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MORPHOLOGICAL ASSESSMENT OF THE REGENERATION OF THE PERIODONTAL LIGAMENTUM IN THE TREATMENT OF GENERALIZED PERIODONTITIS WITH PLASMOGEL FROM PLATELET AUTOPLASMA

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The purpose of the investigation was to study the effect of Plasmogel from platelet autoplasm on the regeneration of collagen fibers of the periodontal ligament in an experimental study on rats with a ligature model of periodontitis. The animals were divided into 2 groups: the first – control of the healing of the pathology without treatment, the second – experimental. For morphological examination, tissue biopsies were taken, Mallory staining was used to determine the fibrous structures of connective tissue. On visual inspection, healing occurred in both groups. However, it didn't go the same way. In Group I, visual recession was noted. In Group II, the recession was not visually identified. In Group I of animals, signs of a chronic inflammatory process were detected, and stromal fibrosis was revealed. The largest area occupied by collagen fibers in the periodontal ligament was recorded in Group II of animals, which is 61.93%. In Group I animals, indicator of the area of collagen fibers significantly lower – 35.44%. Plasmogel is used to create a framework and microenvironment for the growth of collagen fibers of the periodontal ligament, which increases the regenerative capabilities.

Key words: biomaterials, periodontitis, ligature model, plasma therapy, collagen fibers.

Г.О. Вишнеvsька, С.А. Шнайдер, О.Е. Рейзвїх, Г.О. Бабеня, М.Т. Христова МОРФОЛОГІЧНА ОЦІНКА РЕГЕНЕРАЦІЇ ПЕРІОДОНТАЛЬНОЇ ЗВ'ЯЗКИ ПРИ ЛІКУВАННІ ГЕНЕРАЛІЗОВАНОГО ПАРОДОНТИТУ ПЛАЗМОГЕЛЕМ З ТРОМБОЦИТАРНОЇ АУТОПЛАЗМИ

Метою дослідження було вивчення впливу Плазмогеля з тромбоцитарної аутоплазми на регенерацію колагенових волокон періодонтальної зв'язки в експериментальному дослідженні на щурах з лігатурною моделлю пародонтиту. Тварини були розділені на 2 групи: перша – контроль загоєння патології без лікування, друга – експериментальна. Для морфологічного дослідження виконували забір фрагментів щелеп, використовували фарбування по Маллорі для визначення волокнистих структур сполучної тканини. При візуальному огляді загоєння відбулося в обох групах. В першій групі візуально відзначалася рецесія та ознаки хронічного запального процесу, виявлено фіброзування строми. У групі II рецесія не ідентифікувалась. Найбільша площа займана колагеновими волокнами в періодонтальній зв'язці зафіксована в II групі тварин, що становить 61,93%; в I групі – 35,44%. Плазмгель використовується для створення каркаса і мікросередовища для зростання власних колагенових волокон періодонтальної зв'язки, що підвищує її регенеративні можливості.

Ключові слова: біоматеріали, пародонт, лігатурна модель, плазмотерапія, колагенові волокна.

The work is a fragment of the research project: "Correction of pathogenetic mechanisms of metabolic disorders in the oral cavity tissues in patients depending on environmental and alimentary factors affecting carbohydrate and lipid metabolism", state registration No. 0118U006966.

In recent decades, generalized parodontitis occupies a leading position in the structure of dental morbidity. Parodontitis, one of the diseases of the oral cavity that has a high prevalence among all segments of the population. Severe periodontitis destroys periodontal tissues and leads to teeth loss [10], a sharp deterioration in the quality of life and disorders of general health. Patients with a history of parodontitis have an unfavorable prognosis for various systemic diseases, such as diseases of the cardiovascular system, cancer, and metabolic disorders [6, 9, 11]. Proceeding from the above, the search for effective and safe methods of parodontitis treatment is one of the urgent tasks of modern dentistry.

The development of therapy methods for the regeneration of parodontal tissues, including the alveolar bone, root cementum and the periodontal ligament continues [5]. At the moment, the "gold" standard of treatment is a multidisciplinary integrated approach. The main part of non-surgical treatment procedures is aimed at eliminating the etiological microbial factor (professional oral hygiene, smoothing the root surface, teaching individual oral hygiene, etc.). However, with the removal of parodontal pathogens and pathological tissues, only a small amount of parodontal tissues can be restored [10]. Using more complex surgical methods

of treatment, such as directed tissue regeneration, it is possible to restore soft tissues and alveolar bone, but the results are not always predictable and have satisfactory long-term treatment results. Regenerative techniques are also widely used to restore lost periodontal structures with preparations complex obtained from the patient's own plasma. This technique stimulates a better delivery of high concentrations of platelet growth factors, which affects the healing of both soft and bone tissues [13]. Modern biomaterials containing growth factors have improved regenerative techniques for the restoration of parodontal defects. However, the use of these materials in clinical practice is still controversial, and the regeneration of the parodontal complex is a difficult task for a specialist. These techniques require more detailed study in experimental researches to confirm their effectiveness at the cellular level [14]. Also, today, an active search continues for new conservative methods aimed at regeneration and sustention of metabolic balance in the parodontium [7]. Recognition of parodontal conservative therapy as a regenerative procedure requires demonstration of tissue repair of the parodontal complex.

The purpose of the study was to investigate the effect of plasmogel from platelet autoplasm on the regeneration of collagen fibers of the periodontal ligament in an experiment in rats.

Materials and methods. The protocol for experimental research using animals approved by the Commission on Bioethics of State Establishment "The Institute of stomatology and maxillo-facial surgery National academy of medical sciences of Ukraine" in accordance with the rules of the International Convention for the protection of vertebrate animals used for experimental and other scientific purposes (No.97 of 12.09.2018).

All manipulations with the animals were carried out under general anesthesia in a clean operating room in compliance with the "Rules for working with experimental animals".

For experimental studies, 24 white Wistar rats of herd breeding, of both sexes, 2.5-3 months of age, weighing 250-300g were used. All animals were kept on a standard vivarium diet. The animals were divided into 2 groups of 12 animals each.

In an experimental study, parodontal defects in all animals were modeled using a ligature model, by imposing a ligature on the maxillary incisor in the gingival sulcus for 14 days. After 14 days, all animals were removed from the ligatures and treated. Ligatures imitated the role of dental plaque and contribute to the accumulation of microbial biofilm [14].

In Group I (n=12), after removing the ligatures, the gums were treated with a gauze swab moistened with 0.9% NaCl solution, 2 times with an interval of 7 days. This group was the control for evaluation of natural healing.

In Group II (n=12), treatment was carried out by applying a plasmogel from platelet autoplasm to the gums, 2 times with an interval of 7 days. Plasmogel was applied to the area of pathologically altered tissues, covered with a parodontal "Reso – Pac" dressing, for 6 hours until the periodontal dressing was self-absorbed.

Plasmogel was obtained according to the following scheme: blood was taken from each rat from the tail vein in an amount of 2 ml, blood was collected in a test tube with 0.2 ml of heparin solution, centrifuged at 1000 rpm for 5 minutes, the resulting plasma fraction was taken from the test tube with a syringe, which was placed in a TDB-120 thermostat to prepare a plasmogel, at a temperature of +80° C for 7 minutes, then it was cooled at room temperature for 10 minutes.

For the purpose of morphological assessment of the regeneration of collagen fibers of the periodontal ligament, the rats were removed from the experiment by euthanasia 3 weeks after the second injection of the plasmogel. The animals were removed from the experiment under thiopental anesthesia (20 mg/kg), after which biopsies of the gums and jawbones were taken, fixed in 10% neutral formalin. Decalcification was performed in a commercial solution SoftDec (Biovitrum, Russia) according to the manufacturer's protocol. Samples were fixed in 10% formalin for 7-10 days with further washing in running water for 1 hour, placed in a decalcifying solution SoftiDec (Biovitrum) for 70-72 hours at a temperature of 4 ° C, then washed again with running water for 30 minutes and 70 ° alcohol for 4-5 days. After embedding in paraffin, serial sections with a thickness of 10 µm were made. The prepared preparations were stained with hematoxylin and eosin, according to Mallory, studied under a microscope with x200 and x400 magnifications. Mallory staining is used to study the fibrous structures of connective tissue, three dyes are used: aniline blue, sour fuchsin and orange. The method is based on the unique property of aniline blue to color collagen fibers in greenish-blue, and of acid fuchsin - elastic fibers in red. As a result of staining, collagen fibers are stained in a dark blue color, cell nuclei – in orange-red, etc.; method proposed by F. Mallory in 1900. Hematoxylin and eosin staining – overview tissue staining [2].

The results were processed by variational and statistical methods of analysis on an IBM PC in SPSS SigmaStat 3.0 and StatSoft Statistica 6.0 software [4].

Results of the study and their discussion. Studies have shown that modeling of parodontitis using a ligature model leads to pronounced inflammatory-dystrophic changes in parodontal tissues. After removing the ligatures, all animals of the studied groups showed: pronounced swelling of soft tissues, bleeding and the presence of loss of epithelial attachment.

On visual inspection, it can be noted that epithelialization occurred in both the first and second groups on days 7-10. The wound surface quickly healed and a pale pink mucosa was formed, while in Group I of animals, healing occurred with subsequent exposure of the roots of the teeth. In Group II of animals, at the healing stage, the gingival mucosa acquired the correct contour and had a tight fit to the surface of the teeth roots. There were no adverse animal behaviors or other treatment-related safety issues throughout the study.

Conducted morphological and morphometric studies showed that a stereotypical tissue reaction occurs in all groups of the studied animals. In experimental Group I, pronounced signs of a chronic inflammatory process were determined in the proper lamina of the gingival mucosa, fibrosis of the stroma was revealed (fig. 1) with the formation of dense bundles of collagen fibers, which stained blue according to Mallory.

Although there are also, some areas of significant expansion of the gap with a damage of the architectonics of the periodontal ligament (fig. 2) and replacement occurs mainly by bundles of randomly oriented collagen fibers, among which fibroblasts and fibrocytes are mainly determined.

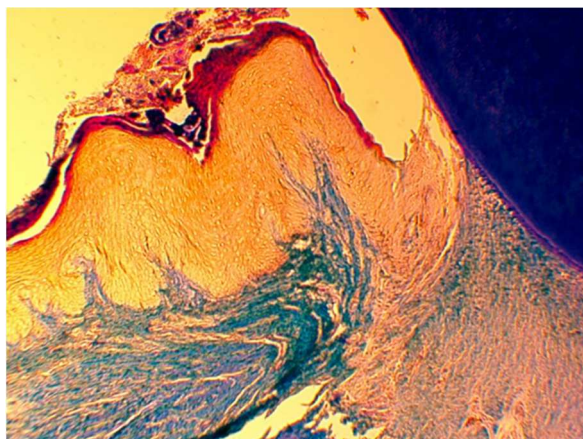


Fig. 1. Collagen fibers of the lamina propria of the gingival mucosa. Group I of animals. Mallory staining. x200.

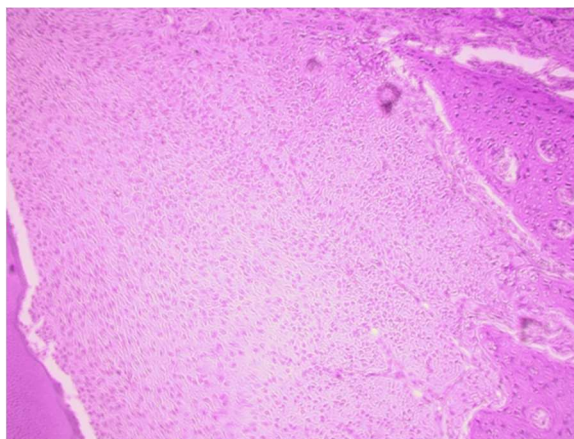


Fig. 2. Periodontal ligament. Group I of animals. Staining with hematoxylin and eosin. x200.

Determination of the area occupied by collagen fibers in the area of the periodontal ligament was carried out on preparations stained according to Mallory. In Group I, the indicator was $35.44\% \pm 6.42\%$ (the norm indicator is $77.9 \pm 0.57\%$ [3]) – the optical density is less than 0.001 c.u.

Changes in the architectonics of collagen fibers of the lamina propria can also be noted in Group II of animals. Collagen fibers form coarse bundles oriented mainly parallel to the surface of the stratified squamous keratinizing epithelium of the gingival mucosa (fig. 3). There is a slight increase in the number of vessels in comparison with the Group I of animals.

The histoarchitectonics of the direction of collagen fibers has slight deviations, cellular elements, such as fibroblasts, are well expressed and thickening of the blood vessel wall is also observed (fig. 4).

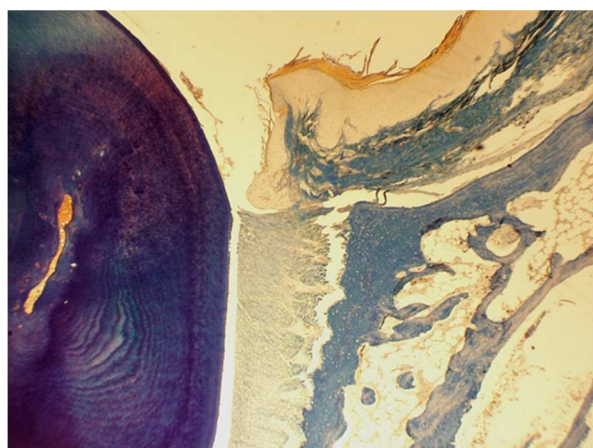


Fig. 3. Collagen fibers of the lamina propria of the gingival mucosa. Group II of animals. Mallory staining. x400.

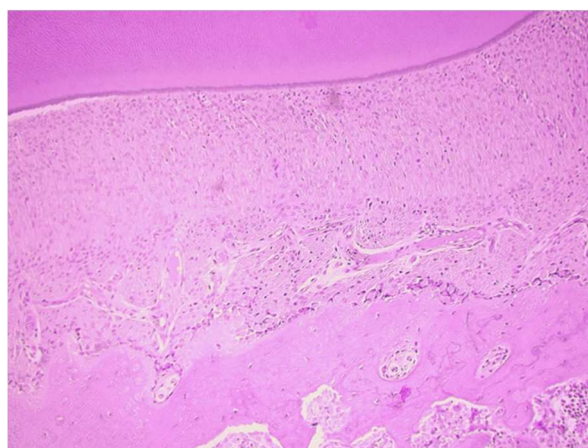


Fig. 4. Periodontal ligament. Group II of animals. Hematoxylin and eosin staining. x200.

The space occupied by collagen fibers was $61.93\% \pm 7.36\%$ in the area of the periodontal ligament. Thickening collagen fibers forming the periodontal ligament should be noted. The optical density of collagen fibers is 0.02 c.u.

Plasmogel is an autologous filler gel, the main component of which is the constituents of its own heat-treated plasma. The presence of active cellular structures in this gel is absent, but at the same time, it has a high density, adhesion and plasticity. When introduced into the periodontal pocket, this biomaterial fills the defect and closes access due to adhesion to the root surface and soft tissues for the aggressive environment of the oral cavity. Plasmogel is used to create a scaffold and microenvironment for the growth of collagen fibers of the periodontal ligament.

The changes in the architectonics of collagen fibers, an increase in the number of vessels and cellular elements in both study groups can be noted when analyzing the state of the tissues of the lamina propria of the gum mucosa.

In the Group I of animals, after healing, the gingival mucosa had a dense structure, but at the same time a decrease in the level of soft tissues surrounding the tooth root (gum recession) was expressed, which makes it possible to assume that the periodontal ligament was repaired. This healing result corresponds in clinical practice to the stage of plaque removal and anti-inflammatory therapy. However, in laboratory animals, after removing the ligatures and removing the retention point for microbial plaque, self-cleaning occurred due to the consumption of solid foods (carrots, cabbage, etc.) and specialized animal feed in the diet. Morphological examination reveals mild signs of a chronic inflammatory process in the lamina propria of the gingival mucosa. The presence of fibrosis of the stroma and the formation of collagen fibers in rough anastomosing bundles makes it possible to make an assumption about the formation of fibrosis of the periodontal ligament and the formation of a scar.

In the Group II of animals, healing after removal of the ligatures occurred without any peculiarities. On examination, in all animals, there was practically no decrease in the volume of soft tissues of the mucous membrane around the roots of the teeth, the gum has a dense structure, but during morphological examination, the histoarchitectonics of the direction of collagen fibers is slightly damaged. However, there is a thickening of collagen fibers in this group, which suggests a more active restoration of the periodontal ligament compared with the Group I.

The index of the area occupied by collagen fibers in the periodontal ligament cannot be restored by 100%, since the fibers of the periodontal ligament functionally transmit the chewing load and, accordingly, there is a daily balancing between the processes of fiber destruction and their restoration. For patients with pathology of parodontal tissues, this is also an important point, since destruction processes prevail in them over recovery processes. The largest area occupied by collagen fibers in the periodontal ligament was recorded in Group II of animals - 61.93%, which significantly exceeds the area of collagen fibers in Group I of animals - 35.44%.

The chosen pathology model makes it possible to evaluate the local systems of plasmogel delivery from platelet autoplasm at the conservative stage of treatment.

When evaluating the results of the study, it is necessary to take into account the fact that periodontal tissues are unique and represent a complicated complex of the surrounding tooth. One of the functions of the parodontium is support-shock-absorbing function, which helps to keep the tooth in the alveolus, distributing the chewing load and regulating the pressure when chewing. In different parts of the periodontal space, bundles of collagen fibers have a different direction, providing a strong connection between the tooth and the alveoli. Daily, due to the chewing load, collagen fibers are destroyed and formed in the periodontal ligament [1]. Parodontal tissue has a high potential to heal itself with proper treatment. For example, in the early stages of parodontitis, a plaque removal procedure can help restore parodontium [11]. The factors affecting parodontal regeneration are quite complex. The correct cell type and sufficient number of cells, a favorable microenvironment with biological signals and the right scaffold matrices are critical for regeneration. Previous studies talk about the ability of the periodontal ligament to regenerate when creating conditions for healing in the form of delimiting the gingival epithelium and connective tissue, using membranes (barriers), from the surface of the tooth root [8]. Biomaterials that have the properties of the patient's own tissues reduce the risks of immune rejection and have great opportunities for tissue regeneration [12]. It is also possible not only options in which biomaterials containing stem cells and growth factors are used, but also materials imitating the structure of the parodontal tissues themselves. This can help create a microenvironment for further regeneration. Evaluation of this method using the rabbit parodontal defect model showed that the cell-free construct induced complete tissue regeneration in the parodontium. [15].

Based on the foregoing, plasmogel can be attributed to biomaterials that have no risks for use in patients with a pronounced immune response, since there are practically no risks of rejection and allergic reactions. When injected locally, there was no local negative reaction to the injection in the studied animals. Also, importantly the gel is easy to use and has good plastic properties, does not spread and is well retained in the tissue defect area.

Sallum E, Ribeiro FV et al. in their research note that the modern concept of tissue engineering creates new opportunities in the field of parodontal tissue regeneration. In recent years, more and more fundamental research in the field of cell biology has been carried out. The creation of new biomaterials raise a great interest [14].

Tissue engineering is based on a combination of three key elements: cells, signaling molecules, and scaffolds or support matrices to repair lost tissue. The use of tissue engineering products can provide a large degree of influence on factors associated with the process of tissue healing, to achieve regeneration. In this context, the researchers [6] hypothesize that the biomaterial acts as a scaffold that holds cells that will form new tissues along with biological signaling molecules that instruct these cells to form the desired tissue type. In our study, a plasmogel acts as a kind of scaffold. It can be assumed that being an autologous material when injected into a parodontal defect, its own cells quickly contribute to its biodegradation, triggering a cascade of regenerative reactions in the parodontal tissues. However, in this study, we studied only the effect on the restoration of collagen fibers of the periodontal ligament, which suggests the regenerative potential of the plasmogel. In further studies, it is also necessary to study its effect on the components of the parodontal complex, such as the alveolar bone and cement of the tooth root.

Biomaterials containing mesenchymal stem cells are also very promising for further research. In a recent *in vitro* study, Silvério KG et al. [10], showed that although donor age may affect periodontal ligament cells, highly purified subpopulations of mesenchymal progenitor cells can be obtained from periodontal ligament cells in both temporary and permanent teeth. Today, in Ukraine, this kind of technologies is quite complicated, from the point of view of legislation, and expensive to manufacture, which makes them unavailable for widespread use by patients. Therefore, the plasmogel preparation can be considered as an alternative treatment option that requires low costs for the manufacture of the gel itself from platelet autoplasm. Thus, in a study by Edward J. Dougherty [13], he showed a scheme for calculating the cost of the procedure for treating a diabetic foot using a gel out of plasma enriched with platelet growth factors. The study showed a lower cost compared to other alternative treatment methods. Plasmogel preparation is also less expensive compared to other variants of regenerative methods in parodontology using osteoplastic materials and enamel matrix proteins.

To date, there are no published, peer-reviewed experimental studies demonstrating the effectiveness of the use of plasmogel preparation in parodontology, therefore, it is not possible to conduct a comparative analysis with the studies of other authors on the morphological structure of collagen fibers of the periodontal ligament during treatment with this technique.

Conclusions

On visual examination, healing occurred in both groups. However, it was not the same. In the Group I of animals, a recession was noted. In the second, the recession was not visually identified. Histological analysis showed that complete regeneration of collagen fibers of the periodontal ligament did not occur in any of the groups. The degree of regeneration of the periodontal ligament was higher in the group using the plasmogel preparation made out of platelet autoplasm 61.93% compared with the group without treatment 35.44%. If we follow the principles of tissue regeneration, the use of biomaterials, then the plasmogel preparation can be a matrix for creating a microenvironment and increasing the regenerative capabilities of the periodontal ligament. The technique is safe for use, does not cause allergic reactions and rejection, as it uses autologous material. The bioactive material has perspectives for further study both in experimental studies – the effect on the regenerative potential of the cells of the cement of the tooth root and alveolar bone, and in clinical studies of its use in the complex treatment of parodontitis.

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EFFECT OF THE NEW DENTAL ELIXIR ON THE CONDITION OF RATS' PERIODONTAL TISSUES WITH SIMULATED METABOLIC SYNDROME

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Biochemical changes in the blood serum, liver and gingival tissue were examined during simulation of the metabolic syndrome of alimentary genesis in experiment on 28 white male rats of reproductive age with an average body weight (280 ± 14) g. It was found that keeping animals for ten weeks on a diet high in saturated fats and carbohydrates led to increase in body weight and in level of biochemical markers: insulin resistance, hyperglycemia, inflammation, both at the level of the body and locally in the periodontal tissues. Local application of the new dental elixir in the form of applications to rats gums against the background of metabolic syndrome reduced the level of triglycerides, had an anti-inflammatory and antioxidant effect, reducing contamination by pathogenic microflora (urease activity), lipid peroxidation activity (malonic dialdehyde content), activating locally in the periodontal tissues the antioxidant defense enzyme and nonspecific immunity – lysozyme.

Key words: metabolic syndrome of gingival tissue, inflammation, treatment, dental elixir.

Л.С. Кравченко, О.Л. Аппельханс, А.Є. Поляков, О.В. Пасечник, С.В. Щербаков ВПЛИВ НОВОГО ЗУБНОГО ЕЛІКСИРУ НА СТАН ТКАНИН ПАРОДОНТУ ЩУРІВ З ВІДТВОРЕНИМ МЕТАБОЛІЧНИМ СИНДРОМОМ

В експерименті на 28 білих щурах-самцях репродуктивного віку, середньою масою тіла 280 ± 14 г охарактеризовані біохімічні зміни у сироватці крові, печінці і ясеневій тканині при відтворенні метаболічного синдрому аліментарного генезу. Було встановлено, що утримання тварин протягом десяти тижнів на раціоні з високим вмістом насичених жирів і вуглеводів призводило до збільшення маси тіла та підвищення вмісту біохімічних маркерів інсулінорезистентності, гіперглікемії, запалення, як на рівні організму, так і локально в тканинах пародонту. Місцеве застосування новоствореного зубного еліксиру у вигляді аплікацій на ясна щурів на тлі метаболічного синдрому знижувало рівень тригліцеридів, надавало протизапальну і антиоксидантну дію, знижуючи обсіменіння патогенною мікрофлорою (активність уреаз), активність перекисного окислення ліпідів (вміст малондеальдегіду), активуючи локально в тканинах пародонту фермент антиоксидантного захисту каталазу і неспецифічного імунітету лізоциму.

Ключові слова: метаболічний синдром, тканини ясни, запалення, лікування, зубний еліксир.

The work is a fragment of the research project "Development of new therapeutic and prophylactic agents and pathogenetic justification of their use in inflammatory periodontal diseases against the background of metabolic syndrome", state registration No. 0120U002197.

The influence of pathogenetic changes, that accompany pathological conditions with metabolic disorders, on the formation of inflammatory diseases in the oral cavity, the peculiarities of their course are currently insufficiently studied. It is known that the development of inflammation on the periodontium tissues, the mucous membrane of the oral cavity is associated with systemic processes of an organism, which is characterized by inflammatory manifestations [1]. One of such condition is the metabolic syndrome (MS), which is characterized by various triggers of the disorders cascade (fat, carbohydrate metabolism, vascular endothelial status) with known components: insulin resistance, visceral obesity, the dyslipidemia syndrome, impaired glucose tolerance [2, 6].