INFLUENCE OF BISOPROLOL, THIOTRIAZOLIN AND QUERCETIN ON THE STRUCTURAL CHANGE OF GINGIVAL EPITHELIUM IN RATS WITH SPONTANEOUS ARTERIAL HYPERTENSION

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Abstract

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

Keywords: *arterial hypertension, Bisoprolol, Thiotriazolin, Quercetin, gingival epithelium.*

1. INTRODUCTION

As a result of extensive clinical investigations, significant advances in the study of pathogenesis mechanisms were recorded, and a large number of hypertension drugs for correcting arterial hypertension gained current interest [1]. In relation with these, very actual are the investigations devoted to the use of metabolic drugs in combination with the therapy of hypertension [2,3]. It is assumed that a very convenient and adequate experimental model for investigating antihypertensive drugs influence is spontaneous hypertension (SAH) in SHR rats, which best suits arterial hypertension in humans [4]. In previous investigations we have found out structural changes in the connective tissue [5] and nerves [6] of gingiva of rats with SAG after 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 in relation with the administration of Bisoprolol, Tiotryozalinom and Quercetin.

2. MATERIALS AND METHODS

The study was performed on 30 male rats - SHR (Spontaneously Hypertensive Rats) - divided into 6 groups (5 rats in each group): 1 - control group; 2 - a group of rats which received Thiotriasolin; 3 – rats to which Quercetin was administered; 4 - rats which received Bisoprolol; 5 - rats injected with Bisoprolol and Quercetin; 6 - rats injected with Bisoprolol and Thiotriazolin. All drugs were administered with food (Thiotriasolin and Quercetin in a dose of 25 mg/kg; Bisoprolol 20 mg/kg). Duration of experiment: 90 days. A separate group consisted of 5 normotensive male rats. Care and manipulation of animals were carried out in accordance with the general ethical principles of animal experiments, approved by the First National Congress on Bioethics (Kyiv , 2001), and with the position of the European Convention for the protection of vertebrate animals used for experimental and other purposes (Strasbourg , 1985).

Collecting and processing of material for electron and histological studies (staining half thin sections with toluidine blue, staining of histological sections with hematoxylin and eosin) were carried out by conventional methods. Electron microscope examination was performed in the Department of electron microscopy (supervisor, Professor L.O.Stechenko) at the Institute of Pathology, "O. O. Bohomolets" National Medical University. Histological preparations were examined and photographed with an Olympus BX51 microscope equipped with Olympus C3030 digital camera.

3. RESULTS AND DISCUSSION

The observation was made that, before the experiment, the normotensive rats blood pressure ranged from 100-110 mmHg, remaining virtually unchanged after 90 days. In SHR rats, blood pressure was equal to 160-170 mmHg. Bisoprolol reduced blood pressure up to 120-130 mmHg, Thiotriazolin caused a moderate reduction of blood pressure (on 5-10 mm Hg), and Quercetin had no influence on the high blood pressure.

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

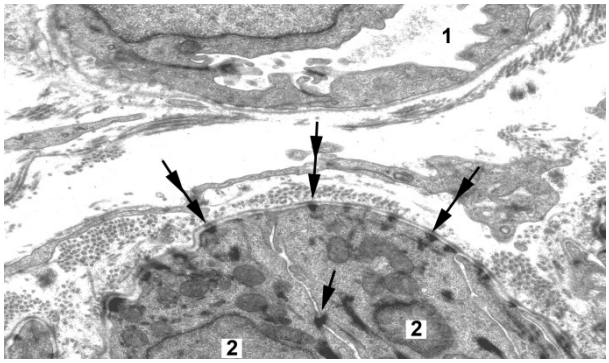

Fig. 1. Electron microphotograph of gingival basal epithelial cells of normotensive rats: 1 – blood capillary; 2 – basal cell nucleus. Arrow – desmosome; double arrow–hemidesmosomes. X8 000

The epithelium basement membrane looks as a continuous uniform layer of an electron density material, in which the high magnification of the electron microscope distinguishes globular (in the form of high electron density granules) and fibrillar (as interwoven fibrils of lower electron density) components. On the external part of the epithelium basement membrane cells there occur adjacent collagen fibers, fibroblasts and blood capillaries of the lamina propria of gingival mucosa.

The gingival epithelial prickle layer of normotensive rats is formed of polygonal-shaped epithelial cells, their bounds being separated from each other by narrow spaces crossed by thin spikes that look like spine. The neighboring epithelial prickle cells are connected by numerous desmosomes (Fig. 2). At high magnification, electron microscopy evidences desmosomes, which form the most symmetrical and strictly parallel area of the packed neighboring plasma lemmae (desmosomes plates). Plates are separated by an interval in which the well-marked central and two lateral fine grain structure lamellae are separated from each other and from plasmo-lemma by lighter intervals. The lateral lamellae are a continuation of the outer leaflet of the cell membrane. The cytoplasm from the lateral side of the desmosomes plate cells contains adjacent tonofilaments connected to it by transverse filaments fibrils.

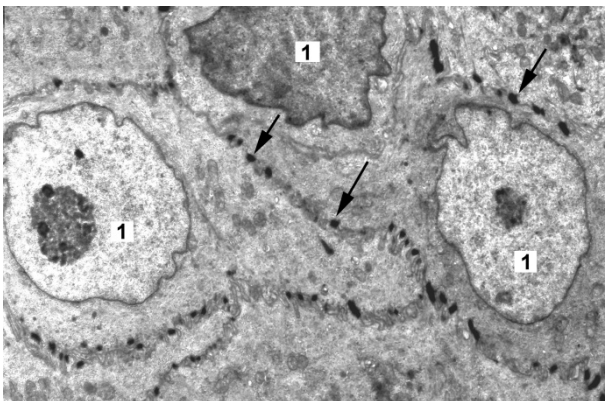

Fig. 2. Electron microphotograph of gingival prickle cells of normotensive rats: 1 – prickle cell nucleus. Arrows – desmosomes. X10 000

Epithelial prickle cells of normotensive rats have a large spherical nucleus, which can be electron light (due to the predominance of euchromatin) or they have moderate electron

density (due to the predominance of heterochromatin). The cytoplasm prickle cells are characterized by a finely developed fibrillar apparatus, which consists of tonofibrils and tonofilaments. The tonofibrils form varying length bundles. The epithelial prickle cells, which are located closer to the granular layer, contain small electron density granules surrounded by a membrane (keratynosomes). The contents of these granules is released into the intercellular space.

The gingival epithelial granular layer cells of normotensive rats differ as to their electron light cytoplasm, which is filled with keratohyaline granules, tonofibrils bundles and granules, similar to the prickle cells granules. The keratohyaline granules not surrounded by a membrane are grouped into different configurations bulks. Keratohyaline masses are associated with tonofibrils bundles, forming tonofibrils-keratohyaline complexes (Fig. 3), often associated with ribosomes.

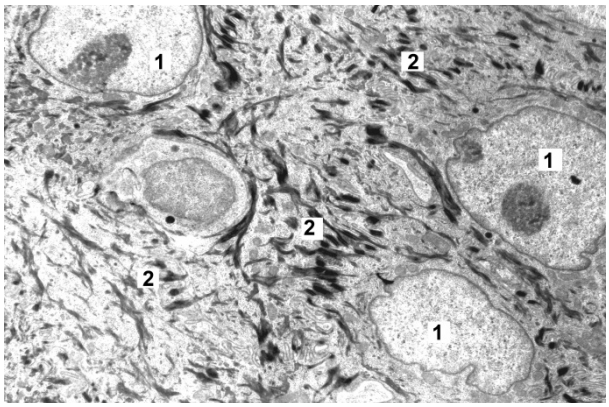

Fig. 3. Electron microphotograph of free gingival granular layer of normotensive rats: 1 – granular layer cell nucleus; 2 – tonofibrils-keratohyaline complexes. X10 000

Tonofibrils-keratohyaline complexes (formed due to the keratohyaline which pervades tonofibrslls) are the direct morphological precursors of keratin fibrils of stratum corneum squames.

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

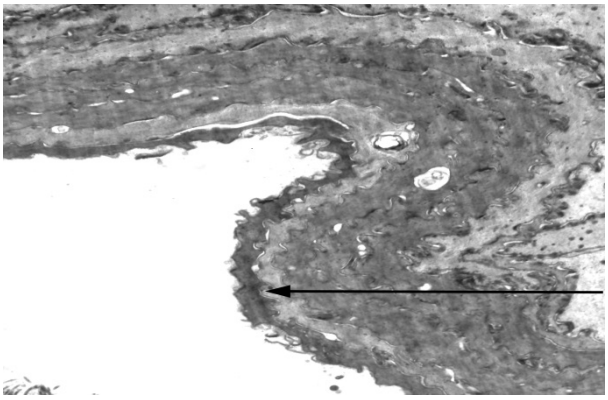

Fig. 4. Electron microphotograph of free gingival keratinous layer of normotensive rats. Arrow – electron density gradient of cytoplasmic matrix of keratinous squames successive stratification. X5 000

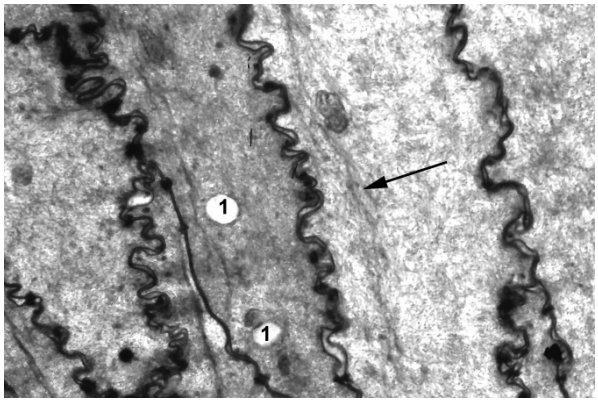

Fig. 5. Electron microphotograph of free gingival keratinous squames of normotensive rats. 1 – remnants of degraded mitochondria. Arrow- keratin fibrils.X20 000.

The gingival epithelium of rats with SAH (groups 1,3-5) not treated with Thiotriazolin is characterized by the reduction (up to a total loss)

in the number of desmosomes and hemidesmosomes in the basal layer, in which some epithelial prickle cells (both with preserved ultrastructure and apoptotic changed cells) were shifted. Loss of desmosomes and hemidesmosomes by epithelial cells, accompanied by degradation of the underlying extracellular matrix, is represented by remnants of the basement membrane in the form of irregular masses of lamellae like and tonofibrils material concentrated near the basal (often spine-shaped) areas of the adjacent epithelial cells. Generally, these changes lead to the disruption of rows and orientation of the epithelial prickle cells and basal layers cells (Fig. 6), while the output enables apoptotic epithelial cells in the loose connective tissue of the lamina propria of the mucosa, where apoptotic cells are subject to phagocytosis.

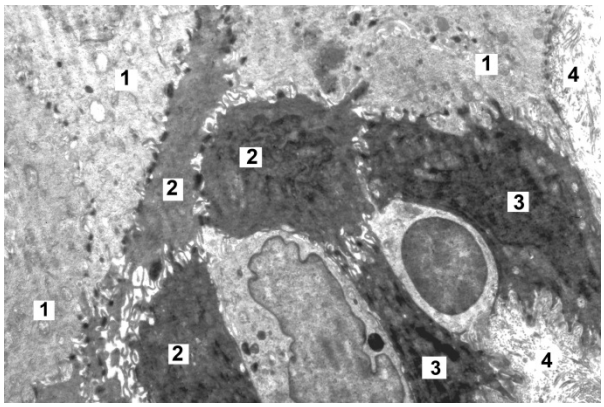

Fig. 6. Electron microphotograph of gingival epithelium of rats group 1 with SAH. Disruption of rows and orientation of epithelial prickle cells (1) as a result of apoptosis (2) and basal layers cells apoptosis (3). 4 – gingival mucosa lamina propria. X8 000

In the gingival epithelium of groups 1,3,5 and especially in group 4, shrinkage and compaction of cytoplasm of prickle epithelial cells occurred. Due to this phenomenon, the intercellular spaces become expanded (Fig. 7), as in the case of spongiosis (intercellular oedema of the prickle cell layer). In different prickle cells, the cytoplasmic matrix shows different electron density but, in some cases, in certain groups of cells it does not differ from the compacted and degraded nuclear matrix. In such modified prickle cells, organelles cannot be distinguished, so that they could be called "cells-shadows", subsequently undergoing apoptosis, following steps change and moving towards the degraded basement membrane.

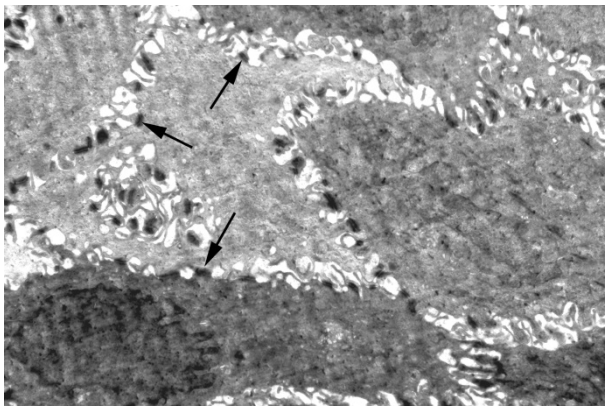

Fig. 7. Electron microphotograph of gingival epithelium of rats group 4 with SAH. Apoptosis changes (shrinkage and compaction of cytoplasm) of prickle epithelial cells. Arrows - desmosomes. X10 000

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

Except keratynosomes, these cells contain keratohyaline granules of different size (up to very large), not surrounded by a high electron density membrane and keratin fibrils. In the cytoplasm of some epithelial cells, the fibrillar component (with "dark" aspect) prevails while, in the cytoplasm of others – the granular component ("light" aspect). The nucleus of these epithelial cells has the usual structure, but, generally, organelles (mitochondria, endoplasmic reticulum, Golgi apparatus) are absent (Fig. 8). As a result of these cells invaginations, the stratum corneum squames exfoliate the formed areas. These squames contain both typical (described above) granules and their transformation products in the form of amorphous masses of high and medium electron density.

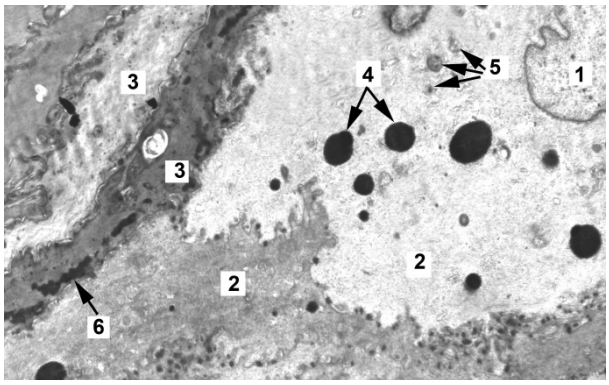

Fig. 8. Electron microphotograph of free gingival epithelium granular layer of rats group 4 at the border with the keratinous layer. 1 – granular cell nucleus; 2 – granular cell cytoplasm; 3 – keratinous sqama; 4 – keratohialine granules; 5 – keratinosomes; 6 – keratohialine high electron density masses. X10 000

In group 4 (rats receiving only Bisoprolol) and group 5 (Bisoprolol treated with Quercetin), the free gingiva keratinizing epithelium restructuration was observed with the movement (going out beyond the usual location) of the components from the stratum corneum. This phenomenon can be considered as a translocation.

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

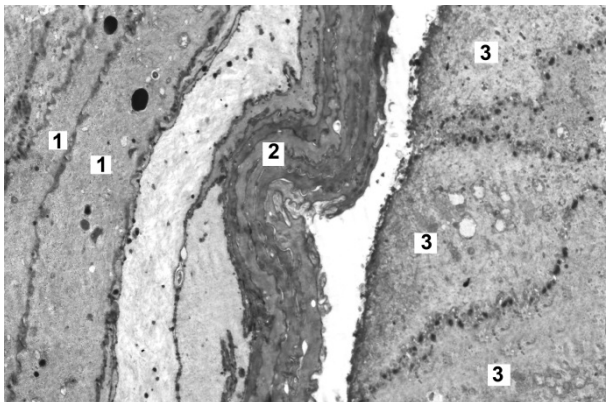

Fig. 9. Electron microphotograph of free gingival epithelium keratinous layer of rats group 4. Translocationof the stratum corneum. 1 – granular layer cell; 2 – keratinous sqames; 3 –prickle cell. X5 000.

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

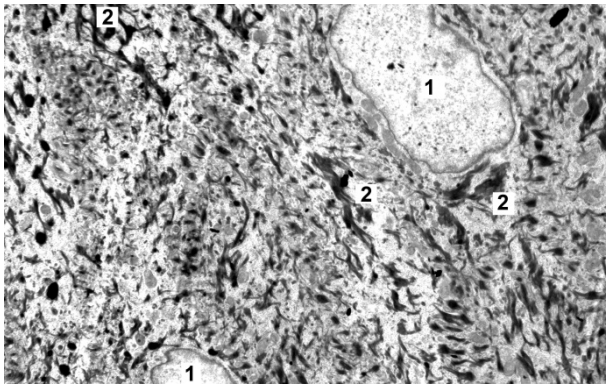

Fig. 10. Electron microphotograph of free gingival epithelium granular layer of rats group 5. Renewed typical structure of epithelial granular layer cells. 1 – granular layer cell nucleus; 2 – tonofibrils-keratohyaline complexes. X10 000.

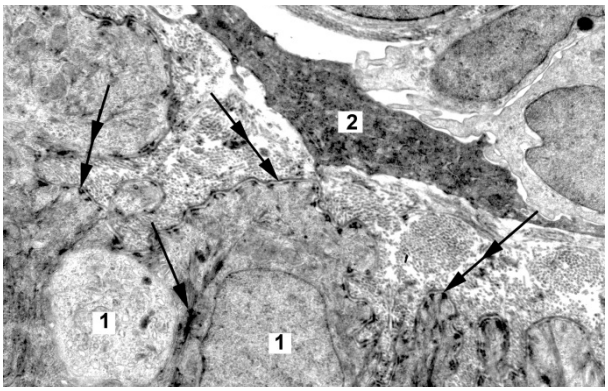

Fig. 11. Electron microphotograph of free gingival epithelium granular layer of rats group6. Renewed typical structure of epithelial basal layer cells.1 – basal layer cell nucleus; 2 apoptotic epithelial cells body in gingival mucosa lamina propria. Arrow - desmosome; double arrow – hemidesmosomes. X8 000.

4. CONCLUSIONS

The characteristic features of the gingival basal epithelium of SAH rats were 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, and disintegration of desmosomes and hemidesmosomes. As known, desmosomes maintain the structural integrity of the epithelial cells stitched together whereas, in combination with intermediate filaments, provide epithelium elasticity and maintain its pulling force. Hemidesmosomes appear as specialized epithelial cells contacts, which stabilize the epithelial layer and determine its adhesion to the underlying basement membrane and connective tissue [6,7]. Hemidesmosomes determine the spatial organization of epithelial cells, maintaining the system forming function, namely that of providing a set of contacting cells and their return into the tissue system. An important role in this process is played by the basement membrane, a supramolecular complex that influences the differentiation, proliferation, organization and attachment of cells.

In view of the above data, destruction of cell-to-cell contacts and connections with the basement membrane (also through its degradation) causes apoptosis following single source update gingival epithelium, which represents the basal epithelial cells, leading to violations of updates and changes of the general cyto-architectonics of the epithelium.

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

In the free gingiva of SAH rats, violation of epithelium keratinization, which is a clear manifestation of the epithelium keratinous layer translocation in rats group 4 treated with Bisoprolol, was observed. Today, scientific literature considers epithelial keratinization as a classical type of apoptosis. It was recognized that maturation of keratin occurs through the chemical transformation of cytoplasmic proteins, the process being considered [8-10] as differentiation or physiological distrophy. The paradox of the situation lies in the fact that the latter would have to be accompanied by necrosis while, in fact, it is accompanied by apoptosis.

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

Thus, the apoptotic changes of epithelial basal and prickle cells occur according to the classical scheme of apoptosis: loss of specialized cell-to-cell contacts; shrinkage and compaction of the cytoplasm and nucleus; their division with the formation of apoptotic bodies. However, the epithelial granular cells followed a different path, the observed changes being possibly considered as an invariant apoptosis. This course includes: convertion of epithelial granular cells into 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 eliminates the phenomenon of stratum corneum translocation and the degradation of the epithelium basement membrane, which is an important prognostic sign of the pharmacotherapeutic action of Bisoprolol side effects.

The prospects for further research in this aim at clarifying the molecular aspects describing the apoptotic gingival epithelial cells, for a further rational use of antiapoptosis agents in the treatment of hypertension complications.

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