells it is not differed from compacted and degraded nuclear matrix. In such way modified prickle cells it is impossible distinguish organelles, so they can be called "cells-shadows", which subsequently undergo apoptosis following steps change and move towards the degraded basement membrane.

In the epithelium granular layer of the free gingiva of rats with SAH (on the border of the keratinous layer) there are located granular epithelial cells, in the cytoplasm there are no typical tonofibrils-keratohyaline complexes. Instead, abundant cytoplasm (Fig. 8) of these cells there are small and medium-sized granules (both are surrounded by a membrane or not) with lamellae like contents of moderite electron density. These granules resemble the structure of keratinosomes granular epidermal cells that are known to contain enzymes and lipids. The contents of described keratynosomes released by exocytosis into the intercellular space, or directly into the cytoplasm of epithelial grained cells.

Except keratynosomes in these cells localized different sizes (up to very large) not surrounded by a membrane keratohyaline granules of high electron density and keratin fibrils. In the cytoplasm of some epithelial cells predominantfibrillar component (and they look "dark") in the cytoplasm of others – granular component(and they look "light"). The nucleus of these epithelial cells has the usual structure, but most general purpose organelles (mitochondria, endoplasmic reticulum, Golgi apparatus) are absent (Fig. 8). Due to the formation of these cells invagination from these cells exfoliate areas that formed the stratum corneum squames. These squames contain both typical (described above) granules and their transformation products in the form of amorphous masses of high and medium electron density.

In rats of group number 4 (receiving only Bisoprolol) and group number 5 (Bisoprolol treated with Quercetin) the free gingivakeratinizing epithelium restructuring was marked at which the movement (going out beyond the usual location) components of the stratum corneum. This phenomenon can be called a translocation.

In translocation areas (Fig. 9) the mature keratinous squames directly adjacent to the gingival epithelium prickle layer, thus violated the above-described gradient of the electron density of cytoplasmic matrix of keratinous squames successive stratification (Fig. 4). To explain such indicated translocation mayby keratinous squames detachment from the granular layer prickle cells, the cells of which are divided into keratinous squames(peculiar apoptotic bodies).

Administration of Thiotriazolin (group number 6 rats) takes the phenomenon of stratum corneum translocation of the free gingiva, with renewed typical structure of epithelial granular layer cells (Fig. 10). In animals of group 6 (compared with animals of groups 4 and 5) significantly reduced expression of apoptosis prickle and basal epithelial cells, and although in the gingival mucosalamina propria revealed remnants of apoptotic epithelial cells as apoptotic bodies, they are separated by a solid homogeneous epithelial basement membrane. The latter evidence of narrowly localized damage to the basement membrane, as well as the effectiveness of the mechanisms of timely updates. Circuit of epithelial cells adjacent to the basement membrane (Fig. 11) is tortuous, but congruent; hemidesmosomes are numerous and retain normal structure.

Conclusion. There was found that the characteristic feature of the gingival basal epithelium of SAHrats the apoptotic changes of varying degrees of severity (higher in rats that did not receive Thiotriasolin), preceded and accompanied by further:1) destruction (loosening, loss of electron density, local lysis) of the epithelium basement membrane; 2) disruption (loss of ordered structure) of the epithelium basal layer, the disintegration of desmosomes and hemidesmosomes. It is known, the desmosomes maintain the structural integrity of epithelial cells stitched them together, and in combination with intermediate filaments provide epithelium elasticity and maintain it pulling force. Hemidesmosomes – a specialized epithelial cells contacts, which stabilize the epithelial layer and determine its adhesion to underlying basement membrane and connective tissue [4]. Hemidesmosomes determine the spatial organization of epithelial cells, keeping the system forming function: providing a set of contacting cells new feature – turning them into tissue system. An important role in this process executes the basement membrane, which is a supramolecular complex that influences the differentiation, proliferation, organization and attachment of cells.

In view of the above data, the destruction of cell to cell contacts and connections with basement membrane (including through its degradation) causes apoptosis following single source update gingival epithelium, which is the basal epithelial cells, which leads to violations of updates and changes of the epithelium general cytoarchitectonic.

In the prickle layer of SAHrats that were not fed Thiotriasolin observed apoptotic changes (early manifestation of which is "psevdospongiosis"), violation of rows and orientation of the epithelial prickle cells. Thus, the main cause of gingival epithelium cytoarchitectonic changes is apoptosis of rat epithelial cells.

In SAHrats in the free gingiva there was observed a violation of epithelium keratinization, which is a clear manifestation of the epithelium keratinous layer translocation in rats group 4 treated with Bisoprolol. Today, scientific literature discusses whether epithelial keratinization classic kind apoptosis. It was recognized matched keratin occurs as a product of chemical transformation of cytoplasmic proteins, and the process considered [5, 10] as differentiation or physiological

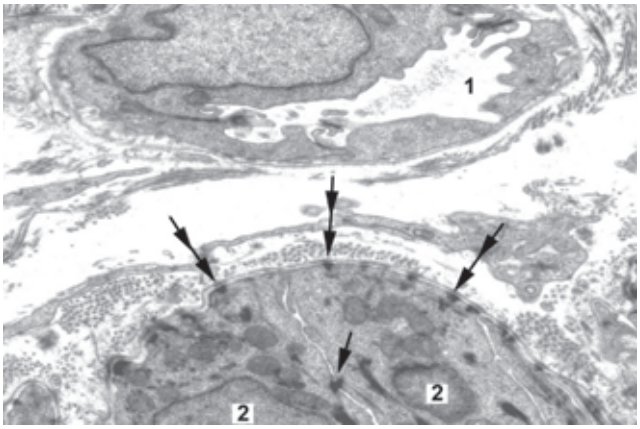

Fig. 1. Electron microphotograph of gingival basalepi the lialcells of normotensiverats: 1 – blood capillary; 2 – basal cell nucleus. Arrow-desmosome; double arrow-hemides mosomes. X8 000.

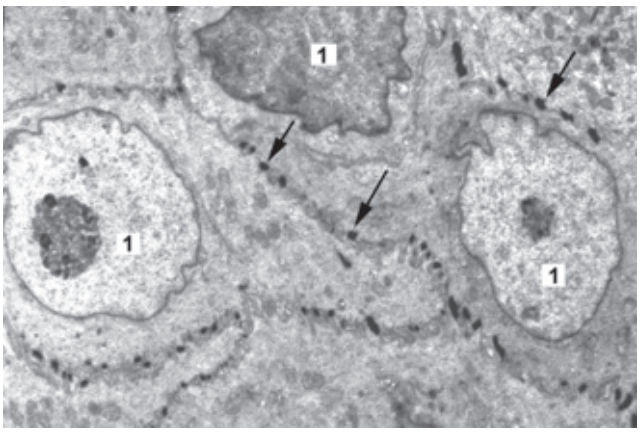

Fig. 2. Electron microphotograph of gingival pricklecells of normotensiverats: 1 – pricklecell nucleus. Arrows – desmosomes. X10 000.

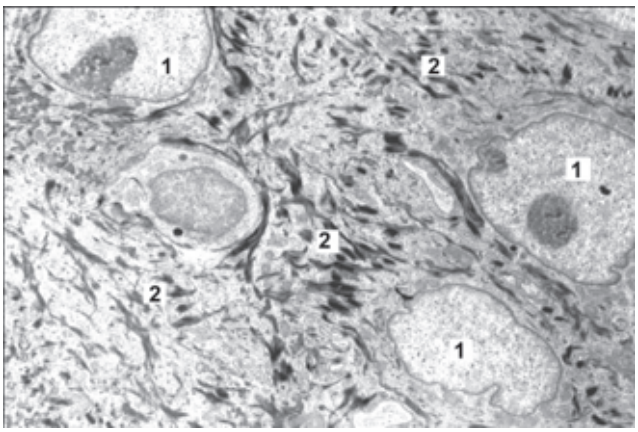

Fig. 3. Electron microphotograph of freegingival granular layer of normotensiverats: 1 – granular layercell nucleus; 2 – tonofibrils-keratohyaline complexes. X10 000.

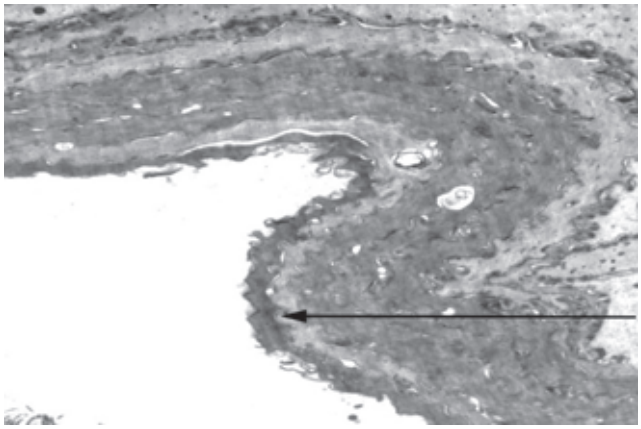

Fig. 4. Electron microphotograph of freegingival keratinous layer of normotensiverats. Arrow – electron density gradient of cytoplasmic matrix of keratinous squamessuccessive stratification. X5 000.

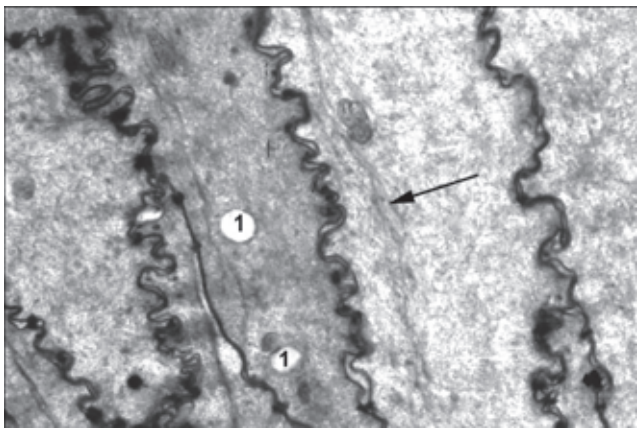

Fig. 5. Electron microphotograph of freegingival keratinous squames of normotensiverats. 1 – remnants of degraded mitochondria. Arrow-keratin fibrils. X20 000.

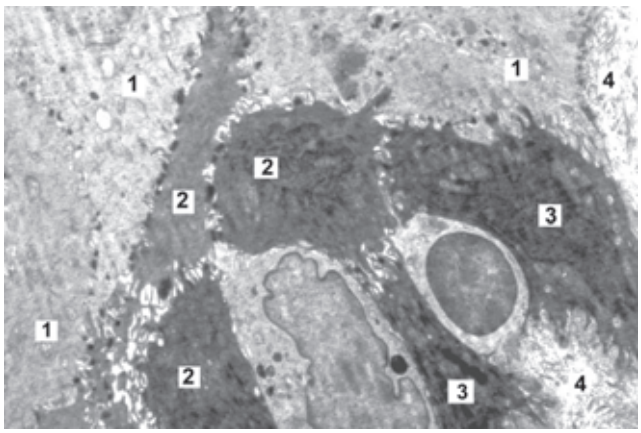

Fig. 6. Electron microphotograph of gingival epithelium of rats group 1 with SAH. Disruption of rows and orientation of epithelial prickle cells (1) as a result of apoptosis (2) andbasal layers cells apoptosis (3). 4 – gingival mucosa lamina propria. X8 000.

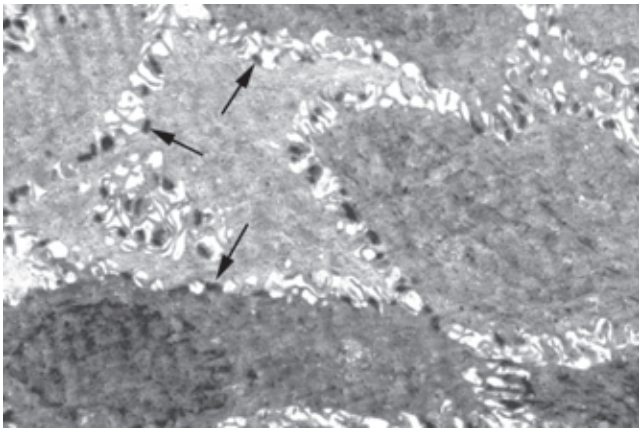

Fig. 7. Electron microphotograph of gingival epithelium of rats group 4 with SAH. Apoptosis changes (shrinkage and compaction of cytoplasm) ofprickle epithelial cells. Arrows – desmosomes. X10 000.

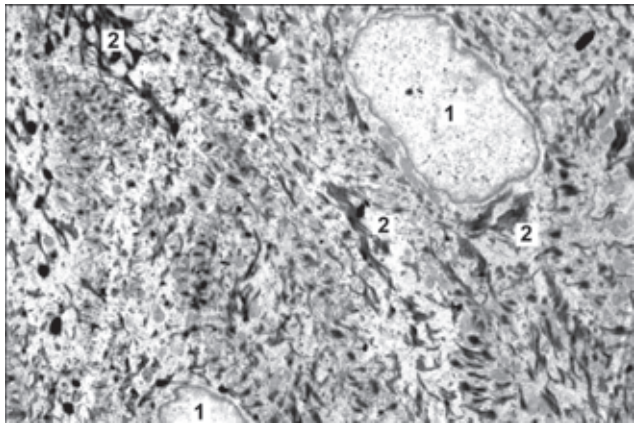

Fig. 10. Electron microphotograph of freegingival epithelium granular layer of rats group 6. Renewed typical structure of epithelial granular layercells. 1 – granular layercell nucleus; 2 – tonofibrils-keratohyaline complexes. X10 000.

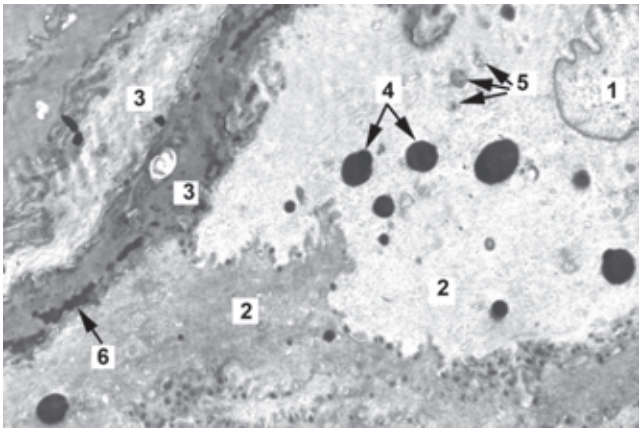

Fig. 8. Electron microphotograph of freegingival epithelium granular layer of rats group 4 at the border with keratinous layer. 1 – granular cellnucleus; 2 – granular cellcytoplasm; 3 – keratinouss quama; 4 – keratohialine granules; 5 – keratino somes; 6 – keratohialine high electron density masses. X10 000.

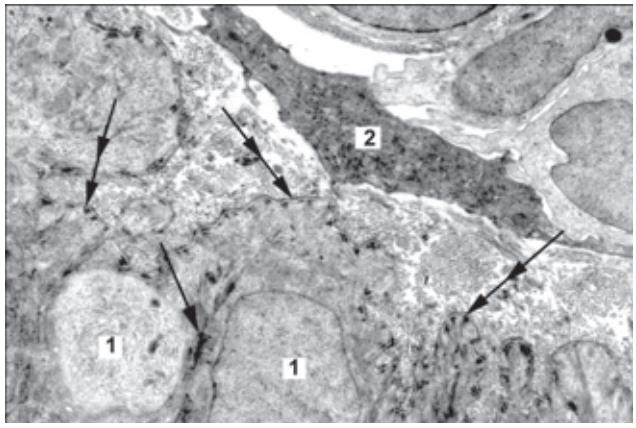

Fig. 11. Electron microphotograph of freegingival epithelium granular layer of rats group 6. Renewed typical structure of epithelial basal layercells. 1 – basal layercell nucleus; 2 – apoptotic epithelial cells body in gingival mucosa laminapropria. Arrow – desmosome; double arrow – hemidesmosomes. X8 000.

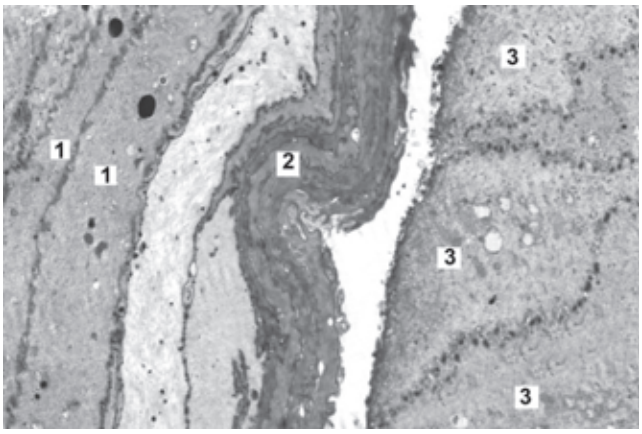

Fig. 9. Electron microphotograph of freegingival epithelium keratinous layer of rats group 4. Translocation of the stratum corneum. 1 – granular layer cell; 2 – keratinous squames; 3 – prickle cell. X5 000.

dystrophy. The paradox of the situation lies in the fact that the latter would have to be accompanied by necrosis and, in fact, accompanied by apoptosis.

The obtained data showed that found in SAH rats morphological signs of keratinization changes and formation of keratinous squames are signs of gingival epithelial apoptosis, which is enhanced under Bisoprololaction.

Thus the apoptotic changes of epithelial basal and prickle cells occur on the classical scheme of apoptosis: loss of specialized cell to cell contacts; shrinkage and compaction of cytoplasm and nucleus; their division with the formation of apoptotic bodies. However epithelial granular cells followed a different path changes can be considered invariant apoptosis. This course includes: converting epithelial granular cells in keratinous sqames; local disappearance of the granular layer; translocation of the stratum corneum.

Administration of Thiotriazolin does not fully reverse the signs of apoptosis of gingival epithelial cells, but removes the established phenomenon of the stratum corneumtranslocation and the degradation of the epithelium basement membrane, which is an important prognostic sign pharmacotherapeutic action of Bisoprolol side effects.

The prospect for further research in this area is to clarify the molecular aspects which had described apoptotic gingival epithelial cells with a view to further rationale for the usage of antiapoptosis agents in the treatment of hypertensioncomplications.

Reviewer: professor V.P. Nespriadko

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СТРУКТУРНІ ЗМІНИ ЕПІТЕЛІЮ ЯСЕН У ЩУРІВ ІЗ СПОНТАННОЮ АРТЕРІАЛЬНОЮ ГІПЕРТЕНЗІЄЮЗА УМОВ ЗАСТОСУВАННЯ ЇЇ ФАРМАКОТЕРАПІЇ

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Резюме. У статті висвітлено дані про вплив бісопрололу, тіотриазоліну та кверцетину на структурні зміни епітелію ясен лінійних щурів із спонтанною артеріальною гіпертензією (SHR). Для гістопатологічних змін в яснах щурів із артеріальною гіпертензією характерним є апоптоз епітелію. В цій ситуації застосований з лікувальною метою тіотриазолін виявив свої якості інгібітораапоптозу.

Ключові слова: артеріальна гіпертензія, бісопролол, тіотриазолін, кверцетин, епітелій ясен.

СТРУКТУРНЫЕ ИЗМЕНЕНИЯ ЭПИТЕЛИЯ ДЕСНЫ У КРЫС СО СПОНТАННОЙ АРТЕРИАЛЬНОЙ ГИПЕРТЕНЗИЕЙ В УСЛОВИЯХ ПРИМЕНЕНИЯ ЕЕ ФАРМАКОТЕРАПИИ

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Резюме. В статье освещены данные о влиянии бисопролола, тиотриазолина и кверцетина на структурные изменения эпителия десны линейных крыс со спонтанной артериальной гипертензией (SHR). Для гистопатологических изменений в десне крыс с артериальной гипертензией характеренапоптоз эпителия. В этой ситуации применен в лечебных целях тиотриазолин проявил свои качества ингибитора апоптоза.

Ключевые слова: артериальная гипертензия, бисопролол, тиотриазолин, кверцетин, эпителий десны.