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STUDY OF MARKERS OF ANTIOXIDANT PROTECTION OF THE ORAL CAVITY IN THE ORAL FLUID OF PATIENTS WITH PERI-IMPLANTITIS AGAINST THE BACKGROUND OF THE TREATMENT AND PREVENTION COMPLEX

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The study was devoted to researching the effect of a therapeutic and prophylactic complex on biochemical markers of bone tissue in rats with peroxidative periodontitis and protein deficiency. The experiment included 30 Wistar male rats divided into three groups: an intact group, a group with combined pathology, and a group with combined pathology receiving the therapeutic and prophylactic complex. In the bone tissue homogenates of the animals, the activity of alkaline and acid phosphatases was determined as markers of osteoblastic and osteoclastic activity, respectively. The therapeutic and prophylactic complex led to a significant increase in the mineralization index and a reduction in alveolar bone atrophy, exceeding the results observed in the group with untreated combined pathology. This improvement in bone tissue indicators suggests the therapeutic complex's potential to restore bone formation processes and suppress resorptive activity, highlighting its applicability for preventing bone tissue degradation under conditions of nutritional deficiencies and oxidative stress.

Key words: periodontitis, bone tissue, rats, experiment, biochemical markers.

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ЕКСПЕРИМЕНТАЛЬНА ОЦІНКА МАРКЕРІВ КІСТКОВОЇ ТКАНИНИ ЩУРІВ НА ТЛІ ПЕРЕКИСНОГО ПАРОДОНТИТУ, АЛІМЕНТАРНОГО ДЕФІЦИТУ БІЛКУ ТА ЛІКУВАЛЬНО-ПРОФІЛАКТИЧНИХ ЗАХОДІВ

Дослідження було присвячено вивченню впливу лікувально-профілактичного комплексу на біохімічні маркери кісткової тканини у щурів з пероксидним пародонтитом та білковою недостатністю. В експерименті взяли участь 30 щурів-самців лінії Вістар, яких поділили на три групи: інтактну, групу з поєднаною патологією та групу з поєднаною патологією, які отримували лікувально-профілактичний комплекс. У гомогенатах кісткової тканини тварин визначали активність лужної та кислої фосфатаз як маркерів остеобластичної та остеокластичної активності відповідно. Лікувально-профілактичний комплекс призвів до достовірного підвищення індексу мінералізації та зменшення атрофії альвеолярної кістки, перевищуючи результати, що спостерігалися в групі з нелікованою поєднаною патологією. Таке покращення показників кісткової тканини свідчить про потенціал лікувально-профілактичного комплексу щодо відновлення процесів кісткоутворення та пригнічення резорбтивної активності, що підкреслює його придатність для профілактики деградації кісткової тканини в умовах аліментарної недостатності та оксидативного стресу.

Ключові слова: пародонтит, кісткова тканина, щури, експеримент, біохімічні маркери.

The work is a fragment of the research project "Improving the prediction of the occurrence and course of dental caries and periodontal disease, schemes for their prevention and treatment", state registration No. 0121U114672.

The study of periodontal tissue disorders, particularly periodontitis, has gained increasing relevance due to its complex pathogenesis and widespread prevalence globally. Periodontitis is a multifaceted disease that affects the supporting structures of teeth, with implications extending beyond oral health to systemic conditions, such as cardiovascular disease and metabolic disturbances [7]. Despite extensive research, the intricate biochemical and cellular mechanisms underlying periodontitis remain partially understood, especially in cases where periodontal pathology is compounded by additional nutritional deficiencies [4, 8]. Addressing this gap, experimental models have been instrumental in exploring the effects of periodontitis, facilitating the identification of key biochemical markers and potential therapeutic targets. These models are invaluable for examining the impact of dietary factors, such as protein deficiency, on periodontal disease progression [6, 10]. However, the consequences of combined periodontitis and nutritional deficiency on bone tissue biomarkers have not been fully elucidated, particularly regarding the synergistic effects that may exacerbate bone atrophy and disrupt homeostasis.

In light of these knowledge gaps, this study was designed to explore the effects of a combined pathology model incorporating peroxidative periodontitis and protein deficiency in rats. By simulating both periodontal disease and protein malnutrition, this model aims to provide insights into the biochemical changes that occur in periodontal bone tissue under these conditions. Alkaline and acid phosphatases, key enzymes involved in bone metabolism, serve as vital indicators of bone formation and resorption processes,

respectively [8]. Previous studies have highlighted the significance of these markers in distinguishing osteoblastic and osteoclastic activities in periodontitis, though investigations combining these markers with nutritional deficiency remain limited [4, 6, 9]. Understanding these dynamics is critical, as the delicate balance between bone resorption and formation directly influences the integrity of periodontal tissue, which is essential for preventing progressive bone loss and supporting long-term periodontal health.

The novelty of this research lies in the use of a comprehensive therapeutic and prophylactic complex (TPC) that targets the biochemical imbalances associated with combined periodontal and nutritional pathology. By examining the effectiveness of this TPC, which includes agents aimed at both osteoblast support and osteoclast inhibition, this study seeks to contribute to the development of new treatment strategies for periodontitis in complex nutritional contexts. Through a systematic evaluation of bone tissue markers and morphological parameters, we aim to clarify the potential for TPCs to mitigate periodontal destruction and promote bone regeneration in settings of dietary insufficiency [9].

The purpose of the study was to evaluate the effect of the drug complex on biochemical markers of rat periodontal bone tissue-mineralisation index and degree of alveolar atrophy against the background of modelling peroxidative periodontitis and alimentary protein deficiency.

Materials and methods. Experimental studies were conducted using 30 male rats of 1 month of age, with an average weight of 60–75 g, of the Wistar line of herd breeding, which was chosen as a model for the study, which is one of the most common lines of laboratory rats for experimental studies. The animals were kept in normal vivarium conditions under natural light and with free access to water and food. Throughout the experiment, the microclimatic conditions of the vivarium environment were strictly observed: temperature (19–23°C) and humidity (50–75 %). Experimental studies were conducted at the Laboratory of Biochemistry and Vivarium of the SE "The Institute of stomatology and maxilla-facial surgery National academy of medical sciences of Ukraine" (SE "ISMFS NAMS"). All experiments on rats were conducted according to standard operating procedures approved by SE "ISMFS NAMS", developed in accordance with the Guidelines of the Pharmacological Committee of the Ministry of Health of Ukraine and the International Regulations for the Use of Laboratory Animals [2, 5].

The animals were divided into 3 groups as follows:

– 1st group – intact, n=10;

– 2nd group – modelling of peroxidative periodontitis and alimentary protein deficiency (combined pathology), n=10;

– 3rd group – combined pathology + TPC, n=10.

Animals in the intact group received balanced feed that fully covered their daily requirements for nutrients, vitamins, minerals and trace elements, as well as disinfected and reverse osmosis-filtered water with free access.

A model of combined pathology – alimentary protein deficiency in rats of the 2nd and 3rd groups was modeled by transferring animals to a diet deficient in proteins, namely essential amino acids (corn – 73.5 %, beetroot – 14.7 %, cabbage – 11.8 %), and these groups were also modelled with experimental periodontitis by adding peroxidised sunflower oil to the daily diet at the rate of 1 ml per animal per day for 60 days. The peroxidised oil was obtained by heating refined sunflower oil in the presence of 2 % $CuSO_4$ for 8–10 hours until the peroxide number reached more than 35 units. The development of the experimental model of periodontitis was based on the modern concept of the development of the disease in humans.

The duration of the experiment was 60 days. Animals were withdrawn from the experiment by an overdose of intraperitoneal anaesthesia using sodium thiopental (at a rate of 40 mg/kg) on day 60 of the experiment by total bleeding from the heart. [1]. Blocks of jaws with teeth were prepared for biochemical studies. In periodontal bone homogenates (75 mg/ml citrate buffer), the activity of alkaline phosphatase (ALP; osteoblast marker) and acid phosphatase (AP; osteoclastic activity marker) was determined. Mineralisation index (MI) was calculated by the ratio of ALP/AP activity. The degree of atrophy of the alveolar process was determined on skeletal lower jaws of rats by the method of Nikolayeva [1].

The results were processed by variational statistical methods of analysis using the Microsoft Office Excel 2016 software. Statistical processing of the experimental study results was carried out by the methods of variation analysis using the Student's test. The difference was considered statistically significant at $p<0.01$ [3].

Results of the study and their discussion. To gain a comprehensive understanding of the impact that peroxidative periodontitis combined with protein deficiency has on bone metabolism and structure, we conducted an extensive analysis of key biochemical markers and morphological changes in the rats' periodontal tissues. We focused on measuring the activities of alkaline phosphatase and acid phosphatase, which are critical enzymes involved in bone formation and resorption, respectively. These enzymes serve as vital indicators of osteoblastic and osteoclastic activities, helping us assess the balance between bone

deposition and degradation under pathological conditions. Additionally, we evaluated the degree of alveolar bone atrophy to quantify the structural damage inflicted by the combined pathology and to determine the efficacy of the therapeutic and prophylactic complex in mitigating these adverse effects. The detailed results of these assessments are presented in Table 1.

Table 1

Effect of the therapeutic and prophylactic complex of drugs on the activity of acid and alkaline phosphatases in bone tissue and atrophy of alveolar bone in rats with combined pathology, M±m

Indices / Groups	AP activity, μ-kat/kg	ALP activity, μ-cat/kg	Mineralisation index, ALP/AP	Atrophy of the alveolar process, %
Intact, n=10	7.15±0.36	58.31±2.52	8.15±0.24	29.3±1.1
Combined pathology, n=10	9.27±0.61 p<0.01	39.20±4.21 p<0.002	4.22±0.18 p<0.001	37.3±1.8 p<0.002
Combined pathology + TPC, n=10	7.40±0.44 p>0.2 p_1<0.01	61.32±2.14 p>0.4 p_1<0.001	8.28±0.29 p>0.7 p_1<0.001	28.2±1.6 p>0.5 p_1<0.02

Note.p – significance of differences to the intact group; p_1 – significance of differences to the "combined pathology" group.

Bone tissue is a unique structure that functions in close connection with other organs and systems. In the context of the development of a pathological process, the pathogenetic pathways of which 'cross' at any level of the skeletal system, bilateral contact is possible with the formation of appropriate reactions from each of them. Bone metabolism is maintained by the balance of bone resorption and formation processes, which reflect changes in bone turnover. Biochemical markers of bone formation include proteins that reflect the activity of osteoblasts, matrix proteins, collagen synthesis by-products and osteoblast enzymes.

Alkaline and acid phosphatases are enzymes involved in bone mineralisation. Alkaline phosphatase catalyses the transfer of phosphoric acid ions from phosphoesters to the components of the organic bone matrix. ALP is localised in osteoblasts and is able to release inorganic phosphate from organic phosphoesters, which is used for the formation and precipitation of calcium phosphate. Acid phosphatase is synthesised by osteoclasts and is involved in bone resorption.

Experimental studies have shown that prolonged (for 2 months) nutritional excess of lipid peroxides and nutritional deficiency of protein in experimental rats leads to significant changes in bone tissue. Under the conditions of modelling the combined pathology, the degree of atrophy of the alveolar process of the jaws of animals of group 2 significantly increased by 27.3 % ($p<0.002$) compared with the intact group. These changes indicate an increase in destructive and resorptive processes in periodontal bone tissue under the influence of lipid peroxides, protein and calcium deficiency.

The presented data indicate that under the conditions of modelling the combined pathology in rats of group 2, the activity of alkaline phosphatase, a marker of osteoblasts, significantly decreased by 32.7 % ($p<0.002$) in the alveolar bone of the jaws of animals. At the same time, an increase in the osteoresorption marker, acid phosphatase activity, which reflects the destruction of hydroxyapatite in bone tissue, was observed by 29.6 % ($p<0.01$) compared to the intact group of experimental animals. At the same time, the degree of bone remodelling, determined by the ratio of ALP/AP activity, which indicates the predominance of one of the components of the bone remodelling process – resorption or osteogenesis against the background of combined pathology, decreased by 18.2 % ($p<0.001$), compared to the intact group, indicating an increase in bone resorption.

Thus, it should be noted that the modelling of combined pathology (peroxidative periodontitis and nutritional deficiency of protein and calcium) leads to inhibition of the main indicators of bone formation: increased destructive and resorptive processes, destruction of hydroxyapatite, inhibition of osteogenesis, increased functional activity of osteoclasts.

Under the conditions of TPC exposure against the background of modelling the combined pathology in animals of group 3, bone formation indicators significantly improved. Thus, a significant decrease in acid phosphatase by 20.2 % ($p_1<0.01$), an increase in alkaline phosphatase activity by 56.4 % ($p_1<0.001$), and a significant increase in the degree of bone remodelling by 96.2 % ($p_1<0.001$) were recorded in animals of group 3, compared to the intact group. Also, this complex of drugs helps to inhibit destructive processes in periodontal bone tissue, as evidenced by a significant decrease in the degree of atrophy of the alveolar process of the lower jaw of rats by 24.3 % ($p_1<0.02$) compared with the intact group, and by 3.8 % ($p>0.5$) this indicator was lower than that of the intact group.

In our study, the model of combined pathology, encompassing peroxidative periodontitis and protein deficiency, yielded significant findings on biochemical and structural markers in periodontal bone tissue, presenting notable deviations in alkaline and acid phosphatase activities and mineralization indices.

This is consistent with prior research, which has shown that periodontitis alone triggers oxidative stress and heightens osteoclastic activity, leading to progressive alveolar bone loss. Similar studies have demonstrated that the presence of nutritional deficiencies, especially protein, exacerbates these destructive mechanisms by limiting the resources necessary for osteogenesis and repair processes, further shifting the balance toward bone resorption. Our data revealed a marked decrease in alkaline phosphatase activity and an increase in acid phosphatase activity in the untreated combined pathology group, aligning with established findings on the effects of oxidative stress on periodontal tissues and supporting the understanding that protein deficiency may amplify the resorptive processes already induced by periodontitis [6, 7, 8]. Interestingly, our results further emphasize that the therapeutic and prophylactic complex effectively counteracted these deleterious effects, as evidenced by a significant restoration of alkaline phosphatase levels and reduction in acid phosphatase activity in the TPC-treated group. This outcome corresponds with previous studies indicating that targeted therapies can mitigate the impact of periodontitis by fostering a more favourable biochemical environment for bone preservation and regeneration. Additionally, the observed improvement in mineralization index with TPC treatment highlights the potential of complex therapeutic approaches to support osteoblastic function, a finding that resonates with the efficacy of similar interventions reported in the literature [4, 9, 10]. Given the multifactorial nature of periodontitis, which involves an interplay of microbial infection, host immune response, oxidative stress, and nutritional status, further research should aim to explore the long-term effectiveness of TPCs in diverse pathological contexts. This includes investigating the efficacy of TPCs under varying degrees of nutritional deficiency, different levels of oxidative stress, and in combination with other systemic conditions that may affect periodontal health. Such studies would provide valuable insights into the adaptability and potential applications of TPCs across a broad spectrum of clinical scenarios. This comprehensive approach would not only deepen our understanding of the pathophysiology of combined periodontal and nutritional pathology but also contribute to the development of more refined, clinically applicable therapeutic strategies. By tailoring interventions to address the specific underlying mechanisms of disease in individual patients, clinicians can improve patient outcomes and potentially reduce the prevalence and severity of periodontitis-related bone loss. Ultimately, this could lead to better management of periodontal diseases and enhance the quality of life for those affected.

Conclusions

1. Combined peroxidative periodontitis and alimentary protein deficiency induce significant alterations in bone metabolism, characterized by increased osteoclastic activity and decreased osteoblastic activity. This is evidenced by a 29.6 % increase in acid phosphatase activity, a 32.7 % decrease in alkaline phosphatase activity, an 18.2 % reduction in the mineralization index, and a 27.3 % increase in alveolar bone atrophy compared to the intact group. These changes indicate enhanced bone resorption, inhibited osteogenesis, and elevated destructive processes in periodontal bone tissue under the modeled pathological conditions.

2. In rats receiving TPC, there was a 20.2 % decrease in acid phosphatase activity and a 56.4 % increase in alkaline phosphatase activity compared to the combined pathology group. The mineralization index improved by 96.2 %, and alveolar bone atrophy decreased by 24.3 %. These findings suggest that TPC effectively enhances osteoblastic activity, suppresses osteoclastic activity, and promotes bone formation, thereby inhibiting destructive processes in periodontal bone tissue.

3. The therapeutic and prophylactic complex demonstrates potential efficacy in restoring bone tissue homeostasis in the context of peroxidative periodontitis and protein deficiency. The normalization of biochemical markers and morphological parameters toward levels observed in the intact group indicates that TPC may serve as a viable intervention for preventing and treating bone tissue damage associated with these pathological conditions. Further research is warranted to explore the clinical applicability of TPC in managing periodontal bone disorders.

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EFFECT OF NEUROPROTECTIVE THERAPY WITH CITICOLINE ON THE COURSE OF EXPERIMENTAL NON-INFECTIOUS ANTERIOR AND INTERMEDIATE UVEITIS

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Non-infectious anterior and middle uveitis is a significant uveitis group, leading to decreased visual acuity and often blindness. The purpose of the study was to determine the features of the clinical course and morphological changes of the choroid, retina, and optic nerve in the early and late stages of experimental non-infectious anterior and middle uveitis under the conditions of neuroprotective citicoline therapy. We studied the clinical course of the disease and morphological changes on the 10–13th, and 33rd days after the administration of the provocative dose. The use of citicoline led to a decrease in inflammation in uveitis. First of all, due to reduced swelling of the cornea and iris. It was confirmed by histomorphological examination. It reduced the violation of the retinal cytoarchitectonic disturbances and degenerative and destructive changes in its neurons. It also reduced the swelling of the retina and optic nerve cells. It activated intracellular compensatory processes in the retina, particularly in the pigment epithelium and optic nerve glial cells.

Key words: anterior uveitis, intermediate uveitis, experimental animal model, choroid, retina, optic nerve, citicoline.

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ВПЛИВ НЕЙРОПРОТЕКТОРНОЇ ТЕРАПІЇ ЦИТІКОЛІНОМ НА ПЕРЕБІГ ЕКСПЕРИМЕНТАЛЬНОГО НЕІНФЕКЦІЙНОГО ПЕРЕДНЬОГО ТА СЕРЕДНЬОГО УВЕЇТУ

Неінфекційні передні та середні увеїти – це значна група увеїтів, що призводить до зниження гостроти зору, а нерідко і до сліпоти. Метою дослідження було визначити особливості клінічного перебігу та мофрологічних змін хоріоідеї, сітківки і зорового нерву на ранніх та пізніх строках перебігу експериментального неінфекційного переднього і середнього увеїту за умов нейропротекторної терапії цитіколіном. Вивчався клінічний перебіг захворювання та мофрологічні зміни на 10–13 та 33 добу після введення провокуючої дози. Застосування цитіколіну призвело до зменшення виразності запалення при увеїті. В першу чергу за рахунок зменшення набряку рогівки та райдужної оболонки. Що підтверджується за допомогою гістоморфологічного дослідження. Це зменшення порушення цитоархітектоніки сітківки, дегенеративних та деструктивних змін її нейронів, зменшення набряку клітин сітківки і зорового нерва; активація внутрішньоклітинних компенсаторних процесів в сітківці, зокрема, в клітинах пігментного епітелію, а також в гліальних клітинах зорового нерву.

Ключові слова: передній увеїт, середній увеїт, експериментальна тваринна модель, судинна оболонка, сітківка, зоровий нерв, цитіколін.

The work is a fragment of the research project "To study the effectiveness of the neuroprotective effect of pyrimidine nucleotides on retinal ganglion cells and optic nerve axons in patients with endogenous anterior uveitis", state registration No. 0119U101224.

Uveitis is a common, vision-threatening inflammatory eye disease with many clinical manifestations. The prevalence of different types of uveitis depends on many factors, such as age, sex, race, geographic distribution, environmental exposure, genetics, and social habits [11]. According to etiology, it is divided into infectious uveitis – more common (30–60 %) in developing countries, non-infectious – this is a significant group of uveitis arising from systemic diseases of the body (about 40 %) – more common in developed countries of the world [11].