რეზიუმე

ადამიანის სისხლში კლოზაპინის თვისობრივი და რაოდენობრივი განსაზღვრის გაზურ ქრომატოგრაფიული – მასსპექტრომეტრული (GC-MS/MS) მეთოდის შემუშავება

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შემუშავებულია და ვალიდირებულია ადამიანის სისხლში კლოზაპინის განსაზღვრის მარტივი, სწრაფი, სელექციური,მგრძნობიარე გაზურ ქრომატოგრაფიული - მასსპექტრომეტრული (GC-MS/MS) მეთოდი. იზოლირებისათვის გამოყენებოდა მყარფაზური ექსტრაქცია. საკალიბრო სტანდარტების კონცენტრაცია მერყეობდა 5-1500 ნგ/მლ-ს შორის.

ანალიზი ჩატარდა გაზურ ქრომატოგრაფზე Agilent 7000A Quadrupole GC-MS/MS შემდეგი პირობებით: კაპილარული სვეტი HP-5MS, 30მ x 0.25მმ, რომელიც დაფარული იყო 0.25 მკმ გარსით, სვეტის ტემპერატურა - 50 - 305°C, რომელიც იზრდებოდა წუთში 10°C-ით, ინჟექტორის და გადამცემის ტემპერატურა - 310°C, ჰელიუმის ნაკადის სიჩქარე - 1.0 მლ/წთ. იონიზაცია მიიღწეოდა დადებითი რეჟიმით (EI+). სკანირება მიმდინარეობდა ჯამური იონური ნაკადით (TIC) და მრავალჯერადი რეაქციების მონიტორინგით (MRM), ელექტრონის იმპულსის 70 ევ. კლოზაპინის შემთხვევაში რაოდენობრივი ანალიზი ჩატარდა იონების აღრიცხვის გზით *m/z* 326 → 256, *m/z* 326 → 243, *m/z* 326 → 192.

მეთოდის ვალიდაცია მოხდა შემდეგ პარამეტრებზე: სელექციურობა, განმეორებადობა, სწორხაზოვნება, სიზუსტე, სისწორე, განსასაზღვრი მინუმუმი (LOD) და აღმოსაჩენი მინუმუმი (LLOQ).

სტატიაში შემუშავებული მეთოდის გამოყენება შესაძლებელია ადამიანის სისხლში კლოზაპინის განსაზღვრისათვის სასამართლო ექსპერტიზის დროს, ასევე, შიზოფრენიით დაავადებულ პაციენტებში თერაპიული დოზის კონტროლისათვის კლოზაპინით მკურნალობის დროს.

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

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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-

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.

Fig. 1. Modeling of chronic recurrent aphthous stomatitis with appearance of ulcerative defects covered whitish film on the oral mucosa of rabbit before treatment (a) preparation (b) and performing (c) ozone therapy after modeling CRAS

The procedure was done strictly in compliance with the Helsinki Declaration, European Convention for the protection of vertebrate animals (18.03.1986), European Economic Society Council Directive on the Protection of Vertebrate Animals (24.11.1986) after approval from the Regional Ethical Review Board at State Establishment "Dnipropetrovsk Medical Academy" protocol № 1 (18.01.2015).

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.

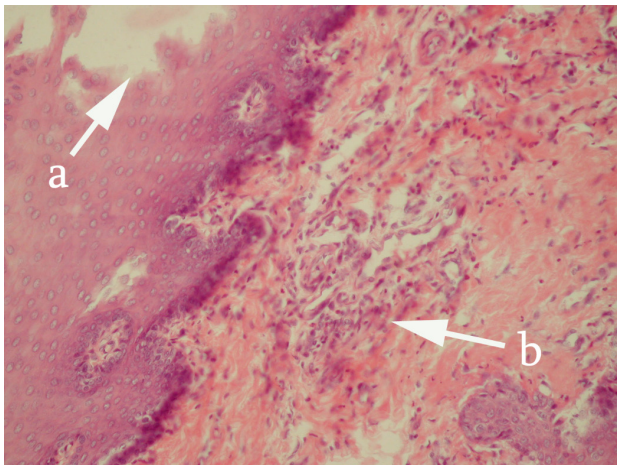

Fig. 2. Formation of erosive-ulcerative defect (a) with focal thinning of the squamous epithelium. The presence of perivascular inflammatory infiltrate (b) in the lamina propria. Group of animals without treatment. H&E stain. Objective 20

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

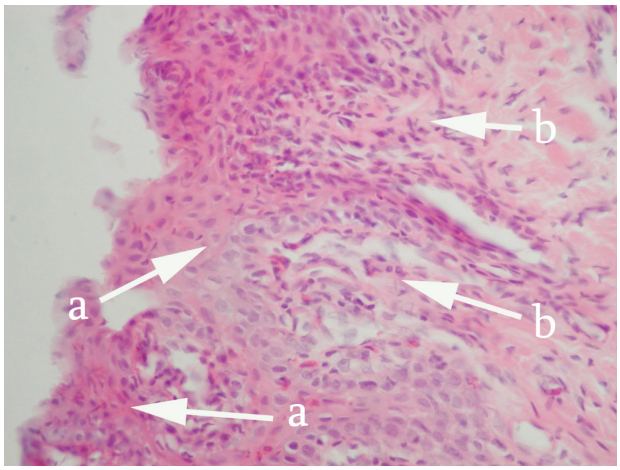

Fig. 3. Aarea with infiltration by inflammatory cells both in the epithelium (a) and lamina propria (b) of the oral cavity with formation of erosive-ulcerative defect. Group of animals without treatment. H&E stain. Objective 20

The examination of animals' oral cavity after ozone therapy revealed reducing of necrobiotic changes in the oral mucosa till

Table. Cellular consist (%) of gingival mucous membrane

	Comparison group (modeling CRAS)	Group of animals treated by ozone therapy
Histiocytes	4.62±0.21	32.21±2.42*
Young fibroblasts	17.02±1.20	13.47±1.42
Fibrocytes	19.91±1.42	27.42±1.43*
Lymphocytes	4.68±0.25	6.84±0.63
Plasma cells	4.83±0.24	4.31±0.67
Macrophages	4.72±0.38	6.02±0.42
Neutrophils	38.30±2.46	6.34±0.63*
Eosinophils	5.49±0.23	2.87±0.05*

** - changes are reliable, p<0,05*

disappearance of visible pathological changes. There are isolate mucosal erosions, with absence of ulcers or aphthous defects in majority of experimental animals; there are isolate no 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 simultaneously 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. Acantotic strips of lamina propria are pronounced (Fig. 4).

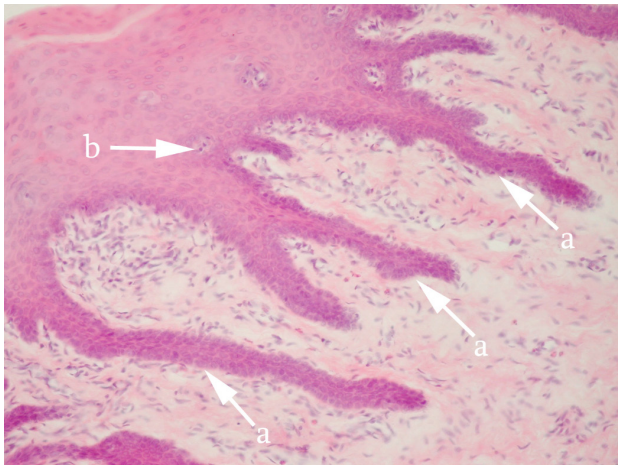

Fig.4. Well pronounced epithelialization in place of aphthous defect. Pronounced akantotic bands (a). Disappearance of necrobiotic processes in epithelium with isolate inflammatory cells (b). Restoration of the cellular layers of the epithelium. Moderately pronounced sclerosis of the papillary layer 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 x20

Superficial papillary layer of the lamina propria consists of loose connective tissue which is represented mainly elastic fibers (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 akantotic 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.

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SUMMARY

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

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Chronic 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 tissual 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

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

Целью исследования явилось определение влияния озона на морфофункциональные особенности мягких тканей ротовой полости при моделировании хронического реци-

дивирующего афтозного стоматита. Проведено экспериментальное исследование для изучения морфофункционального состояния тканей слизистой оболочки полости рта при ХРАС на основе ранее предложенной и широко применяемой модели с использованием овальбумина и гидроксида алюминия. Сформированы две группы животных (голландские кроли, самцы, возраст три месяца, вес 2-2,4 кг). I группа из 8 животных с изменениями слизистой оболочки составила группу сравнения. II группа из 8 животных с изменениями слизистой оболочки ротовой полости получала озонотерапию. Проведено гистологическое исследование.

Микроскопическое исследование тканей показало, что озонотерапия уменьшает признаки воспаления, отека и способствует заживлению язвенных дефектов: наблюдается как исчезновение некробиотических процессов, эпителизация афтозных поражений, акантоз, выраженное уменьшение воспалительных клеток и изменение клеточного отношения - нейтрофилы с 38,30±2,46% до 6,34±0,63%, эозинофилы - с 5,49±0,23% до 2,87±0,05%, восстановление клеточных слоев эпителия, умеренно выраженный склероз сосочкового слоя собственной пластинки. Полученные результаты позволяют заключить, что озонотерапия способствует коррекции тканевых изменений при хроническом рецидивирующем афтозном стоматите.

რეზიუმე

პირის ღრუს ქსოვილზე ოზონოთერაპიის ზემოქმედება
ქრონიკული რეციდივირებული აფტოზური სტომატიტის დროს

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ქრონიკული რეციდივირებული აფტოზური სტომატიტი (ქრას) განეკუთვნება პირის ღრუს ლორწოვანი გარსის ქრონიკულ, ანთებით, წყლულოვან დაავადებათა რიცხვს. კვლევის მიზანს წარმოადგენდა პირის ღრუს რბილის ქსოვილების მორფოფუნქციურ თავისებურებებზე ოზონის ზეგავლენის განსაზღვრა ქრას მოდელირების პირობებში. ქრას დროს პირის ღრუს ლორწოვანი გარსის ქსოვილის მორფოფუნქციური მდგომარეობის შესწავლის მიზნით ჩატარებულია ექსპერიმენტული გამოკვლევა ადრე შემოთავაზებულ და ცნობილ მოდელზე ოვალბუმინის და ალუმინის ჰიდროქსიდის გამოყენებით 3 თვის მამრ ბაჭიებზე. ცხოველები დაყოფილი იყო ორ ჯგუფად: I (შედარების) ჯგუფი (8 ბაჭია) რებულობდა ქრას სააამკურანალო ტარდიციულ თერაპიას; II (ძირითადი) ასევე 8 ბაჭიისაგან შემდგარი ასევე პირის ღრუს ლორწოვანი გარსის ცვლილებებით დამატებით ღებულობდა ოზონოთერაპიას. შეამდეგ ჩატარდა ჰისტოლოგიური გამოკვლევა, რომელმად აჩვენა, რომ ოზონოთერაპია ამცირებს აბთების ნიშნებს, შეშუპებას და ხელს უწყობს წყლულოვანი დეფექტების შეხორცებას ხდება ნეკრობიოტიკური პროცედების ალაგება,აფტოზური დაზიანების ეპითელიზაცია,აკანტოზი, ანთებითი უჯრედების გამოხატული შემცირება, უჯრედული თანაფარდობის ცვლილება - ნეიტროფილების 38,30±2,46% დან 6,34±0,63% მდე, ეოზინოფილების 5,49±0,23% დან 2,87±0,05% მდე; ხორციელდება ეპითელიუმის უჯრედოვანი ფენების აღდენა.

EFFECT OF ARSENIC EXPOSURE ON BEHAVIOR OF RATS OF VARIOUS AGE GROUPS

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Arsenic (As) is ranked first among toxicants posing a significant potential threat to human health based on known or suspected toxicity [11,15]. Currently, the permitted concentration of arsenic in water is 10μ g/L (10 ppb). However, people worldwide are exposed to excessive amounts of arsenic via drinking water.

Several regions (including Lukhuni region of Ambrolauri district and Madneuli area in Bolnisi region) known for their reach As deposits, are characterized by significant accumulation of As in ground water. From 8.9 mg/L to13.8 mg/L As content was found in Adjara and Ambrolauri regions (Rioni river). The catastrophic amounts of soluble As ranging from 83-184 mg/L were found in Lukhunisckali river in Lukhuni region known for realgar (arsenic sulfide) and auripigment mining. During the active mining periods in 1980s, the measurements of As in the regions of rivers Lukhunisckali 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 Lukhunistskali 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 dia-