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STRUCTURAL AND FUNCTIONAL CHANGES IN TONGUE TISSUES UNDER PER OS ADMINISTRATION OF HIGH DOSES OF LEAD ACETATE

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ABSTRACT — The main purpose of this study was to evaluate the structural transformation in rats' tongue tissue after oral administration of lead salts. Under the conditions of acute experiment during 5 days Wistar rats were orally administered the lead acetate solution in the amount of 24 mg per day. Histological studies were carried out at OSMU upon the completion of the experiment. Effect of toxic doses of lead results in symptoms of both hyperkeratosis and destruction of tongue tissue. It is assumed that the oxidative stress and tissue irritation, induced by lead intoxication, causes pathological changes in the shape, height and number of filiform papillae of the tongue. This results in hyperkeratosis, hydropic degradation of the epithelium and in the presence of an inflammatory infiltrate in the connective tissue.

KEYWORDS — lead salts, toxic doses, tongue tissue, hyperkeratosis, oxidative stress.

RELEVANCE

Lead is not a vital element. It is toxic and is a class I hazardous substance. Non-organic lead compounds disrupt metabolism and are enzyme inhibitors (like most heavy metals). One of the most treacherous consequences of impact of non-organic lead compounds is the lead's ability to replace calcium in bones and to be a permanent source of poisoning for a continuous period of time. Lately the problem of effect this metal has on human health, in particular, on oral cavity, has been a focus of attention for researchers and practitioners. Lead intoxication risk group undoubtedly includes people engaged in metal industries, but today certain diseases, which were previously diagnosed only as a consequence of impact of occupational hazards, are registered among population as well due to environmental pollution [1].

Main sources of lead intake into human body are food, water and inhaled air.

Due to man-made environmental pollution, where lead is considered a priority pollutant, information about its toxic effect on living organisms has been accumulated [2].

Degree of toxicity depends on concentration, physicochemical state and nature of lead compounds. Lead intoxication causes major changes in the nervous system, disrupts thermal regulation, blood flow and trophic processes, changes immunobiological properties of the organism and its genetic apparatus. Increased accumulation of lead in animals leads to disruption in porphyrin and heme synthesis processes, protein synthesis, in particular globulins, growing fragility and decreasing osmotic stability of cellular membranes, changes in enzyme and hormone activity. Immature nucleated red blood cells are found in blood in large numbers [4].

Lead is easily digested in gastrointestinal tract, enters almost all organs and tissues and accumulates mostly in bones and teeth, and is removed through gastrointestinal tract and salivary glands [5–6].

When assessing signs of lead intoxication, the researchers note ulcerative stomatitis, grey spots on the buccal mucosa, dense tongue plaque, increasing viscosity and volume of oral fluid sediment. Occasional symptoms are bluish or dark blue longitudinal pigmentation of the gingival margin, gingivitis, loss of pain sensation and tactile sense, taste reception, increased caries index [1]. That way lead contributes to decrease in dental health of the public. Today a number of researches offer results of histological studies of impact of lead on the oral cavity, but changes in epithelial and connective tissues of the tongue are not sufficiently studied and described.

Purpose:
to investigate structural and functional changes in tongue tissues due to toxic effects of high doses of lead acetate.

METHODS

20 Male Wistar rats weighing 260–300 g were used in the study. White rats were chosen for the study due to resemblance of structure of their oral cavity and

tongue to those of the human being. The difference is that in rats filiform papillae are more pronounced, and clavate papillae are located on the tip of the tongue, whereas in humans they are located on the tip and lateral surfaces of the tongue. Also there are differences in the oral mucosa: surface epithelium in rats is represented by multilayer keratinizing squamous epithelium everywhere, whereas in humans multilayer keratinizing squamous epithelium is located only in the area of the hard palate, gum, linea alba and filiform papillae. Blood supply, lymphatic outflow and innervation of the oral cavity in humans and in rats function similarly.

The animals were allocated to 2 groups with 10 subjects in each group: a control group and an experimental group. Rats in the control group received normal nutrition for 5 days. Rats in the experimental group received a toxic dose of lead acetate solution (24 mg daily — LD50 corresponds to 400 mg/kg) per os for 5 days. Rats were withdrawn from the experiment on day six. Decapitation was performed under ether and chloroform anaesthesia (1:1). Material used was the study was tongue segments in Bouin's fixative. Upon fixation material was washed, dehydrated and embedded in paraffin using generally accepted methods. Sections 4 μm thick were made out of the blocks and further stained with haematoxylin and eosin. After that sections were washed for 20 minutes in several changes of flowing water, dehydrated and put into a balm.

Rats were kept, looked after, fed and withdrawn from the experiment in accordance with requirements to ensuring humane treatment of animals, rules of clinical trials in the Russian Federation, approved by the Ministry of Health of the Russian Federation on December 29, 1998, provisions of the Declaration of Helsinki (2000).

RESULTS AND DISCUSSION

Fig. 1 shows the structure of filiform papillae in tongues of the rats in the control group.

Evenly spread true filiform papillae of regular size, shape and orientation are observed, covered with multilayer keratinized squamous epithelium. They are long and thread-shaped. Arched and concave sides of the papillae are fairly clearly detectible, determining posterior direction of tips of the papillae to the base of the tongue.

At the same time animals in the experimental group demonstrate loss of height of filiform papillae, increasing distance between them, destruction of tips, loss of thread-like shape and partial atrophy of the papillae attributable to oxidative stress caused by lead salts. Formation of free radicals increases whereas activity of endogenous antioxidant enzymes decreases, thus inducing cell damage and aberration of their form and atrophy.

Fig. 1. *Normal structure of filiform papillae. Haematoxylin and eosin staining, × 130*

Our findings include loss of height of filiform papillae, increasing distance between them, destruction of tips, loss of thread-like shape and partial atrophy of the papillae attributable to oxidative stress caused by lead salts. Growing formation of free radicals and declining activity of endogenous antioxidant enzymes leads to cell damage with the aberration of their form and atrophy.

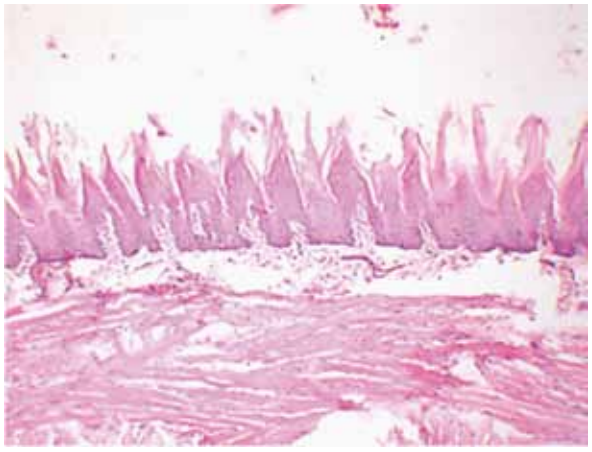

Fig. 2. *Loss of height and atrophy of filiform papillae. Hyperkeratosis. Haematoxylin and eosin staining, × 130*

Hyperkeratosis — extreme thickening of the corneous layer of epithelium — is noted. It is based on intense synthesis of keratin as a result of increasing functional cell activity of granular and spinous layers of epithelium and thickening of those layers induced by lead salts. Clinical signs are thickening of mucosa and whitening.

Inflammatory infiltrate is found in connective tissue. Cells included into inflammatory infiltrate of mu-

cosa are represented mostly by T and B lymphocytes, monocytes and dendritic cells.

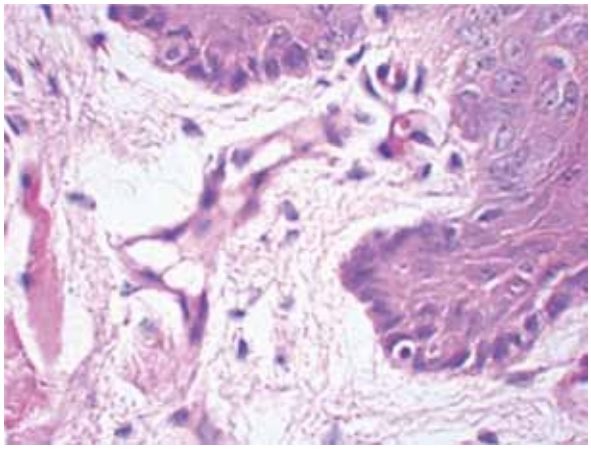

Fig. 3. *Vascular congestion in lamina propria. Moderate focal mononuclear inflammatory infiltration. Haematoxylin and eosin staining. × 900*

We observed a distinct increase in the volume of granular and spinous cell layers of epithelium. Their cytoplasm is filled with vacuoles containing transparent fluid. Nucleus in some cells is shifted to the

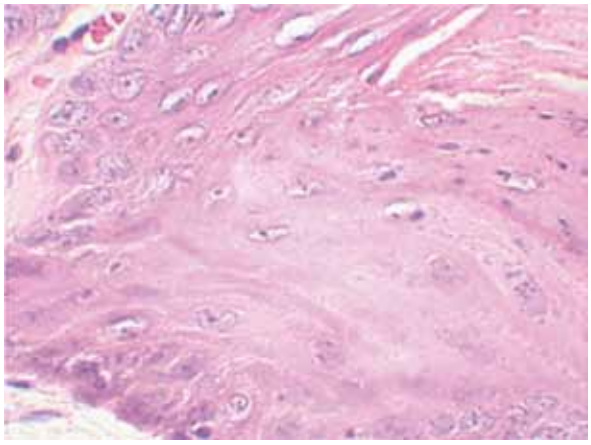

Fig. 4. *Moderate hydropic dystrophy of multilayer squamous epithelium. Haematoxylin and eosin staining, × 900*

periphery. Vacuolization or shrivelling of nuclei is also noted. Growing hydropic effect leads to breakdown of cellular ultrastructures. The mechanism of hydropic degeneration due to lead intoxication of tissues is connected with increased lipid peroxidation and decreased antioxidant capacity. This causes changes in colloid osmotic pressure in the cell, breakdown of membranes, activation of hydrolytic enzymes of lysosomes, which break intramolecular bonds during lysis with water addition [3]. Outcome of progressing hydropic degeneration turns out to be unfavourable in connection with possible total liquefactive cell necrosis.

The findings of the study have showed that highly toxic doses of lead acetate solution have a destructive effect on multilayer keratinizing squamous epithelium in the dorsum of the tongue and cause mononuclear inflammatory infiltration in connective tissue of the lamina propria of the oral mucosa. Oxidative stress and tissue irritation induced by lead intoxication lead to pathological changes in shape, height and number of filiform papillae, hyperkeratosis, hydropic dystrophy of epithelium, and development of inflammatory infiltration in connective tissue. These data corroborate pathological changes in the oral cavity caused by saturnism, which contributes to better understanding of morphofunctional pattern of lead intoxication of tissues, and may be used for prevention, diagnostics and treatment of the described abnormality.

CONCLUSION

Highly toxic doses of lead acetate solution have a destructive effect on multilayer keratinizing squamous epithelium in the dorsum of the tongue and connective tissue of the lamina propria of the oral cavity due to irritation of tissues by a toxic metal and induced oxidative stress.

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