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Стаття надійшла 28.03.2023 р.

DOI 10.26724/2079-8334-2024-1-87-241-245
UDC 611.08-084+612.396.32:611.018.4-599.323.4

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INFLUENCE OF THERAPEUTIC AND PROPHYLACTIC COMPLEX ON BIOCHEMICAL PARAMETERS OF RAT MUCOUS MEMBRANE OF GUMS UNDER MODELING OF EXPERIMENTAL CARIES

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The purpose of this study was to assess the effect of the proposed therapeutic and prophylactic complex on the biochemical parameters of the oral mucosa of rats under conditions of modeling experimental caries against the background of alimentary deficiency of vitamin D. Experimental studies were conducted on 42 one-month-old Wistar rats of both sexes, which were divided into 3 groups. Gingival homogenates were prepared and the level of biochemical markers of systemic inflammation was determined: elastase activity and malondialdehyde content, as well as urease activity, catalase activity and acid phosphatase activity. The therapeutic and prophylactic use of the proposed complex in rats helps to inhibit the detected disorders under conditions of experimental caries against the background of nutritional deficiency of vitamin D, normalizing the studied parameters to the level of intact animals, which indicates the expressed antioxidant, anti-inflammatory and antimicrobial properties of the complex.

Key words: experiment, caries, rats, vitamin D deficiency, biochemical indices.

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ВПЛИВ ЛІКУВАЛЬНО-ПРОФІЛАКТИЧНОГО КОМПЛЕКСУ НА БІОХІМІЧНІ ПОКАЗНИКИ СЛИЗОВОЇ ОБОЛОНКИ ЯСЕН ЩУРІВ ПРИ МОДЕЛЮВАННІ ЕКСПЕРИМЕНТАЛЬНОГО КАРІЄСУ

Метою цієї роботи була оцінка впливу запропонованого лікувально-профілактичного комплексу на біохімічні показники слизової оболонки порожнини рота щурів в умовах моделювання експериментального карієсу на тлі аліментарного дефіциту вітаміну D. Експериментальні дослідження були проведені на 42 одномісячних щурах лінії Wistar обох полів, яких поділили на 3 групи. Готували гомогенати ясен та визначали рівень біохімічних маркерів системного запалення: активність еластази та вміст малонового діальдегіду, а також активність уреази, активність каталази і кислої фосфатази. Лікувально-профілактичне застосування у щурів запропонованого комплексу сприяє гальмуванню виявлених порушень за умов експериментального карієсу на тлі аліментарного дефіциту вітаміну D, нормалізуючи досліджувані показники до рівня інтактних тварин, що свідчить про виражені антиоксидантні, протизапальні та протимікробні властивості комплексу.

Ключові слова: експеримент, карієс, щури, дефіцит вітаміну D, біохімічні показники.

The work is a fragment of the research project "Improving the diagnosis and treatment of diseases of the oral mucosa in people with chronic somatic diseases", state registration No. 0119U003571.

Inadequate health status of children and adolescents is an actual problem of our time. The oral cavity plays an important role in the vital activity of the body, so the study of the state of its structural units in normal and pathological conditions occupies a prominent place in the research of scientists around the world [14, 15]. Pathological changes in the oral cavity in children require correct therapeutic and prophylactic intervention, a prerequisite for the effectiveness of which is a deep knowledge of the relevant etiopathogenetic mechanisms. There are both independent and symptomatic causes and factors that determine the development of dental pathology. For many years, the physiological role of vitamins

important for the functioning of the oral cavity organs has been studied. Particular attention is focused on vitamin D, which is explained by two reasons. Firstly, an extremely high prevalence of overt or latent D-vitamin deficiency (up to 50 % of the total population) has been found in the world's population. Secondly, new data have been established on the effect of vitamin D on the metabolism and regulation of many important physiological processes in different periods of life. Vitamin D affects almost all mechanisms of nonspecific defense against infectious agents and the system of immune specific response [1, 8]. The association of vitamin D deficiency with diseases of the musculoskeletal system, in particular osteoporosis, defects in the development of oral structures, oxidative stress and other processes related to dental pathology, is noteworthy [7, 12]. Therefore, there is a need to conduct appropriate experimental and clinical studies, as well as to develop a well-grounded treatment and prevention complex.

The purpose of the study was to assess the effect of the proposed therapeutic and prophylactic complex on the biochemical parameters of rat gums under conditions of experimental caries modelling on the background of vitamin D deficiency.

Materials and methods. The experimental studies were performed in accordance with the requirements of the Law of Ukraine No. 3447-IV of 21.02.2006 "On the Protection of Animals from Cruelty", the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Purposes and the Procedure for conducting experiments and experiments on animals by scientific institutions in compliance with bioethical standards (Decree of the Ministry of Education and Science of Ukraine No. 249 of 01.03.2012). The work was carried out on the basis of the Department of Biochemistry of the SE "The Institute of stomatology and maxilla-facial surgery National academy of medical sciences of Ukraine" (SE "ISMFS NAMS").

In the experimental studies, 42 white Wistar rats of the herd breeding were used, which were kept in standard vivarium conditions of both sexes with an average body weight of 75±3.

Caries in rats was induced by a diet that is a modified casein-sucrose cariesogenic diet by M.G. Bugayova and S.A. Nikitin [13]. We used table salt instead of the salt mixture, as presented in the work of Volkova O.S. et al. [3] with a reduced content. We also reduced the content of sunflower oil and removed "Undevit" from the diet. The lack of salt and oil was compensated for by increasing the content of sugar and crackers. The decrease in oil content was justified by the use of this oil for dosing retinol acetate for administration to rats, and the removal of "Undevit" was justified by the use of vitamins A and D_3 in the study. Thus, the composition of the carcinogenic diet used in the study was as follows: sugar – 56 %, skim milk cheese – 19 %, white wheat bread crackers of the highest grade – 21 %, unrefined sunflower oil – 3 %, and table salt – 1 %. Retinol acetate was added to the cheese, which was dosed per rat cage in 1 ml of sunflower oil at 48,000 IU per 1 kg of feed. The average feed consumption by rats was 15.9 g per rat. 3 groups of 14 animals (7 of each sex) were formed: Group 1 – intact; Group 2 (DC) – a model of pathology on a D-deficient cariesogenic diet; Group 3 (DC+TPC) – a model of pathology and the use of a treatment and prevention complex.

Table 1 shows the composition of the treatment and prevention complex used in the experimental study.

Animals were euthanized on day 30 of the experiment under thiopental anesthesia (20 mg/kg) by total bleeding from the heart. Gingival homogenates were prepared at the rate of 20 mg/ml of 0.05 M Tris-HCl buffer, pH 7.5. The level of biochemical markers of systemic inflammation was determined: the activity of the proteolytic enzyme elastase and the content of the end product of lipid peroxidation – malondialdehyde (MDH) [2, 11], as well as urease activity (an indicator of microbial contamination), catalase activity (an antioxidant enzyme) and acid phosphatase (AP) [4, 6, 10]. In addition, the antioxidant-prooxidant index (API) was calculated by the ratio of catalase activity and MDH concentration according to the formula: $API=A_{cat}/C_{mdh}\times10$, where A_{cat} is catalase activity, mcg/L; C_{mdh} is MDH concentration, μmol/L [9].

In the statistical processing of the results obtained, the computer program STATISTICA 6.1 was used to assess their reliability and measurement errors. A statistically significant difference between alternative quantitative traits with a distribution corresponding to the normal law was evaluated using the Student's t-test. The difference was considered statistically significant at $p<0.01$ [5].

Results of the study and their discussion. The data presented in Table 2 show that in the gums of the 2nd group of animals in which caries was modelled against the background of nutritional deficiency of vitamin D (DC), the processes of inflammation and lipid peroxidation were intensified: MDH content significantly increased by 24.3 % ($p_1<0.001$) in males and by 23.1 % in females ($p_1<0.001$), and the activity of one of the most important inflammatory markers, elastase, significantly increased by 32,8 % ($p_1<0.001$) in males and by 18.4 % ($p_1<0.02$) in females, compared with the intact group, indicating an increase in inflammatory processes in the mucous membrane of the gums.

Table 1

Composition of the treatment and prevention complex

Used products	Active ingredients	Manufacturer	Mechanism of action
"Sliurem" gel, 1 time per day, 0.3 ml daily per os	sodium carboxymethyl cellulose 2.0–3.5 %; sodium alginate 0.5–1.5 %; glycerin 20–25 %; sodium fluoride 0.5–2.0 %; hydroxyapatite (nanocalcide) 3–5 %; chlorhexidine 1–2 %; sodium benzoate 0.5–1 %; menthol 0.05–0.15 %; flavor 0.8–1.2 %; food coloring 0.001–0.002 %; water – up to 100 %.	Laboratory for the development and research of oral hygiene products of the SE "ISMFS NAMS" (Conclusion of the State Sanitary and Epidemiological Expertise No. 05.05.02-07/36637 dated 25.04.2012., Patent of Ukraine No. 81886 dated 10.07.2013., TC U 20.4-02012-001: 2012).	increase of mineralizing potential of oral fluid, activation of tooth enamel remineralization, has antiseptic effect
Dietary supplement "Zdorovye" "Strong teeth", 10 mg/100 g of rat daily per os	walnut leaves (207.6 mg); vitamin D3 (0.8 mg); vitamin mixture (A, E, C, B12, B2, nicotinamide, folic acid, etc.) (0.1 mg); magnesium oxide (16.5 mg), calcium carbonate (50 mg).	LLC PTC "Farmakom", Ukraine	anti-inflammatory, bactericidal effect; general strengthening and immunostimulating effect on the body. A source of calcium, magnesium and vitamin D
Aquadetrim vitamin D_3 aqueous solution 10 ml, 7.5-8.0 IU/100 g per rat per os	cholecalciferol; 1 ml (30 drops) of the solution contains: cholecalciferol 15000 IU (1 drop contains approximately 500 IU of vitamin D3);	Medana Pharma S. A., Poland	regulation of calcium and phosphate metabolism, promotes proper mineralization and skeletal growth; participates in the functioning of the immune system, affects the production of lymphokines, active anti-rheumatic factor, etc.

The administration of TPC for 30 days in animals of group 3 contributed to an effective reduction in the intensity of lipid peroxidation and attenuation of inflammatory processes. Thus, in the gums of male rats of group 3, there was a tendency of reducing the activity of elastase by 23.7 % ($p_2<0.02$) and by 15.8 % in females ($p_2<0.05$) and a significant decrease in the level of MDH by 19.7 % ($p_2<0.001$) in males and by 18.2 % ($p_2<0.01$), respectively, in females compared to the parameters in group 2, almost completely restoring to the parameters of the intact group. Therefore, the use of TPC indicates an almost complete absence of inflammation in the mucous membrane of the gums of rats on the background of experimental caries, and hence the pronounced anti-inflammatory effect of the complex.

Table 2

Biochemical parameters of rat gums on the background of carcinogenic diet and model of vitamin D deficiency under the influence of treatment and prevention complex, M±m

Animal groups		Indices					
		elastase activity, μ-kat/kg	MDH content, mmol/kg	catalase activity, mcg/kg	API index, units.	AP activity, μkat/kg	urease activity, μkat/kg
Group 1 intact	Males n=7	34.38±2.62	9.15±0.33	3.38±0.15	3.69±0.18	13.75±0.57	0.180±0.016
	Females n=7	39.81±2.55 p>0.2	10.07±0.52 p>0.2	3.09±0.19 p>0.25	3.07±0.15 p<0.01	14.45±0.41 p>0.4	0.173±0.007 p>0.7
Group 2 DC	Males n=7	51.14±3.04 $p_1<0.001$	12.08±0.64 $p_1<0.001$	2.74±0.15 $p_1<0.002$	2.27±0.12 $p_1<0.001$	20.26±0.52 $p_1<0.001$	0.562±0.010 $p_1<0.001$
	Females n=7	48.76±2.96 p>0.6 $p_1<0.02$	13.09±0.50 p>0.25 $p_1<0.001$	2.63±0.12 p>0.6 $p_1<0.05$	2.01±0.14 p>0.2 $p_1<0.001$	20.91±0.60 p>0.8 $p_1<0.001$	0.640±0.027 p<0.01 $p_1<0.001$
Group 3 DC+TPC	Males n=7	39.04±2.37 $p_1>0.25$ $p_2<0.02$	9.70±0.47 $p_1>0.4$ $p_2<0.001$	3.28±0.22 $p_1<0.05$ $p_2<0.05$	3.38±0.20 $p_1>0.25$ $p_2<0.001$	14.70±0.48 $p_1>0.25$ $p_2<0.001$	0.235±0.022 $p_1>0.1$ $p_2<0.001$
	Females n=7	41.04±185 p>0.6 $p_1>0.7$ $p_2<0.05$	10.71±0.62 p>0.25 $p_1>0.7$ $p_2<0.01$	3.24±0.26 p>0.8 $p_1>0.7$ $p_2>0.7$	3.03±0.18 p>0.2 $p_1>0.8$ $p_2<0.001$	15.61±0.44 p>0.2 $p_1>0.1$ $p_2<0.001$	0.201±0.015 p>0.2 $p_1>0.1$ $p_2<0.001$

Note. p – is the index of significance of differences between males and females; p_1 – is the index of significance of differences with group 1 (intact); p_2 – is the index of significance of differences with group 2 (DC)

The activity of the antioxidant defense marker catalase decreased by 18.9 % ($p_1<0.002$) in males and by 14.9 % in females ($p_1<0.05$). Prolonged use of the proposed TPC for 30 days leads to the normalization of catalase activity in rats of group 3 to the level of the group of intact animals. The intergroup difference between the indicators of the 2nd and 3rd groups was 16.5 % in males ($p_2<0.05$) and 18.8 % ($p_2>0.7$) in females.

The functional state of the gums, their resistive capacity is most objectively characterized by the ratio of the activity of antioxidant enzymes and lipid peroxidation products in tissues – the antioxidant-prooxidant index. The API index significantly increased by 32.8 % ($p_2<0.001$) in males and by 33.7 % ($p_2<0.001$) in females of the DC+TPC group compared to the group of rats with modeled pathology.

Urease activity reflects the degree of contamination of the opportunistic microbiota that synthesizes this enzyme in the oral cavity. The level of urease activity can be used to indirectly assess the level of microbial contamination. Its significant increase was found in the modeling of caries in males by 3.1 times ($p_1<0.001$) and 3.7 times ($p_1<0.001$) in females. In rats of the 3rd group, urease activity decreased on the background of pathology modeling and TPC administration, but remained significantly higher than in the intact group by 23.4 % in males ($p_1>0.1$) and by 13.9 % ($p_1>0.1$) in females. The intergroup difference between males of the 2nd and 3rd groups was 58.2 % ($p_2<0.001$), between females – 68.6 % ($p_2<0.001$). It is known that vitamin D activates the synthesis of antimicrobial peptides (the most important link in innate immunity), providing its immunotropic effect.

From the results of the analysis of the gingival mucosa of rats, it is clear that the increased activity of AP (odontoclast marker enzyme) in males of group 2 by 32.1 % ($p_1<0.001$) and in females by 30.9 % ($p_1<0.001$) contributes not only to damage to periodontal tissues, reduces regenerative processes in them, but also contributes to the development of diseases of the oral mucosa. A significant ($p_2<0.001$) decrease in AP activity by 27.4 % in males and 25.3 % in females indicates that the administration of the proposed TPC to rats can inhibit inflammation in the gingival mucosa.

Thus, the results of the experimental studies indicate inflammation, contamination with opportunistic bacteria, reduced antioxidant protection and increased lipid peroxidation in the test material in the modeling of D-deficient caries in rats, as well as inhibition of inflammation of the oral mucosa, which justifies the use of the proposed TPC in the clinic in children with dental pathology.

Our study focuses on the impact of a therapeutic and prophylactic complex on the biochemical parameters of the rat gum mucosa under experimental caries modelling. Special attention was paid to the role of vitamin D in this process. This direction is relevant, as emphasized in studies highlighting the importance of vitamin D for oral health [7, 12]. Comparing our results with the findings of other researchers reinforces the significance of vitamin D in the prevention of dental diseases, particularly in children and adolescents [1, 12]. These results align with our conclusions about the link between vitamin D levels and the development of caries. This underscores the relevance of our direction in developing effective prevention methods. Prevention and treatment of dental diseases require a comprehensive approach, including not only medicinal intervention but also nutrition, lifestyle, and genetic factors [14]. This highlights the importance of our research in developing comprehensive prevention and treatment methods. Further study of the effect of the proposed treatment and prevention complex on the condition of the gingival mucosa will enable doctors to trace the relationship between somatic pathology and the development of dental diseases in children. The effectiveness of these measures depends, first of all, on early diagnosis, which will allow to correct the disorders and ensure normal physiological and dental development of the child. Prospects for further research are to develop an effective method for the prevention of major dental diseases in children, depending on the level of vitamin D in the blood serum, using an appropriate treatment and prevention complex.

Conclusions

1. The analysis of the results of biochemical studies of rat mucous membrane gingival homogenates showed that modelling of experimental caries complicated by nutritional deficiency of vitamin D led to a disorder of the lipid peroxidation-antioxidant system towards the intensification of lipid peroxidation processes (increase in MDH content by 24,3 % and 23.1 %, decrease in catalase activity by 18.9 % and 14.9 %, respectively, in males and females), increased inflammatory processes in the gingival mucosa (increase in elastase activity by 32.8 % and 18.4 %, as well as AP by 32.1 % and 30.9 %, respectively, in males and females) and increased microbial colonization (by 67.97 % in males and 72.97 % in females).

2. The treatment and prophylactic use of the proposed complex in rats helps to inhibit the detected disorders under conditions of experimental caries against the background of nutritional deficiency of vitamin D, normalizing the studied parameters to the level of intact animals, which indicates the pronounced antioxidant, anti-inflammatory and antimicrobial properties of the complex.

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Стаття надійшла 21.03.2023 р.