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FEATURES OF THE DEGREE OF MINERALISATION IN THE PERIODONTAL BONE TISSUE OF RAT PERIODONTAL BONE TISSUE UNDER CONDITIONS OF PEROXIDATION-INDUCED PERIODONTITIS, DIETARY PROTEIN DEFICIENCY, AND THERAPEUTIC-PREVENTIVE MEASURES

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The study was devoted to the researching of the effect of the therapeutic and prophylactic complex on the degree of mineralisation of periodontal bone tissue in rats with peroxidative periodontitis and protein deficiency. The experiment involved 30 male Wistar rats divided into three groups: intact, a group with combined pathology and a group with combined pathology, which received the therapeutic and prophylactic complex. Protein content and calcium level were determined in periodontal bone homogenates, and the degree of mineralisation was calculated from the ratio of calcium to protein content. A marked reduction in bone destruction accompanied by stabilisation of lysosomal membranes and improved mineralisation in rats treated with the complex highlights the potential application of this therapeutic strategy in clinical settings. By eliminating oxidative stress and nutrient deficiencies, the complex may play a key role in preventing osteopenic changes and improving periodontal tissue health in patients at high risk of combined pathologies.

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

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ОСОБЛИВОСТІ СТУПЕНЮ МІНЕРАЛІЗАЦІЇ КІСТКОВОЇ ТКАНИНИ ПАРОДОНТА ЩУРІВ НА ТЛІ ПЕРЕКИСНОГО ПАРОДОНТИТУ, АЛІМЕНТАРНОГО ДЕФІЦИТУ БІЛКУ ТА ЛІКУВАЛЬНО-ПРОФІЛАКТИЧНИХ ЗАХОДІВ

Дослідження було присвячено вивченню впливу лікувально-профілактичного комплексу ступень мінералізації кісткової тканини пародонта 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. В гомогенатах кісткової тканини пародонта визначали вміст білка та рівень кальцію, а по співвідношенню рівня кальцію до вмісту білка розраховували ступень мінералізації. Помітне зменшення деструкції кісткової тканини, що супроводжується стабілізацією лізосомальних мембран та покращенням мінералізації у щурів, які отримували комплекс, підкреслює потенційне застосування цієї терапевтичної стратегії в клінічних умовах. Усуваючи окислювальний стрес та дефіцит поживних речовин, комплекс може відігравати ключову роль у запобіганні остеопенічним змінам та покращенні здоров'я тканин пародонта у пацієнтів з високим ризиком комбінованих патологій.

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

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Periodontitis is a multifactorial inflammatory disease that affects the supporting structures of the teeth, ultimately resulting in alveolar bone loss if left untreated [6, 9]. Recent studies emphasize that both local and systemic factors contribute significantly to periodontal tissue destruction, with an increasing focus on the role of oxidative stress, nutritional deficiencies, and shifts in microbiota composition [8, 10, 12]. In particular, the interplay between dietary protein deficiency and oxidative damage appears to be a critical risk factor for the initiation and progression of periodontitis, as a lack of essential amino acids compromises collagen synthesis, while peroxidation products accelerate inflammatory responses and disrupt bone remodeling [2, 11].

Alimentary protein deficiency can provoke systemic changes that impair the healing potential of periodontal tissues, compromising the structural and functional integrity of the bone [11]. Likewise, peroxidized lipids—generated from prolonged exposure to elevated temperatures and transition metals—exert cytotoxic effects, diminishing bone density and exacerbating inflammatory conditions [10]. Although the detrimental impact of malnutrition and oxidative stress on periodontal health is well recognized, there remains a paucity of studies investigating combined peroxidation-induced periodontitis and dietary protein deficiency in an experimental model. Such a dual-insult scenario mirrors certain real-world situations in

which individuals consume nutrient-poor diets and frequently ingest heated or rancid fats, thereby heightening their susceptibility to bone tissue damage and periodontal disease.

Addressing these gaps is clinically relevant and scientifically novel. By establishing an experimental framework that reproduces both protein deficiency and lipid peroxidation, researchers can more accurately simulate the complex pathophysiology seen in advanced periodontal disease. Moreover, identifying and testing potential therapeutic and prophylactic complexes (TPC) in this context may yield new preventive and treatment strategies, particularly for high-risk groups. Therefore, this study aimed to evaluate the effect of a specialized drug composition on key biochemical parameters of bone tissue mineralization in rat periodontal tissues under conditions of peroxidation-induced periodontitis and dietary protein deficiency. The findings reported herein could guide future translational research and clinical protocols aimed at mitigating bone loss and improving periodontal health in compromised patient populations.

The purpose of the study was to evaluate the effect of the drug complex on biochemical markers of rat periodontal bone tissue – degree of mineralisation 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 [3, 7].

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. The protein content and calcium level were determined in periodontal bone homogenates (75 mg/ml citrate buffer), and the degree of mineralisation was calculated from the ratio of calcium (in grams) to protein (in grams) [2].

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 $p<0.01$ [4].

Results of the study and their discussion. Table 1 presents quantitative data on calcium levels, protein content, and the degree of mineralization in the periodontal bone tissue of the experimental animals.

As demonstrated by the data in Table 1, the second group of animals—those with induced combined pathology—exhibited a pronounced decline in both calcium and protein levels compared to the intact group. Specifically, the mean calcium concentration decreased by 30.9 % (from 59.8±2.2 to

41.3±2.7 mmol/L; p<0.001), while the protein content dropped by 27.4 % (from 24.4±1.4 to 17.8±1.1 g/kg; p<0.002). Although the degree of mineralization—calculated as the ratio of calcium level to protein content—showed a relatively modest decrease of 6.0 % (from 2.47±0.14 to 2.32±0.12), this reduction nonetheless suggests a disruption in normal bone mineralization processes within the periodontal tissues.

Table 1

Protein content, calcium concentration, and degree of mineralization of rat periodontal bone tissue under conditions of combined pathology and following prophylaxis, M±m

Indicators / Groups	Calcium concentration, mmol/L	Protein content, g/kg	Degree of mineralization (Ca/protein), g/g
Intact, n=10	59.8±2.2	24.4±1.4	2.47±0.14
Combined pathology, n=10	41.3±2.7 p<0.001	17.8±1.1 p<0.002	2.32±0.12 p>0.5
Combined pathology + TPC, n=10	62.3±3.4 p>05 p_1<0.001	22.9±1.5 p>0.5 p_1<0.02	2.72±0.13 p>0.25 p_1<0.05

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

These findings underscore the adverse impact of dietary protein and calcium deficiencies, compounded by the prolonged administration of peroxidized oil (with a peroxide value reaching 35 units), on bone homeostasis. Together, these factors appear to impair collagen synthesis—a critical protein matrix component—and reduce hydroxyapatite deposition, ultimately contributing to osteopenic changes in the jaw bones.

In marked contrast, daily administration of the therapeutic and prophylactic composition (TPC) in the third group yielded a substantial improvement in all measured parameters. The calcium level rose by 50.8 % (to 62.3±3.4 mmol/L; p_1<0.001), the protein content increased by 28.7 % (to 22.9±1.5 g/kg; p_1<0.001), and the mineralization index improved by 17.2 % (to 2.72±0.13; p_1<0.05) relative to the second group. These improvements not only reflect an enhancement of collagen synthesis and bone mineral accrual but also point to more effective stabilization of the structural integrity of periodontal bone tissue.

Overall, analysis of these experimental findings indicates that 60-day, regular administration of TPC significantly mitigated the development of pathological processes in the periodontal bone tissue under conditions of periodontitis and dietary protein deficiency. The protective effects are evidenced by reduced bone tissue destruction, stabilization of lysosomal membranes, and enhanced mineralization in periodontal bone tissue, highlighting the potential of this therapeutic approach in preventing and managing osteopenic manifestations in similar combined pathologies.

Calcium is the primary structural component of bones and teeth, exhibits high biological activity, and performs a wide range of functions in the body, including the regulation of intracellular processes, control of cell membrane permeability, regulation of nerve conduction and muscle contraction, maintenance of stable cardiac activity, formation of bone tissue, mineralization of teeth, and participation in blood clotting. Proteins serve as the body's fundamental building blocks. Collagen is a complex protein belonging to the group of glycoproteins; it has a quaternary structure and a molecular weight of approximately 300 kDa. Collagen constitutes 30 % of the total protein in the human body and is characterized by significant microheterogeneity. It is synthesized within various connective tissue cells fibroblasts, osteoblasts, chondroblasts, odontoblasts, and others on ribosomes attached to the rough endoplasmic reticulum (RER) membranes in the form of preprocollagen, which contains an N-terminal signal sequence of approximately 100 amino acid residues [5]. It has been established that the first step in pathological resorption is the degradation of collagen and the intercellular matrix of the periodontal ligament, followed by destructive processes affecting the alveolar bone. The balance between connective tissue synthesis and destruction shifts toward its breakdown first, due to the suppression of fibroblast function and, second, owing to the heightened activity of macrophages, which are capable of destroying the body's own cells [1]. The findings of this study align with previous reports on the multifactorial nature of periodontitis, especially regarding the roles of oxidative stress and nutritional deficits in exacerbating alveolar bone loss [6, 9]. Other investigations have likewise highlighted that insufficient intake of key nutrients disrupts collagen synthesis and hampers bone remodeling, mirroring the protein deficiency effects observed here [11]. In addition, there is growing evidence that peroxidation products, whether introduced externally or formed endogenously, not only weaken the structural integrity of periodontal bone but also intensify inflammatory pathways [10, 12]. The demonstrated success of daily therapeutic-prophylactic composition (TPC) administration in our experiment, which effectively mitigated these pathologic

processes, resonates with the concept that targeted interventions can bolster host defense, enhance collagen matrix formation, and maintain normal bone mineral density in the presence of combined challenges. Notably, the beneficial impact of such compositions holds promise for broader clinical applications, including scenarios where chronic inflammatory conditions and coexisting systemic disorders converge.

Conclusions

1. Under peroxidation-induced periodontitis and alimentary protein deficiency, the rats demonstrated a 30.9 % reduction in calcium concentration and a 27.4 % drop in protein content compared to the intact controls.

2. Daily administration of TPC substantially improved bone tissue parameters over the 60-day period, increasing calcium levels by 50.8 % and protein content by 28.7 %. The mineralization index rose by 17.2 %, indicating enhanced collagen synthesis and hydroxyapatite deposition in periodontal bone tissue.

3. The marked reduction in bone tissue destruction, accompanied by stabilization of lysosomal membranes and improved mineralization in TPC-treated rats, underscores the potential application of this therapeutic strategy in clinical settings. By addressing both oxidative stress and nutritional deficits, TPC can play a pivotal role in preventing osteopenic changes and advancing periodontal tissue health in patients at risk for combined pathologies.

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