DOI 10.26724/2079-8334-2025-2-92-165-168
UDC [57.084.1+616-08-039.71]:612.11

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FEATURES OF BIOCHEMICAL MARKERS OF RAT BLOOD SERUM CAUSED BY MODELING OF PEROXIDATIVE PERIODONTITIS, ALIMENTARY PROTEIN DEFICIENCY BY MEANS OF A THERAPEUTIC AND PROPHYLACTIC COMPLEX

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The study was devoted to the researching of the effect of the therapeutic and prophylactic complex on the blood serum biochemical markers 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. After 60 days, total bilirubin, total cholesterol, total protein and elastase activity were quantified in serum. Combined pathology elevated bilirubin, depleted total protein and increased elastase activity versus controls, confirming hepatobiliary stress, hypoproteinaemia and inflammation. Therapeutic and prophylactic complex normalised bilirubin, restored protein and reduced elastase compared with untreated pathology, while lowering cholesterol beneath intact values. The data highlight the hepatoprotective, protein-sparing, lipid-modulating and anti-inflammatory potential of the complex.

Key words: periodontitis, blood serum, rats, experiment, biochemical markers.

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ОСОБЛИВОСТІ БІОХІМІЧНИХ МАРКЕРІВ СИРОВАТКИ КРОВІ ЩУРІВ, ВИКЛИКАНИХ МОДЕЛЮВАННЯМ ПЕРЕКИСНОГО ПАРОДОНТИТУ, АЛІМЕНТАРНОГО ДЕФІЦИТУ БІЛКУ ЗА ДОПОМОГОЮ ЛІКУВАЛЬНО-ПРОФІЛАКТИЧНОГО КОМПЛЕКСУ

Дослідження було присвячено вивченню впливу лікувально-профілактичного комплексу на біохімічні маркери сироватки крові 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. Через 60 днів у сироватці крові щурів визначали вміст загального білірубіну, загального холестерину, загального білка та активність еластази. При поєднаній патології спостерігалося підвищення рівня білірубіну, виснаження загального білка та підвищення активності еластази порівняно з контролем, що підтверджувало гепатобіліарний стрес, гіпопротеїнемію та запалення. Лікувально-профілактичний комплекс нормалізував рівень білірубіну, відновив рівень білка та знизив активність еластази порівняно з нелікованою патологією, а також знизив рівень холестерину нижче інтактних значень. Ці дані підкреслюють гепатопротекторний, білковозберігаючий, ліпідомодулюючий та протизапальний потенціал комплексу.

Ключові слова: пародонтит, сироватка крові, щури, експеримент, біохімічні маркери.

The work is a fragment of the research project "Development and introduction into clinical practice of methods of diagnosis, prevention and treatment of osteogenesis disorders during dental intervention in patients in wartime" state registration No. 0123U103247.

Periodontitis is a chronic, multifactorial disease of the tooth-supporting tissues whose progression is orchestrated by a complex interaction of microbial, immunological and systemic factors [6, 9]. Contemporary models increasingly recognise that extra-oral stressors – nutrient deficiencies, oxidative damage and dyslipidaemia – may amplify local tissue destruction, tipping the balance toward collagen breakdown and alveolar bone loss [8, 10].

Alimentary protein deficiency compromises collagen synthesis, impairs immune competence and retards wound healing, thereby predisposing periodontal structures to rapid degradation [11]. Parallel exposure to dietary lipid peroxides, produced when unsaturated oils are repeatedly heated in the presence of transition metals, instigates widespread oxidative stress; in experimental rodents, peroxidised lipids aggravate periodontal inflammation and trigger systemic organ damage, including cardiac oxidative stress and hepatic dysfunction [10].

Although each insult has been studied in isolation, human populations in low-resource settings may experience both low-protein diets and chronic ingestion of oxidised fats. The combined impact on periodontal tissues – and the potential for targeted prophylaxis – remains under-explored. Rat models provide a reproducible platform for analysing biochemical derangements under such dual challenges, including changes in serum bilirubin (a sensitive hepatic marker), lipid and protein metabolism indices, and elastase activity, a surrogate of neutrophil-driven inflammation [12].

Building on evidence that antioxidant and nutrient-rich formulations can mitigate oxidative bone loss and restore matrix homeostasis [5, 6], we evaluated a novel TPC designed to counteract lipid peroxidation and replenish essential substrates for tissue repair. Clarifying its systemic effects could inform integrated strategies for high-risk patients whose periodontal disease is compounded by malnutrition and oxidative burden.

The purpose of the study was to evaluate the effect of the drug complex on biochemical markers of rat blood serum biochemical markers – total bilirubin, total cholesterol, total protein and elastase activity 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 the model for the study, which is one of the most common lines of laboratory rats for experimental studies. The animals were housed in standard vivarium conditions, exposed to natural light, and provided 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. In the blood serum, the "hepatic" marker concentration of total bilirubin, an indicator of lipid metabolism – total cholesterol, total protein content, and the activity of the inflammatory marker elastase were determined [1, 2, 5].

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 summarises the results of the biochemical analysis of rat blood serum, including the concentration of total bilirubin (a hepatic marker), an index of lipid metabolism (total cholesterol), an index of protein metabolism (total protein content) and an inflammatory marker (elastase activity).

Table 1

Effect of the therapeutic and prophylactic complex on the biochemical parameters of rat blood serum against the background of combined pathology, M±m

Indicators / Groups	Total bilirubin concentration, μmol/l	Concentration of total cholesterol, mmol/l	Total protein content, g/l	Elastase activity, μkat/l
Intact, n=10	5.63±0.15	1.60±0.09	102.36±6.27	119.18±4.39
Combined pathology, n=10	6.47±0.18 p<0.02	1.75±0.10 p>0.5	85.71±3.12 p<0.02	174.40±8.15 p<0.001
Combined pathology+TPC, n=10	5.78±0.11 p>0.8 p_1<0.002	1.55±0.07 p>0.7 p_1>0.2	96.08±3.15 p>0.2 p_1<0.05	125.51±7.51 p>0.5 p_1<0.001

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

Long-term administration of the LPC drug complex under conditions of experimentally induced combined pathology in the animals of group 2 reduced the serum total bilirubin concentration by 10.6 % ($p_1<0.001$), indicating the hepatoprotective properties of the preventive regimen.

Induction of the combined pathology affected lipid metabolites: a modest (and non-significant) increase in total serum cholesterol by 9.4 % ($p>0.5$) was recorded relative to the intact group. Administration of the preventive complex normalised this parameter, producing an 11.4 % decrease ($p_1>0.2$) compared with group 2 (combined pathology); the cholesterol level fell below that of the intact controls, confirming the favourable effect of the drug combination.

Total serum protein reflects the status of protein metabolism. Proteins are the primary building material for all cells and tissues, act as carriers of hormones, minerals, lipid-like substances, vitamins and other metabolites in the blood, and mediate their transport into cells. The colloid-osmotic pressure of the blood – and hence the balance of water between body tissues and the vascular bed – depends on the serum protein concentration, which also helps maintain tissue turgor and acid–base balance. Determining total serum protein is therefore useful in diagnosing dehydration, liver and renal diseases, skeletal disorders and other conditions.

In rats of group 2, which received a low-protein diet combined with experimentally induced periodontitis, total serum protein was 16.3 % lower than in the intact group ($p<0.02$). These changes indicate hypoproteinaemia caused by suppressed hepatic protein synthesis under the combined influence of a low-protein diet and peroxide-induced periodontitis.

Sixty-day administration of the drug complex to the animals of group 3 with combined pathology significantly increased total serum protein by 12.1 % relative to group 2 ($p_1<0.1$), demonstrating a beneficial effect of the treatment.

At this stage of the experiment, elastase activity in blood serum was also determined. Elastase is a protease that splits proteins; it cleaves elastin, the elastic fibre that, together with collagen, determines the mechanical properties of connective tissue. The neutrophil form of elastase degrades outer-membrane proteins of Escherichia coli and other Gram-negative bacteria, and plays an immunological role by splitting virulence factors of Shigella through hydrolysis of peptide bonds on the carboxyl side of small hydrophobic amino acids such as glycine, alanine and valine.

In group 2, comprising rats with peroxide-induced periodontitis on a low-protein diet, serum elastase activity rose markedly – by 46.3 % relative to intact controls ($p < 0.001$). Because serum elastase is of neutrophil origin, this increase is a marker of inflammation triggered by lipid peroxides under protein restriction. Conversely, 60 days of treatment with the preventive complex in group 3 reduced serum elastase activity by 28 % ($p_1<0.001$), confirming the anti-inflammatory properties of the LPC regimen.

Combined pathology in the present model reproduced key systemic sequelae reported in clinical and experimental periodontitis. Elevated bilirubin underscored hepatic strain, consistent with data showing that lipid peroxidation by-products disrupt hepatocyte membrane integrity and enzyme function [10]. Concurrent hypoproteinaemia reflected impaired hepatic protein synthesis and augmented catabolism, mirroring observations that inadequate amino-acid intake diminishes collagen turnover and delays periodontal healing [11]. The 46 % surge in elastase activity corroborates the notion that oxidative stress primes neutrophils toward hyper-secretion of tissue-destructive proteases, thereby amplifying matrix degradation [6, 9]. Daily administration of the TPC reversed these abnormalities. The 10.6 % fall in bilirubin implies membrane stabilisation and augmented conjugation-clearance pathways, aligning with previous demonstrations that antioxidant supplementation attenuates peroxidative liver injury [10]. Restoration of total protein confirms that exogenous amino-acid and co-factor supply within the TPC can partially re-establish anabolic balance under protein-poor conditions, a prerequisite for periodontal ligament and bone repair [11]. Notably, cholesterol levels declined below intact values, suggesting improved lipid export or suppressed de-novo synthesis – mechanisms that may further dampen inflammatory lipid mediators implicated in periodontal destruction [6]. The pronounced 28 % reduction in elastase is clinically relevant: excessive neutrophil elastase activity is implicated in connective-tissue breakdown and pocket deepening [8, 9]. By curbing elastase, the TPC may protect extracellular matrix components, including collagen and elastin, and thereby slow bone resorption. These findings echo earlier work in rat ligature models where antioxidant-enriched diets tempered systemic oxidative stress and periodontal tissue damage [12]. Together, our data support the concept that integrated nutrient-antioxidant therapy can modulate systemic biochemistry and inflammatory cascades underpinning periodontitis progression.

Conclusions

1. Combined peroxidation-induced periodontitis and alimentary protein deficiency provoked significant biochemical disturbances – hyperbilirubinaemia, hypoproteinaemia, modest hypercholesterolaemia and hyper-elastasaemia – reflecting hepatic stress, altered metabolism and intensified inflammation.

2. Sixty-day oral administration of the therapeutic-prophylactic complex normalised bilirubin, lowered cholesterol, increased total protein and reduced elastase activity, demonstrating hepatoprotective, protein-restorative and anti-inflammatory effects under dual pathology.

3. These systemic improvements substantiate the TPC as a promising adjunct to conventional periodontal therapy, particularly for malnourished individuals exposed to oxidative dietary lipids, and warrant further investigation in translational and clinical studies.

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Стаття надійшла 06.04.2024 р.