The diagnosis and management of the patient with recurrent oral ulceration requires a systematic approach based on the principles of taking an adequate history, clinical examination, investigations as appropriate, institution of management and, finally, review to allow for any necessary modifications of that management [12,16] and creation of new method of treatment. Chronic recurrent aphthous stomatitis (CRAS) belongs to the group of chronic, inflammatory, ulcerative diseases of the oral mucosa. Up to now, the etiopathogenesis of this condition remains unclear; it is, however, considered to be multifactorial [12,14,15].

For today, CRAS is one of the most common types of the inflammatory process in the oral mucosa, with a prevalence of 2% to 10% in Caucasian populations. To treat them properly, physicians should know their clinical appearance and course, conditioning factors, underlying causes, and differential diagnosis [1].

The underlying etiology is not clear, although a number of factors are known to predispose to the occurrence of oral aphthae, including genetic factors, food allergies, local trauma, endocrine disorders, stress, anxiety, smoking cessation, certain chemical products, microbial agents [4,16].

Till now many aspects of chronic recurrent aphthous stomatitis are unexplored and there is a necessity for further experimental investigation to clarify the pathogenesis of this disease for the creation of primary prevention and pathogenetically based treatment of patients with CRAS including their clinical manifestations in the oral cavity [5,9,14].

Various treatment options have been used for healing of the oral tissue in CRAS and other disorders. A range of mouthwash options are used because of the anti-inflammatory, anesthetic, analgesic, antipyretic, and antimicrobial properties. In addition, systemically administered pharmacological agents, such as pentoxifylline, thalidomide, and simvastatin, have been shown to correlate with the development and severity of all the complications reported [3]. Clinical trials have reported that these drugs reduce the frequency and severity of major complications. Despite these treatment options, there is still a need for other cost-effective modalities to prevent disorders of oral cavity [3].

Medical ozone is described as three atom molecules of oxygen known as O₃ and ozone therapy has been proven safe to use in medical treatment because of antimicrobial, disinfectant, and healing properties [7]. In addition, small doses of ozone can ac-

© GMN
tivate biochemical mechanisms and reactivate the antioxidant system. Diseases that can be treated with ozone are infected wounds, circulatory disorders, geriatric disorders, macular degeneration, viral diseases and other [8]. Although ozone treatment has substantial effects, there has been no study in literature about the influence of ozone on CRAS.

The aim of this study was to determine the effects of ozone on the morphofunctional peculiarities of the soft tissues in modeling chronic recurrent aphthous stomatitis.

Material and methods. We performed experimental investigation for study of the morpho-functional state of tissues of the oral mucosa in CRAS (Fig. 1a) with modeling as it had been suggested in the previously proposed and widely used scheme [6,9]; that allows to eliminate the influence of somatic pathology and social factors. Intraperitoneal injection of 1 ml ovalbumin and aluminum hydroxide were performed for modeling CRAS process in young animals (Dutch rabbits, males, aging three-month, weighting 2-2.4 kg) during first 3 days of the experiment. Twice lower dose of ovalbumin was instilled intranasally under local anesthesia five days later (Day 8) with repeated intranasal administration of ovalbumin through on the 16th, 17th, 20th and 21st day of the experiment. Doses of used medicine were determined according to animal body weight. Group of 8 animals with obtained mucosal changes was our comparison group. We formed group of 8 animals also which was treated by ozone therapy (Fig. 1 b,c) with the apparatus "Ozonimed" using (exposure of 40 seconds in each ulcer at the 9th power). The specimens of soft tissues of the oral cavity of were stained with hematoxylin and eosin (H&E) [2] after the routine proceeding. Microspecimens were performed in the Department of Pathological Anatomy of the Kharkov Medical Academy of Postgraduate Education (head of the department Irina Yakovtsova). Morphometric studies were performed.

Results and their discussion. Ulcerative defects of round or oval shape with 5 mm diameter with the imprinting surface and covered with whitish film have had been revealed on examination of the oral mucosa group of animals with modeling CRAS (Fig.1a) in comparison group and investigated group before ozone correction. Used ozone correction (Fig. 1b,c) was realized in reducing or disappearance of visible ulcerative changes. The histological examination of the obtained microspecimens shows that CRAS modeling is realised by a complex of pathological changes in the oral mucosa. Squamous epithelium is characterized by uneven thickness with necrotic, mainly erosive injuries (Fig. 2), but ulcers were detected also. Intraepithelial lymphocytes, eosinophils, signs of proliferation in the basal cellular layer, moderate development of papillomatous changes have been demonstrated in untreated animals. Inflammatory infiltration is expressed in the lamina propria of the oral cavity of animals before start of ozone therapy.

Simultaneously there are areas with infiltration by inflammatory both in the lamina propria and epithelium of the oral cavity (Fig. 3).

The examination of animals' oral cavity after ozone therapy revealed reducing of necrobiotic changes in the oral mucosa till...
disappearance of visible pathological changes. There are isolates mucosal erosions, with absence of ulcers or aphthous defects in majority of experimental animals; there are isolate pronounced erosive changes in 2 rabbits from that group.

Histologically epithelium is uniform in thickness, but there are areas with pronounced thickening. Superficial cells are flat, near the spindle-shaped, the pycnosis phenomenon is not pronounced. The cytoplasm of the superficial epithelial cells is shown as a thin, eosinophilic, intensely stained border. As an approach to basal membrane cells are increased in volume by both the nucleus and the cytoplasm size.

The shape of the cells is changed from oval to elongate with simultaneous changing the orientation of the epithelial cells and the almost vertical position in the basal membrane. The nuclei of the basal epithelial cells are well defined, oval, uniform, hyperchromatic; cytoplasm is moderately basophilic. The location of the basal cell layer is regularly, without “jumping” the cells. Grouped intraepithelial lymph leukocyte elements were not detected. The basement membrane is uneven with uneven thickness. Acanthotic strips of lamina propria are pronounced (Fig. 4).

Reticular layer is located deeper and is represented by rough connective tissue fibers. Cellular consist of gingival mucous membrane is presented in the table 1. Cellular elements between connective tissue fibers (fibroblasts, histiocytes, lymphocytes, mast cells, macrophages) are isolated. Cells of connective tissue are presented by mature cells predominantly present in papillary and reticular layers. Lymphoid elements are dispersed evenly between the connective tissue fibers, without the formation of focal accumulations. Eosinophils are absent; signs of accumulation of inflammatory exudate have not been demonstrated.

Changes which obtained as result of our treatment could be recognized as positive changes [13,17] with healing of injured areas. Our results are combined with studies in literature indicating that ozone treatment reduces oxidative stress, improves wound healing, and increases tissue partial oxygen pressure [18]. Pathogenesis of periodontal inflammation might involve inhibition of cell death, through the apoptotic factors, due to the DNA damage by the product of catalysis [10,11] with highest levels activity found at sites of chronic inflammation. Small doses of ozone can activate biochemical mechanisms and reactivate the antioxidant system.

Changes in cellular component with reducing cells of inflammatory origin prove about positive process in ozone therapy, but connective tissue as fibroblasts, fibrocytes, histiocytes have an important role in wound healing and many studies in literature have examined the effect of different method of therapy on fibroblast cell growth mainly [3]. The results of this study demonstrated that ozone therapy as favorable influence for condition of connective tissue components. Histopathological examination has shown that ozone reduces inflammation and edema and is useful in wound healing in soft tissue.

The data of this study suggest that ozone therapy has positive effects in the treatment of CRAS. These results may be related to the duration and dose of ozone applications. Different duration or dose of ozone application may change the results.

**Conclusion.** Correction of tissual changes in chronic recurrent aphthous stomatitis could be obtained with ozone therapy that is realized morphologically in disappearance of necrobiotic processes, epithelialization of aphthous defect, growth of acanthetic bands, pronounced reducing of inflammatory cells, restoration of the cellular layers of the epithelium, moderately pronounced sclerosis of the papillary layer of the lamina propria.

**Conflict of Interest Statement.** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

<table>
<thead>
<tr>
<th>Table. Cellular consist (%) of gingival mucous membrane</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Comparison group (modeling CRAS)</strong></td>
</tr>
<tr>
<td>Histiocytes</td>
</tr>
<tr>
<td>Young fibroblasts</td>
</tr>
<tr>
<td>Fibrocytes</td>
</tr>
<tr>
<td>Lymphocytes</td>
</tr>
<tr>
<td>Plasma cells</td>
</tr>
<tr>
<td>Macrophages</td>
</tr>
<tr>
<td>Neutrophils</td>
</tr>
<tr>
<td>Eosinophils</td>
</tr>
</tbody>
</table>

* - changes are reliable, p<0,05

**Fig.4.** Well pronounced epithelialization in place of aphthous defect. Pronounced acantotic bands (a). Disappearance of necrobiotic processes in epithelium with isolate inflammatory cells (b). Restoration of the cellular layers of the epithelium. Moderately pronounced sclerosis of the papillary layer of the lamina propria. Superficial papillary layer of the lamina propria consists of loose connective tissue which is represented mainly elastic: H&E stain. Objective х20

Superficial papillary layer of the lamina propria consists of loose connective tissue which is represented mainly elastic fibers (Fig. 4).
REFERENCES


SUMMARY

INFLUENCE OF OZONE THERAPY ON ORAL TISSUE IN MODELING OF CHRONIC RECURRENT APHTHOUS STOMATITIS

Kovach I., Kravchenko L., Khotimska Yu., *Nazaryan R., *Gargin V.

State Establishment "Dnipropetrovsk Medical Academy", *Kharkiv National Medical University, Ukraine

Influencing recurrent aphthous stomatitis (CRAS) belongs to the group of chronic, inflammatory, ulcerative diseases of the oral mucosa. The aim of this study was to determine the effects of ozone on the morphofunctional peculiarities of the soft tissues in modeling chronic recurrent aphthous stomatitis.

We performed experimental investigation for study of the morpho-functional state of tissues of the oral mucosa in CRAS with using of previously proposed and widely used modeling scheme with ovalbumin and aluminum hydroxide. Two groups of animals were formed (Dutch rabbits, males, aging three-month, weighting 2-2.4 kg). Group of 8 animals with obtained mucosal changes was our comparison group. Other group of 8 animals with obtained mucosal changes was treated by ozone therapy. Histological investigation has been performed. Microscopical examination of tissue had shown that ozone therapy reduces inflammation and edema and is useful in wound healing in soft tissue as disappearance of necrobiotic processes, epithelialization of aphthous defect, growth of akantotic bands, pronounced reducing of inflammatory cells and changing of cellular ratio (with of neutrophils part from 38.30±2.46% to 6.34±0.63%, eosinophils from 5.49±0.23% to 2.87±0.05%), restoration of the cellular layers of the epithelium, moderately pronounced sclerosis of the papillary layer of the lamina propria. Described results allow to conclude that correction of tissue changes in chronic recurrent aphthous stomatitis could be obtained with ozone therapy using.

Keywords: chronic recurrent aphthous stomatitis, histology, experiment, ozone.

РЕЗЮМЕ

ВЛИЯНИЕ ОЗОНОТЕРАПИИ НА ТКАНЬ ПОЛОСТИ РТА ПРИ МОДЕЛИРОВАНИИ ХРОНИЧЕСКОГО РЕЦИДИВИРУЮЩЕГО АФТОЗНОГО СТОМАТИТА

1 Ковач И.В., 1 Кранченко Л.И., 1 Хотимская Ю., 1 Назарян Р.С., 2 Гаргин В.В.

1 Днепропетровская медицинская академия; 2 Харьковский национальный медицинский университет, Украина

Хронический рецидивирующий афтозный стоматит (ХРАС) относится к группе хронических, воспалительных, язвенных заболеваний слизистой оболочки полости рта.

Целью исследования явилось определение влияния озоно-на морфофункциональные особенности мягких тканей ротовой полости при моделировании хронического рецидивирующего афтозного стоматита.
Evans et al. (1987) found that arsenicosis leads to the formation of a fibrous scar, which may cause ocular damage.

Ash and Gillow (1989) reported that arsenicosis is associated with the development of cataracts and blindness.

Kocharyan et al. (2010) concluded that arsenicosis is a major cause of blindness in the region studied.

The prevalence of arsenicosis in the study area was high, with 75% of the population affected.

The most common symptoms observed were dry eyes, redness, and pain.

A meta-analysis of several studies concluded that the incidence of arsenicosis in the study area is three times higher than in the general population.


divating aftozous stomatitis. Conducted a prospective study for the identification of morpho-functional characteristics of the skin of different age groups of rats. The study revealed an increased susceptibility of rats to acute respiratory diseases. Arsenic exposure was associated with pathological conditions of the heart, liver, kidney, cardiovascular, reproductive, cancer, and diabetes.

In 1980s, the measurements of As in the regions of rivers Lukhuniskali and Korula revealed 45-170 mg/L of As content in the snow and 10-100 mg/L of As in grass samples [2]. Development of Lukhuni deposit has been ceased since 1985, however waste of the former As industry and the deserted underground excavations, from which mine waters flow into the main hydrographic unit of Lukhuni region, are powerful sources of As accumulation in Lukhuniskali river [21].

The epidemiological studies within the population of these regions revealed the increased susceptibility to acute respiratory disease, pathological pregnancy and premature birth. As compounds are known to induce significant health damage (gastrointestinal, hepatic, renal cardiovascular, reproductive effects, cancer and diabetes).