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# ESTIMATION OF MAST CELLS IN THE RAT GINGIVA MUCOUS MEMBRANE IN THE NORMAL CONDITION AND UNDER THE CONDITIONS OF EXPERIMENTAL ALCOHOL INTOXICATION

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**Abstract:**

*The article investigates the mast cells (histamine, serotonin, catecholamines) in the gingiva structures of 4 groups of rats: group 1 - alcohol dependent, group 2 - alcohol dependent, receiving treatment with cholisal, group 3 - intact rats receiving treatment with cholisal, group 4 – control.*

*An increase of the period of alcoholization up to 3 months in the first study group led to a significant increase of the concentration of histamine in the mucous membrane of epithelial layer (an increase of 6.7 times from 0.28±0.02 to 1.86±0.003 c.u., p<0.001) compared to the underlying layers: the papillary and reticular layers of the mucous membrane lamina propria (3 times in each). This is also confirmed in qualitative terms: with a luminescent-histochemical study, a lemon-yellow and green glow is observed. Catecholamines and serotonin most clearly reflect the inflammation aspect in the mucous membrane deeper layers (lamina propria), the level of which changes 2.5 times in comparison with the control group. It should be noted that no reliably significant changes in the level of serotonin in the lamina propria reticular layer were observed because serotonin is rapidly captured by the terminals of the nervous system that are located in this layer. The differences in serotonin of group 3 and group 4 are also interesting: in the group of intact rats, the serotonin level is 2–3.5 times significantly higher than intact rats receiving preventive treatment with cholisal. We believe that the differences are due to the fact that when eating coarse, solid food, there occur mechanical damage to the oral mucosa of rats, while compensatory defense mechanisms are activated, including the activation of constant opportunistic pathogenic microflora in the oral cavity. Due to the preventive effect of the drug cholisal, the effect of this aspect is neutralized in the 3rd study group. To reduce the level of histamine and bioamines in mast cells and, as a result, to minimize the clinical signs of inflammation in the gingiva, a local application of cholisal, which has anti-inflammatory and antimicrobial effects, impacting the key links of pathogenetic processes, is sufficient.*

***Key words:*** *mast cells, bioamines, histamine, alcohol, gingiva, parodont*

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## INTRODUCTION:

A chronic intake of alcohol in the body, prolonged contact with the oral mucosae, and the deprivation of oral hygiene create a favorable background for the inflammatory diseases of parodontium tissues development [1]. At the same time, destructive changes occur in the epithelium and the gingiva lamina propria. Morphologically, this is resulted in edema and epithelium ecdysis. Further, an inflammatory reaction develops in the subsequent connective tissue [2,3].

Currently, researchers have identified an autoimmune component in the pathogenesis of parodontium inflammatory diseases, i.e. pathology proceeds as a delayed-type hypersensitivity, which contributes to the chronic inflammatory process [4]. Mast cells play the main role in the inflammatory process formation in a parodontium. They are involved in the tissular homeostasis formation, affect the growth and maturation of parodontium fibrous tissue, and are involved in immunopathological processes [5,6]. Mast cells secrete a large part of biologically active substances, which includes various types of compounds such as amines, polysaccharides, peptides, proteins, etc., which most clearly reflect the gingiva state under stress of various etiologies [7,8].

THE STUDY PURPOSE: is anassessment of mast cells (bioamine metabolism) in the mucous membrane of rat gingiva in the normal conditions and under conditions of experimental alcohol intoxication.

## MATERIALS AND STUDY METHODS:

40 outbred rats were taken for study and randomly distributed into 4 groups (10 in each group). All rats were of the same age and weight (190–210 g), were in equal conditions of keeping and feeding.

The basis of the diet for feeding rats of the control and experimental groups was juicy (tuberous roots) and concentrated (fodder grain) feed. Among them are carrots, beets, apples, pears, bananas, grains, walnuts, as well as boiled vegetables: carrots, beets, potatoes.

The first study group according to water intake schedule received a 20% solution of ethanol, as the only source of water.

The parodontium tissues of the second group, while receiving a 20% ethanol solution, were treated with cholisal (composition: the main substances - choline salicylate, cetalkonium chloride, and auxiliary - hyetellose, methyl parahydroxybenzoate, propyl parahydroxybenzoate, glycerol, aniseed seed oil, ethanol, water).This drug is selected due to the requirements for pathogenetic mechanisms necessary, according to the references, for anti-inflammatory, antifungal, and also antimicrobial actions [6,9]. So, with local administration, choline salicylate, which is rapidly absorbed through the oral mucosa, has an analgesic, anti-inflammatory and antipyretic effect: it inhibits COX activity, the functions of macrophages and neutrophils, and the production of interlikins-1 and inhibits the synthesis of prostaglandins. In the acidic and alkaline environment, it also has antimicrobial and antifungal effects. Cetalkonium chloride is an antiseptic that affects the bacteria, as well as fungi and viruses. The gel ethanol-containing adhesive base provides a rapid development of the effect and keeps it on the oral mucosa for a long time.

The third group was the control group (it received water), the fourth group according to water intake schedule received water, while parodontium tissues were treated preventively with cholisal using the application method. The result was evaluated after 20 days, 1 month, 2 and 3 months. In this case, they were guided by the "Working procedures using experimental animals." After the experiment, the animals were withdrawn from the experiment according to the WHO recommendations (table 1).

**Table 1: Characterization of study groups**

| | 1 group | 2 group | 3 group | 4 group |
|---|---|---|---|---|
| Number of animals | n=10 | n=10 | n=10 | n=10 |
| Group characteristics | Alcohol dependent | Alcohol dependent + treatment | Control | Control + treatment |
| Water intake schedule | 20% ethanol | 20% ethanol | Water | Water |
| Diet | Tuberous roots, fodder grain | Tuberous roots, fodder grain | Tuberous roots, fodder grain | Tuberous roots, fodder grain |
| Treatment and preventive measures | No | Yes | No | Yes |
| Treatment method | No | Application | No | Application |

For the study, the gingiva part was removed and the following structures of gingival papilla were investigated: a multilayer squamous keratinized epithelium, a lamina propria, consisting of papillary and reticular layers. The gingival papilla was sampled on the lower jaw between the central incisors. The drugs were frozen and fixed in a cryostat (t = -20C), a histamine content (section thickness 15–20 μm) was determined in mast cells using the Cross-Even-Rost fluorescence-histochemical method [10]. The Falk method was used to determine the catecholamines and serotonin. [11]. The quantitative content of bioamines was determined using cytospectrofluorimetry on a LUMAM-4 luminescent microscope with a FMEL-1A photometric nozzle. To assess the intensity of the histamine glow, a filter No. 7 (wavelength 515 nm) was used. The number of intensity measurements is at least 20 in each section. The results were recorded in luminescence conventional units (conventional units) using a digital multimer.

The obtained results of the conducted clinical and laboratory studies were subjected to statistical analysis using Statistical Program Package Microsoft Office 365 (Microsoft Corporayion, Seattle, USA), Microsoft Excel and the statistical package STATISTICA 8,0.

All data obtained were statistically processed: the significance of differences when comparing the values was determined using the Student-t-test criterion, under a normal distribution, under another distribution - using the Mann-Whitney criterion. To identify and analyze the conjugation of parameter changes, the Pearson's linear correlation analysis (*r*) was used. The critical level of significance in testing statistical hypotheses was taken to be equal to 0.05.

## RESULTS AND DISCUSSION:

Studies have shown that after the gingiva visual inspection, the animals of the first group with alcohol intoxication for a period of 2-3 weeks had gingival hyperemia. After 1 month, the visual signs of inflammation intensified. After 3 months, a pronounced inflammatory reaction of the gingiva was characterized by expressed hyperemia, swelling, bleeding in case of the slightest touch.

In the second group of animals, after a month of the study, visual signs of inflammation are minimal during the visual examination - slight hyperemia, which disappeared after 3 months of the experiment. In the third and fourth groups no visual signs of inflammation were observed.

When assessing the gingiva structures using the luminescent-histochemical method, the extracted histamine, combining with ortho-phthalic aldehyde, forms a highly fluorescent compound of imidazolylethylamine derivatives. Moreover, the epithelium and connective tissue of the mucous membrane lamina propria have a different glow. Thus, a high content of compounds with histamine is noted in a multilayer squamouskeratinizedepithelium, which is clearly outlined by the basement membrane, and the accumulation of histamine compounds in the latter is maximal - a lemon-yellow glow is observed.The papillary layer accumulates the formed histamine complexes in a slightly smaller amount, which is reflected in the green glow during luminescent-histochemical studies. The reticular layer also shows the maximum levels of histamine contained in the gingiva structure mast cells. A low histamine level is further noted in the underlying tissues - a blue (dark-blue) glow (Fig. 1).

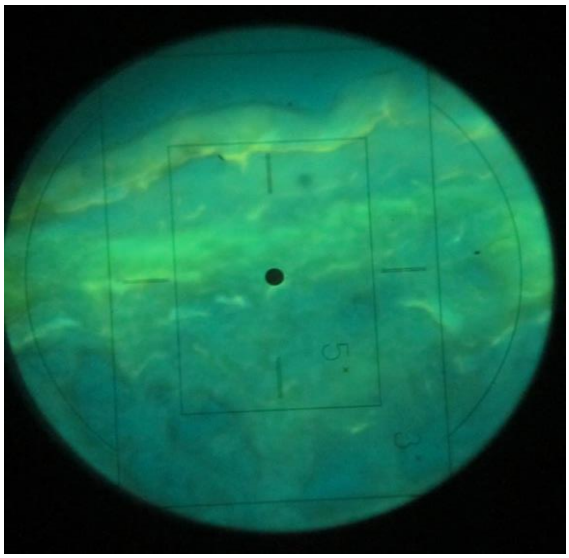

**Fig. 1 The epithelium and lamina propria glowing in the 1st study group after 3 months of observation**

This is also confirmed by the quantitative measurement of histamine level in mast cells. So, in the multilayer squamous keratinized epithelium after 1 month of observation, a sharp increase in the histamine level in mast cells of the first and second study groups was revealed. A significant difference in the first and second study groups compared with the control ones (third and fourth) was observed after a month of observation ($p<0.001$), an increase of 4-6 times (Table 2).

**Table 2: The histamine level in the multilayer squamous keratinized epithelium in the study groups after 20 days, 1, 2 and 3 months of the study (M±m), in c.u.**

| | Prior to study | 20 days | 1 month | 2 months | 3 months |
|---|---|---|---|---|---|
| 1 group | 0.28±0.02 | 0.25±0.01 | 1.11±0.22 | 1.62±0.04 | 1.86±0.003 |
| 2 group | 0.30±0.003 | 0.25±0.005 | 1.35±0.1 | 0.6±0.01 | 0.67±0.16 |
| 3 group | 0.37±0.02 | 0.42±0.056 | 0.27±0.039 | 0.27±0.04 | 0.42±0.15 |
| 4 group | 0.32±0.03 | 0.29±0.01 | 0.47±0.3 | 0.34±0.13 | 0.37±0.02 |

After 2 months, against the background of the drug cholisal local administration, a significant decrease in histamine levels from 1.35 ± 0.1 c.u. up to 0.6 ± 0.01 c.u. ($p<0.05$) is noted in the second group, whereas in the first group (without treatment) this indicator increased by another 1.5 times - up to 1.86 ± 0.003 c.u. from 1 to 3 months of the study. The initial histamine level in the mucous membrane lamina propria papillary layer in all groups also did not differ significantly and varied at the level of 0.47 ± 0.01 c.u. - 0.52 ± 0.02 c.u. When comparing the groups, it was found that in rats of the 1st group, a significant increase in histamine levels in mast cells was observed over time: in 20 days, 0.68 ± 0.06 c.u., after 2 months this indicator was already 1.29 ± 0.26 c.u. (a 2-fold increase), reaching a maximum after 3 months - 1.79 ± 0.005 c.u. ($p<0.001$), which indicates an intense inflammatory reaction in the gingiva. In the second group, a slight increase in histamine level (1.5 times) was also observed during the first month, from 0.95 ± 0.01 c.u. up to 1.4 ± 0.12 c.u., however, against the background of the drug use, after 2 and 3 months of the experiment, this indicator equalized to the level of the control groups (0.59 ± 0.15 c.u. and 0.52 ± 0.19 c.u. respectively), Fig. 2.

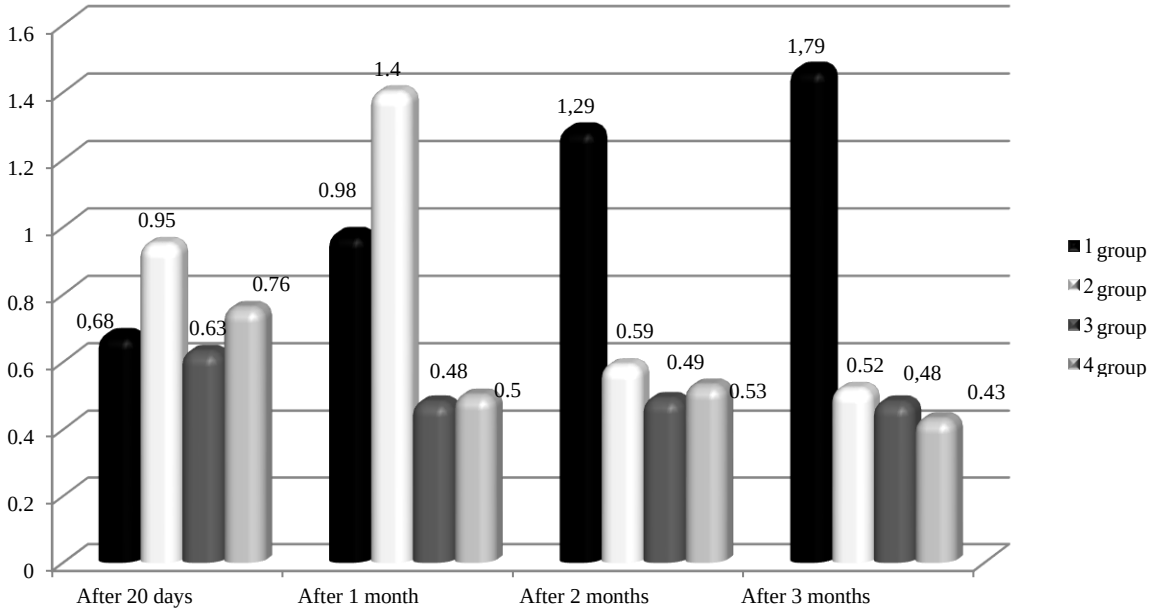


**Fig. 2 The histamine level in the mucous membrane lamina propria papillary layer after 20 days, 1, 2 and 3 months in the study groups (in c.u.)**

A similar situation can be observed in the reticular layer of the mucous membrane lamina propria: the histamine content in the gingiva tissue in case of periodontium of alcoholic etiology is significantly higher than in rats with an intact periodontium. So, in the first group, the histamine level before the study was 0.61 ±, 0.04 c.u. after a month - 1.08 ± 0.09 c.u., 2 months - 1.44 ± 0.1 c.u., in 3 months this indicator was 1.47 ± 0.12 c.u. (2-fold increase), $p<0.001$.In the second group, the histamine level before the study was 0.38 ± 0.04 c.u. after a month - 0.97 ± 0.22 c.u., 2 months - 0.62 ± 0.05 c.u., after 3 months of the study the amount of histamine corresponded to the control groups (0.24 ± 0.13 c.u. and 0.25 ± 0.006 c.u.), which indicates the effectiveness of the treatment applied.

The serotonin level in intact rats (study group 4) ranged from 0.08 ± 0.002 to 0.12 ± 0.009 c.u. In the alcohol dependent (first) group of the study, in multilayer squamous keratinized epithelium, the serotonin level after a month of the study decreased to 0.012 ± 0.006 c.u. followed by a steady increase in 2 and 3 months of observation (0.34 ± 0.001 and 0.56 ± 0.001, respectively).In the papillary layer, the serotonin level in the first group after 1 month of observation compared to intact rats increased 2 times and amounted to 0.16 ± 0.001, $p<0.05$. After some time (3 months of observation), this indicator decreased to 0.06 ± 0.001, $p<0.001$. We believe that this happened as a result of the so-called migration (redistribution) of mast cells from the papillary layer to the multilayer squamous keratinized epithelium.

A visual representation of the preventive treatment effectiveness and the effect of the latter on serotonin levels is observed in the second study group. So, in the multilayer squamous keratinizing epithelium, the serotonin level after 1 month of observation increased 18.5 times in comparison with the normal rate and amounted to 0.37 ± 0.003 c.u., $p<0.001$. Against the background of preventive treatment, to the second month of observation, a sharp drop in serotonin level to 0.05 ± 0.0018 c.u., $p<0.001$, this level was preserved at the observation period of 3 months.

The mucous membrane lamina propria papillary layer confirms a cell stress condition in chronic alcohol intoxication. So, after a month of the experiment, the serotonin level in the second study group increased to 0.53 ± 0.004 c.u. (an increase of 5.3 times compared with the normal rate, $p<0.001$), with a sharp decrease in this indicator after 2 and 3 months of observation to indicators of intact rats.

No reliably significant changes in the serotonin level in the mucous membrane lamina propria reticular layer are observed. We believe that this is due to the fact that the serotonin released during mast cell degranulation is rapidly captured by the nervous system terminals located in this layer.

It should be noted that there is a slight difference in the serotonin level between the control group (group 4) and the group of intact rats receiving preventive treatment with cholisal - observation group 3. So, in the multilayer squamous keratinized epithelium, the serotonin level after 1.2 and 3 months of observation was at the level of 0.01 ± 0.0003 c.u., which is 2 times less than the indicator of intact rats. During all observation periods, the amount of serotonin in the papillary layer was at the level of 0.02 ± 0.0004 c.u., which is 3.5 times less than the indicator of intact rats in this layer ($p<0.001$). A similar picture is observed in the mucous membrane lamina propria reticular layer: after 1 month, 0.015 ± 0.0004 c.u., after 2 and 3 months of observation, 0.025 ± 0.0005 and 0.02 ± 0.0003 c.u. respectively. We believe that the presented picture is due to the fact that in all rats, when eating coarse, solid food, a mechanical damage to the oral mucosa occurs, while compensatory defense mechanisms are activated, including the constant opportunistic pathogenic microflora activation in the oral cavity. Because of the preventive effect of the drug cholisal, which has anti-inflammatory and antimicrobial effects in the study group 3, the effect of this aspect is neutralized.

In the multilayer squamous keratinized epithelium, the content of catecholamines didn't differ significantly in the second, third and fourth groups (variability from 0.07 ± 0.002 to 0.15 ± 0.01 c.u.; $p>0.05$). In the first group, against the background of chronic alcohol intoxication, the level of catecholamines increased almost 2.5 times: from 0.1 ± 0.01 to 0.24 ± 0.03 c.u. ($p<0.05$). A similar situation is noted in the mucous membrane lamina propria papillary layer.

When analyzing the level of catecholamines in the mucous membrane lamina propria reticular layer in the first group, an increase of 3.5 times was observed: from 0.08 ± 0.002 to 0.29 ± 0.02 c.u. (after 3 months of the experiment), $p <0.001$. In the second group, the level of catecholamines after a month of observation significantly increased to 0.5 ± 0.006 c.u. (an increase of 5 times). It should be noted that against the background of treatment, the concentration of catecholamines reached the level of the control group only after 3 months of the experiment - 0.13 ± 0.004 (Fig. 3). The third and fourth groups didn't differ significantly ($p>0.05$), the indicator of catecholamines level ranged from 0.07 ± 0.001 to 0.13 ± 0.005.

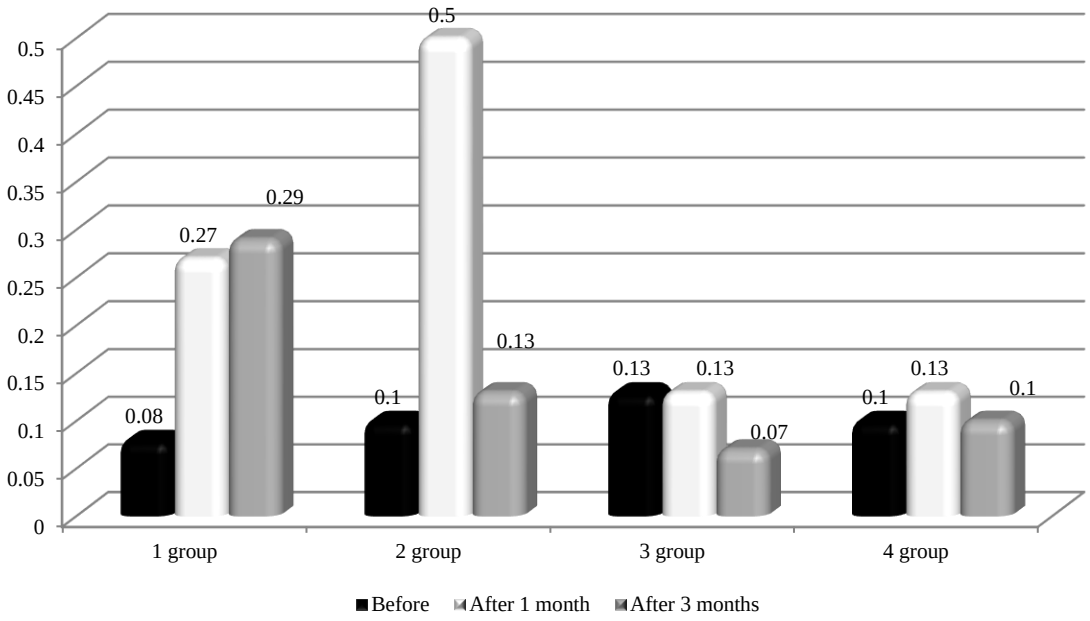


**Fig. 3 The level of catecholamines in the mucous membrane lamina propria reticular layer in observation groups: before the study, after 1 and 3 months of the experiment**

In the group with an intact periodontium, a direct tight connection was established (r=+0.9) between catecholamines and serotonin. In rats with chronic generalized periodontitis of alcoholic etiology, this relationship is reversed and weak (r=-0.02). Serotonin displaces noradrenaline from tissue reservoirs.

Thus, an increase in the period of alcoholization up to 3 months leads to a gingiva structure stress in all its layers, which is determined both at the macro– and microscopic, as well as biochemical levels. The concentration of histamine in the gingival mucosa mast cells under the conditions of experimental alcohol intoxication increases from the inside out. The most indicative is the multilayer squamous keratinized epithelium: the increase in histamine level in the first group is 6 times higher than in intact animals. The local administration (by application method) of the drug cholisal contributes to the histamine level decrease in the second group after 2 months of the experiment. Serotonin is most indicative in the mucous membrane lamina propria papillary layer. And the level of catecholamines is maximal in the deeper, reticular layer of the mucous membrane lamina propria.

## CONCLUSION:

A chronic alcohol intoxication proceeding with chronic generalized periodontitis leads to a clearly visible mast cell reaction. So, in the epithelial layer, the histamine content is determined at the maximum concentration (6-fold increase) in comparison with the underlying layers: the mucous membrane lamina propria papillary and reticular layers (3 times each), and catecholamines and serotonin, on the contrary, in the deeper layers of the mucous membrane (lamina propria), the level of which changes 2.5 times in comparison with the control groups. At the same time, serotonin displaces noradrenaline from tissue reservoirs.

To reduce the level of histamine and bioamines in mast cells and, as a result, to minimize the clinical signs of inflammation in the gingiva, a local administration of cholisal, which has anti-inflammatory and antimicrobial effects, impacting the main links of pathogenetic processes, is sufficient. This allows reducing the signs of inflammation, improving the biochemical parameters of the oral mucosa in the region of a healthy periodontium, and, as a result, restoring the functions of the oral mucosa as a mechanical, immune, absorption, and defense mechanism in general.It is assumed that non-steroidal anti-inflammatory drugs that reduce the level of biogenic amines can also be used as applications. The information obtained is recommended to justify the local administration of antihistamines and anti-inflammatory drugs, as mast cell stabilizers, in the integrated treatment of patients with periodontium pathology of alcoholic etiology.

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