MORPHOLOGICAL CHANGES IN THE GUMS AND LIVER OF EXPERIMENTAL ANIMALS UNDER THE INFLUENCE OF ALCOHOL INHALATION

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Abstract

Introduction. The periodontal disease is one of the leading challenges in modern dentistry, due to its high prevalence in people of all ages, to the lack of methods for nosological diagnosis and to effective prevention and treatments. Undoubtedly, excessive alcohol consumption has a detrimental effect on the body, in particular on the liver. We have proven that not only alcohol consumption, but also prolonged inhalation of alcohol, occurring during alcohol production, lead to liver disfunction and to hepatitis. At the same time, the condition of the liver significantly affects the condition of other organs and systems, including the tissues of the oral cavity ("hepato-oral syndrome"). **Aim.** To determine the characteristic features of pathomorphological changes in the gums and liver of experimental animals under the influence of alcohol inhalation. **Materials and methods**. White Wistar rats were used in the experiments. 4 experimental groups were formed. Experimental periodontitis in rats was modelled by converting the animals to a peroxide model of periodontitis with the addition of the usual diet of peroxidized sunflower oil, at a dose of 1 ml per animal. The material of the study was provided by the biopsies of the mucous membrane of the gums and liver tissue of experimental rats. General histomorphology was studied on drugs stained with Ehrlich hematoxylin – eosin, by the method of van Gizon. **Results and discussion.** Pathomorphological changes in the gums of animals from the experimental group IV show the presence in the gums of rats of focal wet and dry necrosis of their epithelial layer. The epithelial areas become homogeneous and intensely stained blue with hematoxylin. Hyperplasia and desquamation of the superficial layers of the epithelium are observed near the areas of necrosis. The necrotic areas are separated and ulcers are formed in their place. In such places, an inflammatory infiltration of the subepithelial connective tissue, a sharp dilation of blood vessels, hemorrhage, may be observed. Histological examination of the liver evidenced that animals treated with alcohol inhalation showed perivascular lymphoid infiltration of the liver of moderate severity. Focal vacuolar degeneration of the small groups of hepatocytes is also characteristic. **Conclusions.** The pathomorphological changes in rats with simulated periodontitis caused by inhalation exposure to ethanol vapor revealed vacuolar epitheliocyte distrophy, hyperplasia and desquamation of the surface layers of the epithelium.

Keywords: *histomorphology, gums, liver, alcohol inhalation.*

1. INTRODUCTION

The periodontal disease is one of the leading challenges in modern dentistry, due to its high prevalence in people of all ages, to the lack of methods for nosological diagnosis and of effective prevention and treatments [1,2]. Generalized periodontal diseases are characterized by steady progression of the inflammatory-destructive process which, with age, leads to complete destruction of the tooth-retaining apparatus and premature tooth loss, caused by local factors, including microorganisms and their products, traumatic occlusion and general pathology of organs and systems, as well as by the influence of climatic-geographical and ecologically dangerous factors [3,4]. Prolonged intake of chemical industrial substances, in particular alcohol vapors, causes metabolic disorders of systems and organs in the macroorganism, which in turn affects the periodontal tissues, leading to increased morbidity and complication of their course [5,6].

There is no doubt that an excessive alcohol consumption has a detrimental effect on the body, in particular on the liver [7]. The pathogenic effect of excessive alcohol consumption primarily affects the state of lipid metabolism, especially in the liver, causing steatosis or steatohepatitis which, in most of the cases, ends in liver cirrhosis [8,9]. We have proven that not only alcohol consumption, but also prolonged inhalation of alcohol during alcohol production lead to liver disfunction and hepatitis. At the same time, the condition of the liver significantly affects the condition of other

organs and systems, including the tissues of the oral cavity ("hepato-oral syndrome") [10]. A previous work of ours, and other similar studies showed that not only oral consumption of alcohol, but also a prolonged exposure to alcohol vapors during alcohol production may have a significant impact on the state of the immune system, in particular on the content of cytokines and C-reactive protein [11,12].

The **objective** of the present study was to determine the characteristic features of the pathomorphological changes observed in the gums and liver of the experimental animals under the influence of alcohol inhalation.

2. MATERIALS AND METHODS

The white Wistar rats used for the study were included in 4 experimental groups, as follows: Group I - (intact rats); Group II (experimental model of periodontitis); Group III (healthy rats + inhalation of ethanol), group IV (experimental model of periodontitis + inhalation of ethanol vapors).

Experimental periodontitis in rats was modelled by converting the animals to a peroxide model of periodontitis with the addition of the usual diet of peroxidized sunflower oil, at a dose of 1 ml per animal [13,14]. To study the effects of ethanol vapors upon the body of rats, healthy rats and animals with experimental periodontitis were kept daily for 3 hours, along 2 weeks, in a special chamber supplied with air with ethanol vapors [15]. The groups of animals were simultaneously removed from the experiment. Euthanasia was performed under ether anesthesia, by bleeding from the heart [16,17].

The materials of the study were provided by the biopsies of the mucous membrane of the gums and liver tissue of the experimental rats [18]. General histomorphology was studied on drugs stained with Ehrlich hematoxylin – eosin, by the method of van Gizon [19,20]. The research was conducted in compliance with the general rules and provisions of the European Convention for the Protection of Vertebrate Animals [21], applied for research and other scientific purposes (Strasbourg, 1986).

3. RESULTS

Macroscopic examination of periodontal tissues in healthy animals of group I showed that the mucous membrane of the gums is pale pink, moderately moist. No pathological changes were visualized. The histological structure of the mucous membrane of the gums of rats consists of its own plate and epithelium, separated by a basement membrane. The gums are covered with a multilayered squamous epithelium with signs of keratinization or parakeratosis (Fig. 1).

Stratum corneum is represented by non-nuclear flat cells. The cytoplasm of cells of the granular layer, looking flattened, contains granules of keratohyalin. The spinous layer is represented by several rows of cells of irregular shape, with a small inclusion of glycogen. The basal layer is represented by cells located on the basement membrane, showing high mitotic activity, which indicates a high level of metabolic processes, which assure epithelial regeneration. The attached part of the gums is covered by a keratinized multilayered epithelium. In the own plate of a mucous membrane, connective tissue papillae are well-expressed. Rat periodontium is represented by a dense connective tissue, the bundles of collagen fibers which fill the periodontal gap being intertwined with the cementum of the tooth root, while also penetrating periosteum of the cell process. Between the collagen fibers, layers of loose connective tissue with blood and lymphatic vessels, cellular elements, and nerve receptors may be observed.

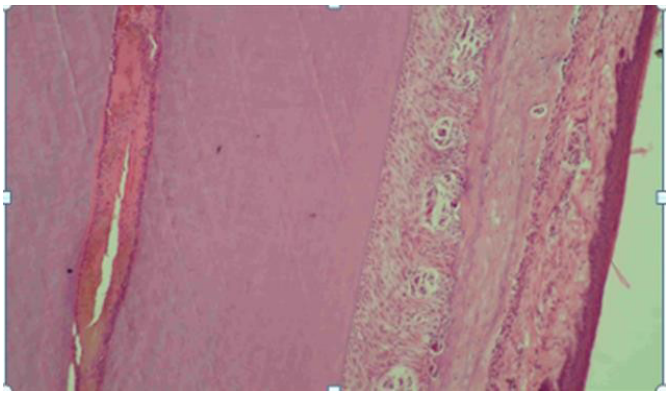

Fig. 1. Condition of periodontal tissues in healthy rats of the first group (stained with hematoxylin and eosin, x oc. 10, obj.20)

During the periodontal preparations of rats of group II, on the 60th day of the experiment hyperemia and edema of the interdental papillae and marginal part of the gums were detected by objective examination and microscopically - significant morphological changes in the structure of periodontal soft tissues, in particular, and microcirculatory vessels and bone tissue of the cellular part of the lower jaw, characteristic to generalized periodontitis. As a matter of fact, the bone tissue of the periodontium is already destroyed in the early stages of generalized periodontitis, lacunar resorption occurs, gradually leading to the formation of periodontal pockets. Thus, the epithelial layer of gums was only partially preserved; areas of erosion covered with granulation tissue, noticeable hyperplasia of basal cells (Fig. 2), still remained.

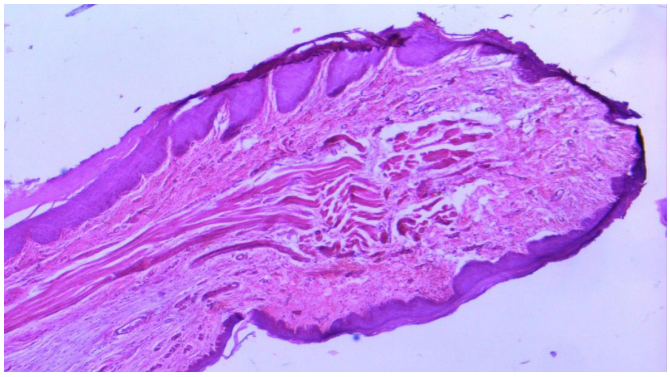

Fig. 2. Changes in the periodontal tissues of the experimental group of animals with simulated periodontitis (staining with hematoxylin and eosin, x oc. 10, obj.4)

Figure 3 shows the uneven keratinization of epitheliocytes in group III of experimental animals, exposed to inhalation of alcohol vapors

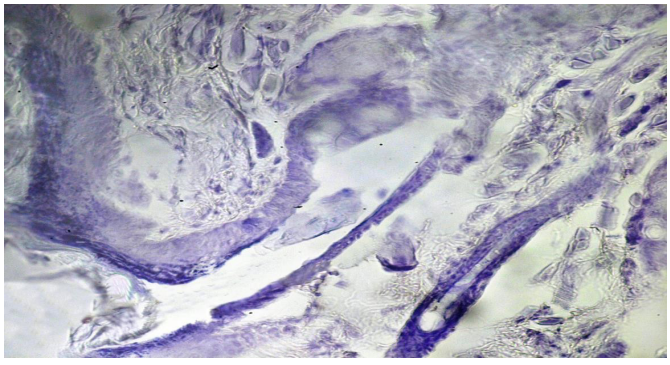

Fig. 3. Clear rats of group III after inhalation of alcohol for 60 days (staining with hematoxylin and eosin, x 120)

Such changes are accompanied by edema of the stroma and its lymphoid infiltration. In some places, significant changes in the subepithelial tissue, in the absence of epithelial erosion, may be noticed.

Vacuolar distrophy of epitheliocytes, epithelial detachment and uneven pathological keratinization of epitheliocytes of all layers were also observed.

Figures 4 and 5 show the pathomorphological changes in the gums of animals of experimental group IV, in which periodontopathogenic phenomena were combined with the inhalation effect of ethanol vapors.

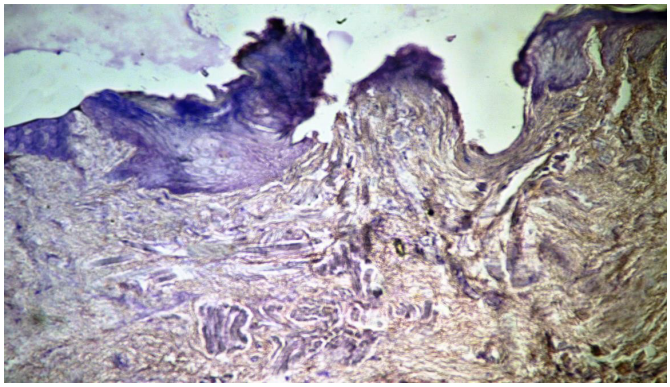

Fig. 4. Clear rats of group IV after alcohol inhalation for 20 days (staining with hematoxylin and eosin, x 120)

Figure 4 illustrates the presence, in the gums of rats, of focal wet and dry necrosis of the epithelial layer of gums. The epithelial areas become homogeneous and intensely blue-stained with hematoxylin. Hyperplasia and desquamation of the superficial layers of the epithelium are observed near the areas of necrosis. Necrotic areas are separated and ulcers are formed in their place. In such places, inflammatory infiltration of the subepithelial connective tissue, sharp dilation of blood vessels, and hemorrhage occur.

Also present is the distrophy of the alveolar-tubular glands, which are located in the subepithelial tissue. In other places of a mucous membrane of gums, phenomena of atrophy or hypertrophy of a multilayered flattened epithelium are observed. Significant hemorrhages, accompanied by reactive proliferation of fibroblasts, deposition of hemosiderin grains, are investigated (Fig. 5).

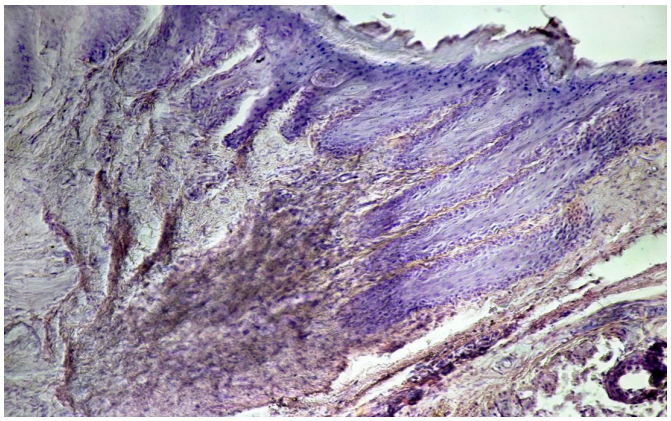

Fig. 5. Clear rats after inhalation of alcohol for 20 days (staining with hematoxylin and eosin, x 120)

Histological examination of the liver showed that the animals subjected to alcohol inhalation showed perivascular lymphoid infiltration of the liver of moderate severity (Figs. 6,7).

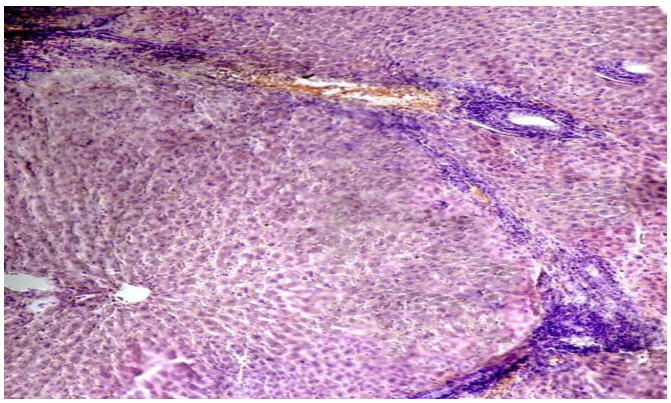

Fig. 6. Liver of rats of group IV. Histoarchitectonics of the liver is observed. Perivascular lymphoid infiltration. Diffuse vacuolar degeneration of hepatocytes (hematoxylin-eosin x 120)

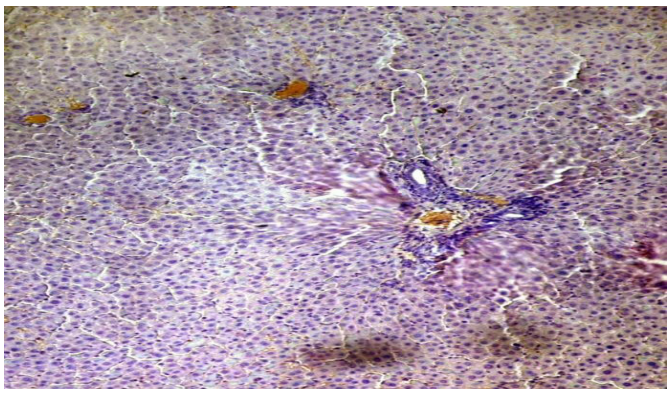

Fig. 7. Liver of rat in group IV. Lymphoid perivascular infiltration. Most pronounced phenomena of edema and vacuolar degeneration of hepatocytes near inflammatory infiltration (hematoxylin-eosin x 120)

Focal vacuolar degeneration of small groups of hepatocytes is also characteristic (Fig. 8).

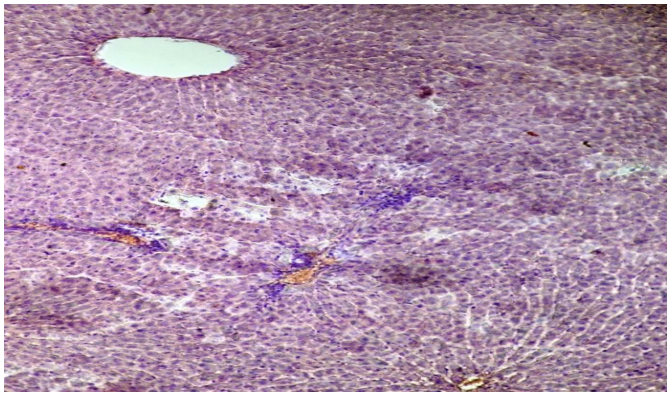

Fig. 8. Liver of rats of group IV. Vacuol degeneration of hepatocyte groups (hematoxylin-eosin x 120)

Vacuol degeneration of hepatocytes is observed on the periphery of liver particles near the interparticle connective tissue and portal veins. At the same time, hepatocytes increased in size. The sinusoids between the platelets are dilated and filled with fluid.

4. DISCUSSION

Experimental studies have shown that, in animals with simulated periodontitis, under the influence of ethanol vapors inhalation, the biochemical markers of inflammation (elastase activity, urease, MDA) were significantly increased. There was also an increase in the degree of atrophy of the alveolar process of jaws, depletion of the antioxidant system, and a decrease in the concentration of protective factor (lysozyme and hyaluronic acid in gum homogenates), comparatively with intact animals and experimental rats with simulated periodontitis.

When exposed to ethanol vapors, the experimental animals showed an increase in their hepatic markers of inflammation (bilirubin, alanine transaminase and alkaline phosphatase). The detected biochemical changes in the homogenates of gums and liver were significantly adjusted by our proposed treatment and prevention complex. Pathomorphological changes in rats with simulated periodontitis, on the background of inhalation exposure to ethanol vapors, revealed vacuolar distrophy of

epitheliocytes, hyperplasia and desquamation of the surface layers of the epithelium.

5. CONCLUSIONS

The pathomorphological changes in rats with simulated periodontitis induced by inhalation exposure to ethanol vapor revealed vacuolar epitheliocyte distrophy, hyperplasia and desquamation of the surface layers of the epithelium.

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