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EXPERIMENTAL SUBSTANTIATION OF APPLICATION GEROPROTECTORS FOR PREVENTION PERIODONTITIS IN THE ELDERLY

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Abstract. The article presents the results of studies of the tissues of the alveolar and femoral bone of rats and gingival tissue in experimental periodontitis and its prevention by a complex of preparations containing vitamins, minerals and adaptogens. It is proved that the proposed complex significantly inhibits resorption of the alveolar bone of rats, increases the non-specific resistance and antioxidant status of animals under conditions of an alimentary excess of lipid peroxides.

Keywords: experimental periodontitis, atrophy, prophylaxis, adaptogens, minerals, vitamins, geroprotectors.

Relevance. According to many researchers, the prevalence of periodontal disease in elderly and senile people is about 100% [2,4], and periodontal lesions in them have a more severe course than in middle-aged people, are characterized by progressive destruction of the periodontal and bone, and the prevalence of the intensity of major dental diseases has a direct correlation with the age of patients [7]. All this leads to the loss of teeth, which ranges from 81.6% in the age group 60-69 years old to 95.4% in the age group 90 years old and older [1].

In elderly people, the onset of periodontitis is associated with many factors, among which, of course, the aging process itself, which causes changes in both structures and functions [4].

Many scientific works have been devoted to the problems of treatment and prevention of periodontitis in elderly and senile patients. Since periodontitis is accompanied by a violation of almost all types of metabolism, difficulties arise in the choice of medication both for the elimination of inflammatory phenomena and for prevention. The proposed medications do not always provide a stable remission and stabilization of the process in the periodontium.

All of the above led to the need to search for a complex of medications for the prevention of periodontitis in elderly and senile people.

The purpose of our work was the experimental substantiation of the use of a complex of adaptogens, vitamins and minerals for the prevention of periodontitis in old age.

To achieve this goal, the following tasks were set:

1. To investigate changes in the bone tissue and tissues of the gums of rats during experimental periodontitis.
2. To study the effect of a complex of adaptogens, vitamins and minerals on biochemical parameters in blood serum, tissues of the gums and femur.

Materials and research methods. In the experimental work, 24 Wistar rats of herd breeding males at the age of 16 months with an average weight of 456 ± 19 g were used. Periodontitis was modeled by adding peroxidized sunflower oil to the animal feed at the rate of 1.5 ml per rat. The peroxide value of the used oil was 44.6 mmol / kg [9].

The experimental animals were divided into three groups of 8 rats each:

- 1 - intact (healthy control);
- 2 - periodontitis model;
- 3 - model of periodontitis + treatment and prophylactic complex.

The treatment and prophylactic complex consisted of bioaron (manufactured by Fitofarm Klenka S.A., Poland), calcicor (calcium citrate, manufactured by NPA Odessa Biotechnology, Ukraine), alphabet (complex of vitamins and minerals, manufactured by ZAT AKVION, Russia), quertulin (quercetin, inulin, calcium citrate, NPA "Odessa Biotechnology", Ukraine). The drugs were administered orally in the morning on an empty stomach 2 hours before feeding, starting from the first day of modeling the pathology at doses of Bioaron 3.6 ml / kg, Calcicor 500 mg / kg, Alphabet 150 mg / kg, and Quertulin 100 mg / kg. Doses of drugs are equivalent to human doses.

The duration of the experiment was 30 days, after which the rats were removed from the experiment under thiopental anesthesia (20 mg / kg) by bloodletting from the heart. Blood was collected to obtain serum, the gums and femur were isolated, which were frozen at -35 ° C until the study. Jaws were also isolated to calculate the degree of atrophy of the alveolar bone [12]. In the blood serum, the activity of catalase [6], the total proteolytic activity of OPA [3], the content of the trypsin inhibitor IT [5] and the content of malondialdehyde MDA [13] were determined. In gingival homogenates (20 mg / ml 0.05 M Tris-HCl, pH 7.5), the content of MDA [13], the activity of catalase [6], elastase [8], alkaline (AP) and acid phosphatases (AP) [ten]. In homogenates of the femur of animals (75 mg / ml 0.1 M citrate buffer, pH 6.1), the activity of proteolytic enzymes (OPA and elastase), alkaline and acid phosphatase was determined [10]. The IT / OPA index was calculated as the ratio of the IT content in g / L to the OPA in nkat / L of blood serum. The antioxidant-prooxidant index (API) was calculated using the formula:

$$API = \frac{A_{кат.}}{C_{MDA}} \cdot 10$$

where is Akat. - catalase activity ($\mu\text{at} / \text{kg}$); SMDA is the concentration of MDA in mmol / kg [11].

Research results and their discussion. The results obtained indicate that the addition of peroxidized oil to the diet of rats for 1 month did not significantly affect the high resorption of the jaw bone tissue ($P > 0.05$) (Table

Table 1. Influence of peroxidized oil and prophylaxis with a complex of drugs on atrophy of the alveolar ridge in old rats

№	Groups of rats, n = 8	The degree of atrophy of the alveolar process of the lower jaw of rats, %
1	Intact	58,0 ± 2,0
2	Periodontitis model	61,3 ± 3,2 P > 0,05
	Periodontitis model +	53,1 ± 1,4
3	Prophylactic complex	P > 0,05
		P ₁ < 0,05

Note. P - reliability of differences from the indicator in the intact group; P₁ - reliability of differences from the indicator in the group "model of periodontitis"

It should be noted that this indicator in young animals is 1.5 ... 2.5 times lower, depending on age. At the same time, in rats of the 3rd group, who received a complex of prophylactic drugs daily against the background of peroxidized oil, resorption of the alveolar bone was inhibited, since the degree of atrophy was significantly reduced ($P_1 < 0.05$).

Based on the data obtained, it is possible to draw conclusions about the antiresorption effectiveness of the developed complex containing vitamins, minerals and adaptogens.

This was confirmed by studies of the activity of proteinases and phosphatases in the femoral tissue. The results of these analyzes are summarized in Table 2. Modeling of periodontitis by long-term intake of peroxidized oil by rats causes a significant increase in the activity of proteolytic enzymes OPA and elastase ($P < 0.001$) in the tissue of the femur of animals. Since these enzymes are involved in the hydrolysis of the organic basis of bone tissue, an increase in their activity may indicate an intensification of bone tissue resorption processes under the influence of an alimentary excess of lipid peroxides.

Reproduction of pathology also led to an increase in alkaline phosphatase (ALP) activity by 48.5% ($P < 0.002$). At the same time, the activity of acid phosphatase increased by almost 40% ($0.05 < P < 0.1$), which indicates an increase in the processes of hydrolysis of the mineral components of bone tissue (Table 2). As for alkaline phosphatase, the activity of which reflects the functional activity of osteoblasts, which synthesize new bone tissue, the intensification of its activity can be explained by a compensatory response to the activation of bone tissue resorption enzymes.

Administration of the prophylactic complex to rats completely prevented an increase in the activity of elastase, alkaline phosphatase and OPA, which means the ability of the studied drugs to inhibit pathological resorption of bone tissue induced by excess

alimentary peroxides. The level of activity of all enzymes in bone tissue corresponded to that in intact rats (Table 2). The results obtained on the activity of enzymes in the bone tissue can also explain the inhibition of the degree of atrophy of the alveolar bone in rats receiving the prophylactic complex against the background of periodontitis modeling.

Table 2. Activity of proteinases and phosphatases in homogenates of the femur of rats in modeling periodontitis and its prevention

The investigated indicators	Groups of rats, n = 8		
	Intact	Periodontitis model	Periodontitis model + Prophylactic complex
OPA, nkat / kg	14,93 ± 1,12	24,10 ± 2,15 P < 0,001	17,35 ± 1,50 P > 0,25 P! < 0,02
Activity elastase, mk-cat / kg	10,62 ± 1,07	15,64 ± 0,55 P < 0,001	11,33 ± 0,99 P > 0,6 P! < 0,01
ALP activity, µat / kg	29,0 ± 2,5	43,08 ± 3,68 P < 0,002	32,88 ± 2,74 P > 0,2 P! < 0,05
CF activity, µat / kg	2,13 ± 0,29	2,96 ± 0,29 0,05 < P < 0,1	2,70 ± 0,24 P > 0,2 P! > 0,6

Note. P - reliability of differences from the indicator in the intact group; P! - reliability of differences from the indicator in the group "model of periodontitis".

Long-term consumption of lipid peroxides had a negative impact on certain parameters in the blood serum of rats (Table 3). During the reproduction of periodontitis in the blood serum, a significant increase in APA (P < 0.002) was noted, which may indicate the development of a general inflammatory reaction in the body of animals. The content of trypsin inhibitor (IT) in the blood serum of rats with periodontitis did not change (P > 0.2). Nevertheless, the index, indirectly reflecting the state of nonspecific resistance of IT / OPA in rats with periodontitis, decreased from 0.27 to 0.17. Against this background, the development of pathology was accompanied by an intensification of lipid peroxidation (LPO), which was concluded by a significant increase in the MDA level in the blood serum of animals with periodontitis (P < 0.001), as well as a decrease in the activity of the body's antioxidant defense in connection with a significant decrease in the activity of the main antioxidant enzyme catalase (P < 0.05). As a result, the API index, which characterizes the state of the antioxidant-prooxidant system, has almost halved (Table 3).

Table 3. Indicators of antioxidant-prooxidant and proteinase-inhibitory systems in the blood serum of rats during the modeling of periodontitis and its prevention

The investigated indicators	Groups of rats, n = 8		
	Intact	Model periodontitis	Periodontitis model + prophylactic complex
OPA, nkat / kg	1,95 ± 0,20	3,04 ± 0,23 P < 0,002	2,06 ± 0,18 P > 0,7 P! < 0,002
Trypsin inhibitor content, g / l	0,528 ± 0,002	0,518 ± 0,007 P > 0,2	0,526 ± 0,008 P > 0,8 P! > 0,5
IT / OPA	0,27	0,17	0,25
Catalase activity, µat / l	0,231 ± 0,018	0,172 ± 0,020 P < 0,05	0,210 ± 0,22 P > 0,5 P! > 0,2
MDA content, mmol / l	0,73 ± 0,06	1,06 ± 0,04 P < 0,001	0,76 ± 0,03 P > 0,7 P! < 0,001
API	3,16	1,62	2,76

Note. P - reliability of differences from the indicator in the intact group; P! - reliability of differences from the indicator in the group "model of periodontitis"

Prevention of periodontitis by daily administration of the complex (Bioaron, Alphabet, Calcior and Quertulin) to rats significantly improved the disturbed parameters in the blood serum of animals. Thus, under the influence of the prophylactic complex, OPA, catalase activity and MDA content normalized. The IT / OPA and API indices also corresponded to the level in healthy rats (Table 3). The data of the analysis of blood serum of animals confirm the prophylactic effect of the proposed complex of drugs showing anti-inflammatory and antioxidant efficacy under conditions long-term consumption of lipid peroxides with food.

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Modeling of pathology also caused biochemical disturbances in the tissues of the gums of animals (Table 4). All investigated markers of inflammation significantly increased in homogenates of the gums of rats that received peroxidized oil with the diet for 1 month: elastase activity - by 35.3%, CP activity - by 33.5%, MDA content - 113.1%. The established changes indicate the activation of inflammatory processes, LPO and disruption of the integrity of the cell membranes of the gum tissue. Against this background, the activity of catalase ($P > 0.4$), and hence the antioxidant protection, slightly decreased, which confirms the decrease in the API index by 2.37 times (Table 4).

Table 4. Parameters of inflammation and antioxidant system in rat gingival homogenates in modeling periodontitis and its prevention

The investigated indicators	Groups of rats, n =		
	Intact	Model periodontitis	Periodontitis model + prophylactic complex
Elastase activity, mkat / kg	0,051 ± 0,005	0,069 ± 0,005 $P < 0,02$	0,060 ± 0,005 $P > 0,25 P_1 > 0,25$
Acidic activity phosphatase, μ at / kg	12,79 ± 1,10	17,08 ± 1,57 $P < 0,05$	13,29 ± 1,41 $P > 0,8$ $0,05 < P_1 < 0,1$
Content of MDA, mmol / kg	13,45 ± 1,12	28,66 ± 2,74 $P < 0,001$	17,35 ± 0,98 $P < 0,02 P_1 < 0,001$
Catalase activity, mkat / kg	5,47 ± 0,51	4,90 ± 0,42 $P > 0,4$	5,10 ± 0,48 $P > 0,6 P_1 > 0,7$
API	4,06	1,71	2,94

Note. P - reliability of differences from the indicator in the intact group; P1 - reliability of differences from the indicator in the group "periodontitis model".

In the gum tissue of rats, which were daily injected with a complex of adaptogens, vitamins and minerals against the background of periodontitis modeling, the activity of elastase and CP as a result of a decrease corresponded to the level in intact rats. At the same time, the MDA content, despite a significant drop under the influence of the prophylactic complex ($P_1 < 0.001$), remained high compared with that in healthy animals ($P < 0.02$). The API index in the gum tissue of the rats treated with the complex of drugs also increased, but did not reach the normal level (Table 4).

Conclusions.

1. Alimentary excess of lipid peroxides leads to the activation of resorption processes of both mineral and organic parts of bone tissue, induces inflammation and lipid peroxidation in the gum tissue and the whole body.

2. The proposed complex of adaptogens, vitamins and minerals (bioaron, alphabet, calcior and quertulin) effectively prevents the established violations of biochemical parameters in blood serum, gum and femur tissues. As a result of the use of the drugs, the resorption of the alveolar bone of rats is significantly inhibited, the nonspecific resistance and antioxidant status of animals increase in conditions of an alimentary excess of lipid peroxides.

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