DOI 10.26724/2079-8334-2022-2-80-194-198 UDC [616.314+616.311.2-002]:796.332+616.31-08-039.71

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## EXPERIMENTAL EVALUATION OF THE EFFECTIVENESS OF THE USE OF A DRUGS COMPLEX DEVELOPED FOR THE PREVENTION OF MAJOR DENTAL DISEASES IN YOUNG ATHLETES

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Dental morbidity in athletes, including children who play sports, comes out on top against other categories of the population, so the issue of improving the prevention of major dental diseases in children-athletes remains relevant. The aim of the work was to evaluate experimentally the effectiveness of the treatment and prevention complex, which has immunomodulatory, adaptogenic, detoxifying and anti-dysbiotic effects in the combination of malnutrition and physical exercise. The experiment was performed on 25 white rats of herd breeding, divided into 3 groups. The results of experimental studies indicate a high caries-prophylactic and periodontal-protective effectiveness of the proposed treatment and prevention complex in the conditions of experimental pathology. The obtained results must be taken into account when developing treatment and prevention measures for children-athletes.

Key words: rats, inadequate nutrition, physical activity, basic dental diseases, treatment and prevention complex.

## О.В. Дєньга, А.О. Осадча, О.А. Макаренко, Г.О. Бабеня, В.Н. Горохівський, С.А. Шнайдер ЕКСПЕРИМЕНТАЛЬНА ОЦІНКА ЕФЕКТИВНОСТІ ВИКОРИСТАННЯ КОМПЛЕКСУ ЗАСОБІВ, РОЗРОБЛЕНОГО ДЛЯ ПРОФІЛАКТИКИ ОСНОВНИХ СТОМАТОЛОГІЧНИХ ЗАХВОРЮВАНЬ У ДІТЕЙ-СПОРТСМЕНІВ

Стоматологічна захворюваність у спортсменів, в тому числі і у дітей, що займаються спортом, виходить на перше місце проти інших категорій населення, тому питання підвищення ефективності профілактики основних стоматологічних захворювань у дітей-спортсменів зберігають свою актуальність. Метою роботи була експериментальна оцінка ефективності використання лікувально-профілактичного комплексу, що має імуномоделюючу, адаптогенну, детоксикаційну та антидисбіотичну дію в умовах поєднання неправильного харчування та фізичного навантаження. Експеримент проведено на 25 білих щурах стадного розведення, розподілених на 3 групи. Результати експериментальних досліджень свідчать про високу карієспрофілактичну та пародонтопротекторну ефективність запропонованого лікувально-профілактичного комплексу в умовах експериментальної патології. Отримані результати необхідно враховувати при розробці лікувально-профілактичних заходів для дітей-спортсменів.

**Ключові слова**: щури, неадекватне харчування, фізичні навантаження, основні стоматологічні захворювання, лікувально-профілактичний комплекс.

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

In recent years, the number of publications in the field of dental health devoted to the preservation of dental health during sports has increased [7, 11, 13, 14].

Many researchers have noted that dental morbidity in athletes, including children who play sports, not only remains at a high level, but also comes out on top against other categories of the population, so the issue of improving the prevention of major dental diseases in children-athletes remain relevant [5, 15].

Intense physical and psycho-emotional loads to which professional athletes are exposed in the process of systematic training, lead to the development of overtraining syndrome due to reduced immunological reactivity and adaptive capacity of the body, which is characterized by imbalance in acid-base balance, microbiocenosis, antioxidant system and is accompanied by frequent disturbances in the functional state of the nervous, endocrine and cardiovascular systems, which often leads to chronic foci of inflammation, which under favorable conditions and against the background of fatigue, especially in the pre-competition period, affect physical performance and athletic performance [2, 8].

Contributes to the emergence of stress immunodeficiency and malnutrition of the athlete [10].

Some authors attribute the growing prevalence of dental caries and periodontal disease to the widespread use of energy sports drinks, training complexes and sports nutrition concentrates [7, 9].

Therefore, there is a need to develop comprehensive measures in the program of prevention of major dental diseases, taking into account the peculiarities of their pathogenesis in this category of children, namely children athletes.

**The purpose** of this study was an experimental evaluation of the effectiveness of the treatment and prevention complex, which has immunomodulatory, adaptogenic, detoxifying and antidisbiotic effects in the combination of malnutrition and exercise.

**Materials and methods**. The experiment was performed on 25 white rats herd breeding (males, 1 month, average weight 34±5 g), divided into 3 groups: 1st – intact control (7 rats). 2nd - inadequate nutrition (9 rats): 30 % sugar, peroxidized oil (1 ml/rat), daytime Coca-Cola (The Coca-Cola Company), evening to morning – water, caffeine was given in the last week (30 mg/kg, Ahrofarm LLC). 3rd – inadequate nutrition, treatment and prevention complex *per os* (9 rats).

As part of the treatment and prevention complex, rats of the 3rd group received daily: 1. Egg albumin plus, 1 g/kg (AIE "Odeska biotekhnolohiya", Ukraine). 2. Biotrit-C, 300 mg/kg (AIE "Odeska biotekhnolohiya", Ukraine). 3. Baktoblis, 300 mg/kg (Bluestone Pharma, Switzerland). 4. Apple pectin, 450 mg/kg (Pektowin, Poland). 5. Vitamin and mineral complex "Alfavit", 225 mg/kg (PJSC "Vitaminy", Ukraine).

In the process of conducting the experiment to assess the endurance of rats was used test of behavioral despair by the method of Porsolt in the modification of Karkishchenko V.M. et al., which involves the study of endurance and speed of fatigue of animals on the basis of multiple presentation of swimming load with a weight of 10 % of body weight [1].

The duration of the experiment was 50 days. On day 51, rats were euthanized under thiopental anesthesia (20 mg/kg) by total cardiac bleeding. The jaws with teeth were isolated, in which carious cavities and their depth were counted, the degree of atrophy of the alveolar process was determined in the jaws by the method of Nikolaeva A.V. In the homogenate of periodontal bone tissue, the calcium content, the activity of alkaline (ALP) and acid (AP) phosphatase, and elastase activity were determined. The mineralization index was determined from the ALP/AP ratio [4].

In the homogenates of the oral mucosa (20 mg/ml Tris-HCl buffer, pH 7.5), the level of malonic dealdehyde (MDH), the activity of elastase, catalase [15], the activity of lysozyme, and urease were determined, and the antioxidant-prooxidant index (API) was calculated and degree of dysbiosis [3].

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.

**Results of the study and their discussion**. The development of the carious process in rats was assessed by the number of carious cavities (average per 1 rat) and the depth of caries lesions. As can be seen from the data in table 1, long-term consumption of excess sugar and lipid peroxide by rats in combination with Coca-cola contributed to an increase in the number of carious lesions by 32.2% (p<0.01) and their depth by 36.4% (p<0.05) in animals of the second group.

Table 1

Indices of the carious process and the degree of the alveolar process atrophy of the lower jaw in rats
of different groups

Indices	Number of carious cavities,	Depth of tooth decay,	Atrophy of the alveolar
Groups	average per 1 rat	points	process, %
Intact	5.9±0.5	6.6±0.8	24.5±1.4
Inadequate nutrition	7.8±0.2	9.0±0.7	27.4±1.3
	p<0.01	p<0.05	p>0.05
Inadequate nutrition+ prevention	4.0±0.6	4.3±0.7	16.9±0.6
	p<0.05	p<0.05	p<0.001
	$p_1 \le 0.001$	$p_1 \le 0.001$	$p_1 \le 0.001$

Note. p – the index of the reliability of differences relative to the "intact" group;  $p_1$  – the index of the reliability of differences relative to "inadequate nutrition" group.

Daily administration of a complex of therapeutic and prophylactic drugs on the background of inadequate nutrition in rats of the 3rd group showed a pronounced caries-prophylactic effect: the number of carious cavities significantly decreased by 48.7 % compared to the level of this indicator in the 2nd group ( $p_1$ <0.001), the depth of the lesion - by 52.2 % ( $p_1$ <0.001). It should be emphasized that in rats of the 3rd group both indicators were significantly lower than the corresponding values in intact animals (p<0.05 and p<0.05, respectively).

In addition, the consumption by rats of excess sugar and peroxidized oil caused a tendency to increase the degree of atrophy of the alveolar process of the mandible of rats of the 2nd group ( $p_1>0.05$ ). After the use of a complex of vitamins, minerals, adaptogen, complete protein, probiotic and sorbent in rats of the 3rd group atrophy of the alveolar process decreased significantly – by 38.3 % compared with the 2nd group ( $p_1<0.001$ ) and – by 31.0 % in relation to the values of intact animals (p<0.001).

The next step was to study biochemical markers of bone resorption and osteogenesis in the alveolar bone of experimental rats. From the results shown in table 2 it is seen that both resorption parameters are significantly increased in the bone tissue of the alveolar process of animals of group 2, which received inadequate nutrition: elastase activity increased by 54.7% (p<0.001), and acid phosphatase activity - by 42.9% (p<0.002).

Table 2

Indices of resorption in the bone tissue of the alveolar process in rats on the background of inadequate nutrition and its correction

Indices Groups	Elastase activity, μ-cat/kg	Acid phosphatase activity, μ-cat/kg
Intact	15.02±1.06	7.24±0.41
Inadequate nutrition	23.24±1.57 p<0.001	10.35±0.79 p<0.002
Inadequate nutrition+ prevention	18.13±1.10 0.05 <p<0.1 p<sub>1</sub>&lt;0.01</p<0.1 	8.62±0.65 p >0.1 p <sub>1</sub> >0.2

Note. p – the index of the reliability of differences relative to the "intact" group;  $p_1$  – the index of the reliability of differences relative to "inadequate nutrition" group.

The established changes indicate an increase in the processes of resorption of the bone tissue of the alveolar process with the participation of elastase – the collagen matrix, and under the influence of acid phosphatase – the main mineral of the bone tissue – hydroxyapatite. An increase in the activity of these bone enzymes can explain the trend towards increased atrophy of the alveolar process in rats that received inadequate nutrition.

Prophylactic administration of a complex of vitamins, minerals, protein, adaptogen, probiotic and natural sorbent effectively prevented the activation of destructive enzymes in the bone tissue of the alveolar process in rats of the 3rd group. As shown in table 2, the level of elastase activity decreased and approached the norm (0.05 , and acid phosphatase corresponded to normal values. <math>(p > 0.1).

The content of calcium in the alveolar bone of animals of the 2nd group on the background of inadequate nutrition (by 14.2 %, p>0.2). This is a consequence of increased activity of acid phosphatase, which destroys hydroxyapatite. Carrying out prophylaxis with a complex of drugs stabilized the level of calcium in the bone tissue of the alveolar process of animals of the 3rd group (p>0.7 and  $p_1>0.25$ ).

The marker of osteoblasts (alkaline phosphatase activity) increased in the alveolar bone of rats with inadequate nutrition by 26.9% (p<0.05), which can be explained by the compensatory response to increased resorption of bone tissue under the influence of adverse factors. Administration of vitamins, minerals, protein, adaptogen, sorbent and probiotic to animals of the 3rd group contributed to an even greater increase in bone alkaline phosphatase activity – by 13.5% compared to the level of this indicator in rats of the 2nd group receiving defective nutrition (p<0.001 and p<sub>1</sub>>0.25). A significant increase in alkaline phosphatase activity in the bone tissue of the jaw indicates the stimulation of the therapeutic and prophylactic complex of osteogenesis under adverse nutritional conditions and explains the significant inhibition of atrophy of the alveolar process in animals of group 3.

The state of bone tissue remodeling reflects the mineralizing index, which is calculated as the ratio of alkaline to acid phosphatase activity (ALP/AP). The value of this index is shown in fig. 1, which shows a slight decrease in ALP/AP in the bone tissue of the alveolar process of rats of the 2nd group by 11.3 % (p>0.2), which is consistent with an increase in alveolar bone atrophy in these animals under influence of inadequate nutrition. Carrying out prophylaxis with a complex of drugs contributed to a significant increase in the mineralizing index (p<0.05 and  $p_1$ <0.002), due to which the atrophy of the alveolar process in rats of the 3rd group slowed down sharply.

The next step was to investigate the condition of the gums of experimental animals.

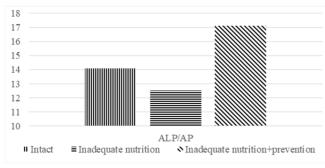
The activity of elastase, the source of which is neutrophils, in the gums of rats of the second group increased by 40.8% (p<0.002), which indicates the development of inflammatory processes under the influence of malnutrition. The introduction of treatment and prevention complex to animals of the 3rd group somewhat prevented an increase in the activity of elastase, although its level exceeded the value in intact control (p<0.05).

Another marker of inflammation - the level of malonic dialdehyde (MDH), which reflects the degree of lipid peroxidation in tissues, had a tendency to increase in the gums of rats with inadequate nutrition (p>0.25) and normalization after treatment with prophylactic drugs (p>0.7 and  $p_1>0.2$ ).

The activity of one of the main enzymes of antioxidant protection of catalase tissues in the gums of rats of the second group increased by 19.9 % (p<0.02). The established fact can be regarded as a

compensatory activation of this link of antioxidant protection in response to a slight increase in lipid peroxidation under the influence of inadequate nutrition. The level of catalase activity in the gums of rats of the 3rd group, which additionally received a complete protein, a vitamin-mineral complex, an adaptogen, a probiotic, a sorbent, and purified water, occupied an intermediate position between the indicator in the gums of intact control and the "inadequate nutrition" group (p>0.4 and  $p_1>0.2$ ).

The antioxidant-prooxidant index (API), indicating changes in the antioxidant-prooxidant system, did not undergo significant changes in the gums of rats of the 2nd and 3rd groups (fig. 2).



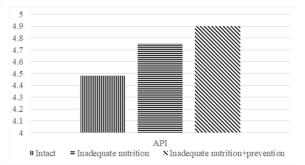


Fig.1. Indicator of osteogenesis in the bone tissue of the alveolar process of rats on the background of inadequate nutrition and its correction

Fig.2. Biochemical index of inflammation in the gums of rats on the background of inadequate nutrition and its correction

This indicates that the endogenous antioxidant protection of rat gum tissues in response to inadequate nutrition is able to compensate for existing oxidative disorders, at least at this stage of the study.

The biochemical marker of microbial contamination (urease activity) increased in the gums of animals of the second group by 36.0% (p<0.01).

This increase can be explained by a decrease in the main antimicrobial factor in the oral cavity-lysozyme, the activity of which decreased in the gums of rats that received inadequate nutrition by 30.0% (p<0.002). As a result of these changes, the degree of dysbiosis in the gums of animals of the 2nd group increased by 1.9 times (p<0.001).

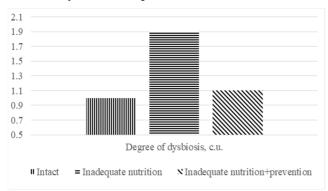


Fig.3. Biochemical index of dysbiosis in the gums of rats on the background of inadequate nutrition and its correction

In the gums of rats of the 3rd group, which were additionally injected with egg white, vitamin-mineral complex, adaptogen, probiotic, sorbent and purified water, a decrease in lysozyme activity was prevented (p>0.4 and  $p_1$ <0.002); increase of urease activity (p>0.8 and  $p_1$ <0.05) and the degree of dysbiosis (p>0.5 and  $p_1$ <0.001). The obtained results indicate the antidysbiotic efficacy of the studied therapeutic and prophylactic complex of drugs used in the 3rd group of rats.

As evidenced by the results of scientific research conducted by scientists in the field of

sports dentistry in recent years, the negative changes that we studied in our work can act as a decisive etiological factor in the occurrence, development and prevalence of major dental diseases among people involved in high-performance sports [8, 9, 10, 12]. The conducted experimental study shows that longterm use of excess sugar, lipid peroxide in combination with "Coca-cola" induced the development of a carious process and resorption of the bone tissue of the alveolar process in experimental animals. Enhanced resorption of the bone tissue of the jaws was carried out due to the activation of hydrolytic enzymes of acid phosphatase and elastase, as a result of which the level of calcium in the jaw tissue of rats decreased. Inadequate nutrition also stimulated the development of inflammation, a decrease in antimicrobial and antioxidant protection, an increase in microbial contamination and the degree of dysbiosis in the gums and mucous membranes of the oral cavity of rats, as judged by increased activity of elastase, urease and MDA content on the background of a decrease in the activity of lysozyme and catalase [6]. Prophylactic administration of a complete protein, vitamin-mineral complex, adaptogen, probiotic, sorbent and purified water on the background of inadequate nutrition not only prevented the established disorders, but also significantly inhibited the natural degree of development of the carious process and resorption of the alveolar bone. The results obtained, in our opinion, should be taken into account when developing therapeutic and preventive measures to accompany the dental treatment of child athletes.

## Conclusions

- 1. Prophylactic administration of the prophylactic complex on the background of inadequate nutrition prevented the established disorders and significantly inhibited the natural degree of development of the carious process and resorption of the alveolar bone, which are characteristic of healthy animals.
- 2. Inadequate nutrition stimulated the development of inflammation, a decrease in antimicrobial and antioxidant protection, an increase in microbial contamination and the degree of dysbiosis in the gums and mucous membranes of the oral cavity of rats.
- 3. Long-term use of excess sugar, lipid peroxide in combination with "Coca-cola" induced the development of a carious process and resorption of the bone tissue of the alveolar process in experimental animals.

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Стаття надійшла 24.05.2021 р.