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Ultra-structure of the oral cavity epithelial cells in white rat after experimental application of Naran S preparation

Two main components could be differentiated in the histological structure of the gum: stratified squamous epithelium and the connective tissue, which creates the barrier between the external environment and the internal part of the organism. Gum epithelium is constantly exposed to the activity of mechanical factors such as food mastication; to chemical factors such as: drug or condiments intake, toothpaste application and others as well as inflammatory ones such as dental plaque, for example. In the epithelium one can observe, on the one hand, the desquamation of highly differentiated cells and on the other – constant keratinocytes' renewal (clonal proliferation) and their differentiation (2).

Connective tissue structures, on which the epithelium rests, are connected with the synthesis of the ground substance and the connective tissue fibres and the transport of nutrition substances from the vascular rete to the epithelium. Chronic irritation may result in changes in the process of keratinocytes' differentiation in the form of an excessive or incomplete epithelial keratosis. In pathologic conditions one may observe changes in the structure of connective tissue such as: fibrosis (3) and the disturbances of collagen distribution (6).

The aim of this study was the experimental research of the impact of Naran S natural preparation on the ultra-structure of normal and inflammatory mucous membrane of the oral cavity.

MATERIAL AND METHODS

The experiment that lasted four weeks was conducted on Wistar male rats from the inbred breed of the average body mass amounting 200 g. The animals were divided into two control groups I and III and two experimental groups – II and IV with 10 animals in each group. The animals were fed with standard fodder for rodents. All animals, both experimental and the control ones were subjected to injection performed intra-gingivally into the interdental papilla of the lower incisors. After that the oral cavities of the animals were rinsed two times in 24-hour period with the natural drug (two sachets of the mixture were inundated with 250 ml of water in temperature of 80° C and left for cooling) or with physiological salt according to the following scheme:

Group I – injection of 0.025 ml of 0.9% NaCl, rinsing – 0.9% NaCl
Group II – intra-gingival injection of 0.025 ml of complete Freund (AF)(Calbiochem)-adjuvant for inducing the topical benign gingivitis, rinsing with 0.9% NaCl Opinion number 347/2002
Group III – injection of 0.025 ml of 0.9% NaCl, rinsing with Naran S preparation (20 ml)
Group IV – intra-gingival injection of 0.025 ml of complete Freund adjuvant, rinsing with 20 ml of Naran S

The study material was taken after 24 hours' time after injection and after the 1st, 2nd and 4th week past the experiment.

Ultra-structural examination was conducted on the specimens taken from the experimental and control animals. The specimens of about 1 mm^3 surface were fixed in 4% glutar aldehyde buffered with 0.1 M of phosphate buffer of 7.4 pH. After rinsing, the specimens were placed in 1% solution of osmium tetroxide. Next they were dehydrated in the number of alcohols of the increasing concentration. The material was embedded in "Spurr" resin; the blocks were polymerized at the temperature of 60° C, and cut on Reichert Ultracut S ultra-microtome. The semi-thin scrapes of 1μm thickness were stained with methylene blue and azure II. The preparations were then studied under the light microscope. Ultra-thin scrapes were contrasted with uranyl acetate and lead citrate according to the Reynolds method. The observation and the pictures were performed by the use of TESLA BS-500 transmission electron microscope of the Laboratory of Electron Microscopy in the Department of Embryology and Histology of the Medical University of Lublin.

RESULTS

Observation of the specimens under transmission electron microscope from groups I and III presented a typical image of the epithelium cells ultra-structure and the mucous membrane of the oral cavity regardless of the time elapsed from the beginning of the experiment.

The greatest changes were observed in the specimens from experimental group II. The mucous membrane contained great quantities of the amorphous fluid and disorderly arranged thin collagen fibres. Many cells of the inflammation infiltration localized just under the epithelium were observed. Epithelial cells presented cell oedema, numerous mitochondria occurring in the spinous layer of the epithelium displayed oedematic mitochondrial matrix and blurred mitochondrial crests. Cell nuclei contained electron–bright chromatin and nuclear areola created numerous indentations to the inside of the cell nucleus. The changes observed subsided with time (Fig. 1).

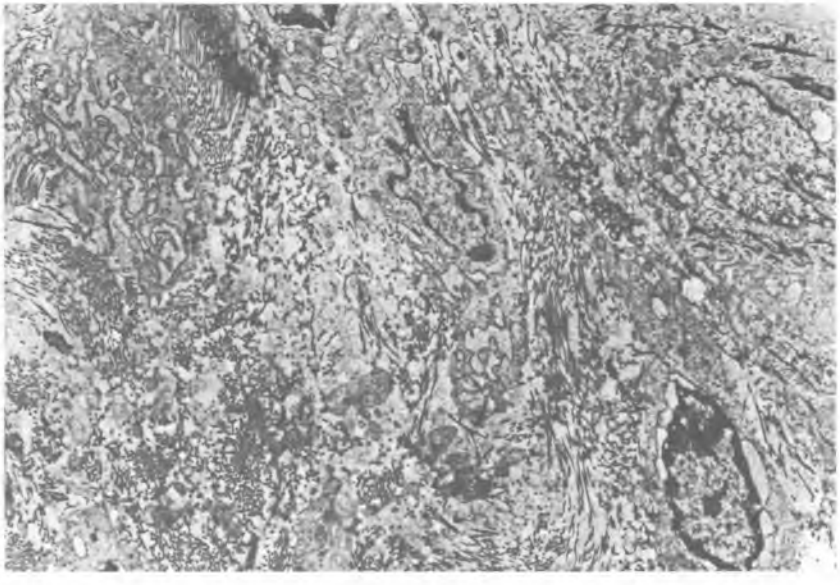

Fig. 1. TEM x 4000. Benign inflammation in the mucous membrane in the process of treatment

The studied group IV, in which the application of Naran S rinsing was effected, was similar to group II in its cellular ultra-structure. The changes observed subsided faster, and it was just after one week after application start. Epithelium cells created compact layers electron-connected with dense desmosomes; cellular nuclei were big, oval with dispersed nucleus chromatin (Fig. 2). In the cytoplasm of the granular layer cells, big, dense granules were observed, the size of which was increasing with the time of medicament application (Fig. 3).

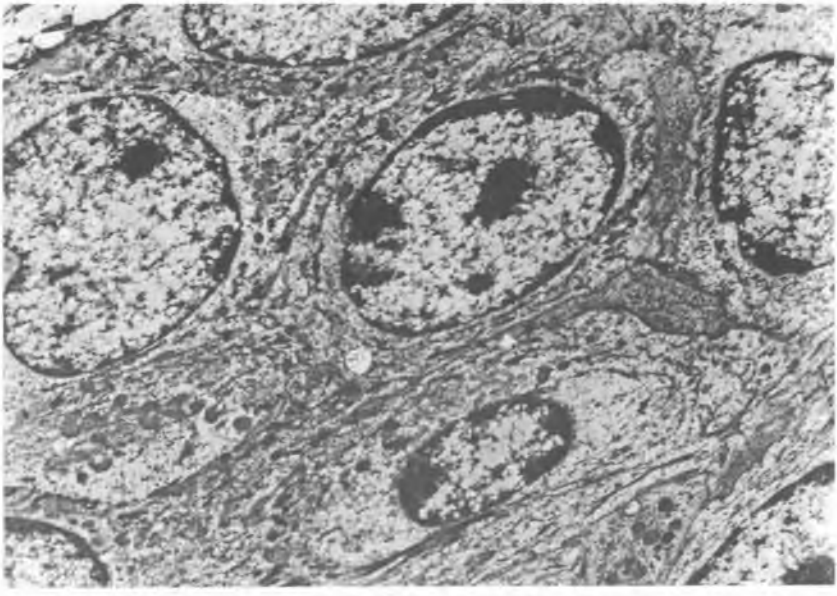

Fig. 2. TEM x 4000. The mucous membrane epithelium after treatment

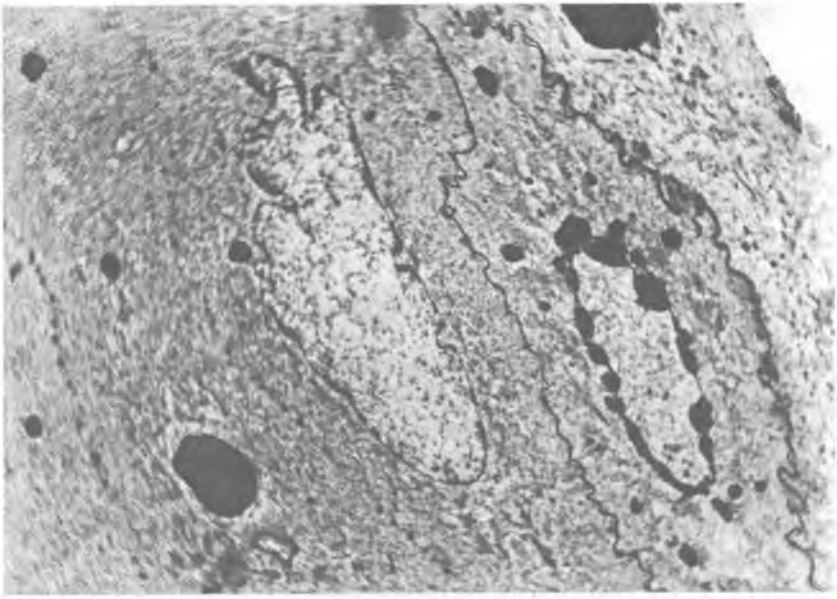

Fig. 3. TEM x 4000. Granular character of the natural dye from the drug in the epithelial cells

DISCUSSION

The general time cell transition from the reproductive layer to the cornification layer of the epithelium takes from 14 to 28 days and, among others, is dependent upon the age, hormonal impact and exogenous factors. The epithelium is constantly renewed due to the process of mitosis. Lamina propria of the mucous membrane of the gum is composed of fibrous and flaccid connective tissue, the basic component of which are the collagen fibres, constituting approximately 60% of the tissue volume. 5% is constituted by the fibroblasts and the remaining 35% – by the blood vessel and nerves and the remaining constituents of the matrix (8).

The dominating type of the cells in the gum are the fibroblasts, accounting for 65% of all cells, whose function is to create a variety of connective tissue fibres (collagen, reticular, oxytalanic and elastic ones) and the participation in the synthesis of the ground substance. One may also find in it a tiny group of inflammatory cells – most often: macrophages, neutrophil granulocytes, lymphocytes and plasmatic cells; in subepithelial cells of connective tissue in vicinity of the bottom of the gingival fissure these cells could create small aggregations. They could also be spotted around the blood vessels.

In the gingival connective tissue and other locations of the oral cavity there are also mast cells that can also be localized in paravascular regions (1, 7). Mast cells are a dominant component of the immunological system of the oral cavity tissues where they appear in twofold larger quantities than in the duodenal mucous membrane (1). They are the constant element of the gum structure and appear regularly regardless of age (7). It is shown that the number of mast cells increases in the operated sites and in the inflammatory foci. This increase is proportional to the level of fibrosis in the connective tissue (3). In the fibrosis region, one can spot the fibroblasts with impaired phagocytosis ability, which may result in disturbing collagen distribution and may eventually lead to fibrosis (6).

In the early experimental period of gum inflammation, parallel to the inhibition of division activity of the epithelial cells, mast cells in the connective tissue are shifted under the epithelium. One may suppose that these cells and strictly speaking – the substance excreted by them affect the epithelial cells and remain in connection with the spreading of the inflammatory condition in the gingival region (5).

CONCLUSIONS

1. Application of Freund adjuvant caused changes in the ultra structure of the epithelium of the oral cavity, which are characteristic of the topical, benign inflammation.

2. Application of a natural drug – Naran S caused the change of the cell ultra structure to the normal state.

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SUMMARY

The effectiveness of the natural drug – Naran S was studied in the therapy of the topical inflammation of the oral cavity mucous membrane. The experiment was conducted on male Wistar rats that were subjected to induction of inflammatory condition by injecting Freund adjuvant and rinsing oral cavity with the infusion of the drug for the period of 28 days. The analysis of the ultra structure of the epithelium cells was conducted with the use of transmission electron microscope. It was observed that under the influence of the drug, the epithelium undergoes renewal, the cells are joined with thick desmosomes, the nuclei have the proper areolas and the mitochondrium matrix is not oedematic.

Ultrastruktura komórek nabłonka jamy ustnej szczura białego po doświadczalnym zastosowaniu Naranu S

Badano skuteczność leku naturalnego Naranu S w terapii miejscowego stanu zapalnego śluzówki jamy ustnej. Doświadczenie przeprowadzono na szczurach rasy Wistar, którym wywołano stan zapalny kompletnym adjuwantem Freunda, a następnie przepłukiwano jamy ustne naparem leku roślinnego przez 28 dni. Przeprowadzono analizę obrazu ultrastrukturalnego komórek przy pomocy mikroskopu elektronowego transmisyjnego. Zaobserwowano, że pod wpływem leku dochodzi do odbudowy prawidłowego nabłonka: komórki połączone są gęstymi desmosomami, jądra mają prawidłową otoczkę, a macierz mitochondrialna nie jest obrzęknięta.