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Реферати

ЕКСПЕРИМЕРТАЛЬНО-МОРФОЛОГІЧНЕ ОБГРУНТУВАННЯ ОБЛІТЕРАЦІЇ ТРУБЧАСТИХ БІОЛОГІЧНИХ СТРУКТУР ШЛЯХОМ ВИСОКОЧАСТОТНОГО ЕЛЕКТРОЗВАРЮВАННЯ ТКАНИН

Кобзар О.Б., Дорошенко С.В., Пархоменко М.В., Хворостяна Т.Т., Примаченко В.І.

В експерименті на трупному матеріалі проведено дослідження впливу високочастотного струму ендоскопічним способом на змодельований канал нориці травного каналу. Визначені оптимальні параметри генератора ЕКВЗ-300 Патонмед необхідні для руйнування слизової оболонки. Морфологічними макро- та мікроскопічними методиками підтверджено ефективність впливу струму для руйнування слизової оболонки нориці.

Ключові слова: електрозварювання біологічних тканин, морфологічні зміни, кишкова нориця.

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ЕКСПЕРИМЕРТАЛЬНО-МОРФОЛОГИЧЕСКОЕ ОБОСНОВАНИЕ ОБЛИТЕРАЦИИ ТРУБЧАТЫХ БИОЛОГИЧЕСКИХ СТРУКТУР ПУТЕМ ВЫСОКОЧАСТОТНОЙ ЭЛЕКТРОСВАРКИ ТКАНЕЙ

Кобзарь А.Б., Дорошенко С.В., Пархоменко М.В., Хворостяная Т.Т., Примаченко В.И.

В эксперименте на трупном материале проведено исследование влияния высокочастотного тока эндоскопическим воздействием на смоделированный канал свища пищеварительного тракта. Определены оптимальные параметры генератора ЕКВЗ-300 Патонмед необходимые для разрушения слизистой оболочки. Морфологическими макро- и микроскопическими методиками подтверждена эффективность воздействия тока для разрушения слизистой оболочки свища.

Ключевые слова: электросварка биологических тканей, морфологические изменения, кишечные свищи.

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INVESTIGATION OF CALCIUM- AND HYALURONIC ACID-CONTAINING DRUGS OSTEOPLASTIC ACTIVITY IN RATS WITH PERIODONTITIS

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The aim of the research was to investigate the effect of subgingival injection of the solutions of calcium and hyaluronic acid on the condition of the bone tissue of the alveolar process of the lower jaws of rats with experimental periodontitis. The conducted experimental trials confirmed the intensification of the resorption processes in the jaw bone tissue of rats, which were fed peroxidized oil for a long time. Thus, the alimentary intake of lipid peroxides led to an increase in atrophy of the alveolar process of the lower jaw of rats and the loss of calcium in the bones of the jaws. Hydroxyapatite injections had a slight effect on the studied parameters, since the atrophy of the alveolar bone could not be slowed down, and the biochemical indicators, despite some improvements, did not reach normal values. Calcium in combination with unstabilized hyaluronic acid turned out to be much more effective. The authors insisted that further experimental trials are required in order to determine the frequency of subgingival calcium injections of calcium hydroxyapatite with non-stabilized hyaluronic acid.

Key words: experimental periodontitis, bone tissue, blood, hyaluronic acid, calcium hydroxyapatite, subgingival calcium injections.

This work is a fragments of the research project "To study disorders of mineralization and collagen formation in oral cavity in dental pathology and to improve the methods of these disorders early diagnosis and correction", state registration No. 0116U004077.

The bone tissue is a special type of connective tissue and the main component of human bones [9, 10]. The body is constantly undergoing renewal processes and the bone tissue is not an exception. Diseases, injuries or age-related changes can disrupt these processes and lead to the changes in the bone tissue, which can adversely affect the quality of life. For example, resorption of the bone tissue of the facial skeleton with age progression leads to changes in the individual bone structures and to a shift in the attachment of muscles and ligaments and, as a result, to the age-related changes in the face [14].

The possibility of strengthening the structure of the bone tissue would solve many questions in various fields of medicine (traumatology, dentistry, aesthetic medicine, and others). Is there any opportunity to strengthen the bone structure from the outside? Let us try to figure it out.

The inorganic composition of bone tissue includes hydroxyapatite and calcium phosphate crystals, and the composition of the organic matrix of the bone tissue includes collagen and glycosaminoglycans. It is believed that glycosaminoglycans are directly related to the ossification [7].

The purpose of the work was to study the effect of subgingival injection of the solutions of calcium and hyaluronic acid on the state of the bone tissue of the alveolar process of the lower jaws of rats with experimental periodontitis.

Materials and methods. The study was conducted on 63 Wistar rats of the herd breeding, females aged 6-7 months with an average weight of 285±34 g. The animals were randomized into 4 groups:

1. Intact rats
2. Rats with periodontitis
3. Rats with periodontitis who received calcium hydroxyapatite injections
4. Rats with periodontitis who received "calcium hydroxyapatite + non-stabilized hyaluronic acid" injections

Experimental periodontitis was reproduced by adding peroxidized sunflower oil to the rats' meal at the rate of 1 ml per animal per day [12].

The following medications were used in the experiment:

• Calcium hydroxyapatite - Crystalys ("Luminera" company, Israel)

• Calcium hydroxyapatite and non-stabilized hyaluronic acid - Crystalys + Luminera Hydryal 2% (("Luminera" company, Israel).

The medications were administered once a day at a dose of 0.1 ml on the 21st day of modeling of the pathology of periodontitis in the gums of the lower molars of rats.

The rats were removed from the trials with the use of thiopental anesthesia by heart bleeding in three stages: 2 weeks, 4 weeks and 6 weeks after the medications' injections. The lower jaw was allocated and thoroughly cleaned of soft tissues. The method of biometrics was used to calculate the degree of atrophy of the alveolar process by determining the linear dimensions of the exposure of the roots of molars [6].

The determination of the calcium content in the jaw bone hydrolyzate (50 mg/25 ml 0.2 n HCl) was carried out via the use of arsenase reagent according to [12]. In bone homogenates (75 mg / ml 0.1 n citrate buffer pH 6.1), elastase activity was determined via hydrolysis of N-t-BOC-L-alanin-p-nitrophenyl ester [11].

The data obtained were analyzed and calculated via One-way Analysis criteria (standard ANOVA method) [3]. $P<0.05$ was used as the statistically significant difference criteria.

Results of the study and their discussion. First of all, it should be noted that the duration of the full cycle of "resorption-formation" of bone tissue, including jaws, takes about 150 days, i.e. 2-3 cycles per year, while in rats the duration of this cycle is 40 days (9 cycles per year), i.e. three times as intense. As a result, 2 months of modeling of periodontal pathology in rats is equivalent to the semiannual duration of the same process in humans.

Table 1 shows the data for determining the degree of atrophy of the alveolar process of rats. The assessment of the dystrophic process in the alveolar bone of experimental animals showed a significant increase in the degree of atrophy in the group with peroxide periodontitis by 38.5% after 7 weeks ($p<0.05$) and by 47.2% after 10 weeks ($p<0.001$). The obtained results allow us to make conclusions about the activation of resorption processes in the bone tissue of the lower jaws of rats under the influence of lipid peroxides obtained from food, which is aggravated over time (table 1).

Table 1

Effect of calcium and hyaluronic acid on the degree of atrophy of the alveolar process of the lower jaw of rats with peroxide periodontitis (%)

N	Groups	4 weeks after injections (7 weeks of periodontitis)	6 weeks after injections (9 weeks of periodontitis)
1	Intact	26.5±2.2	
2	Periodontitis	36.7±1.9 $p<0.05$	39.0±2.0 $p<0.001$
3	Periodontitis + hydroxyapatite	33.2±2.2 $p<0.05$ $p_1>0.05$	36.3±2.0 $p<0.01$ $p_1>0.05$
4	Periodontitis + calcium + HA non-stabilized	30.2±1.8 $p>0.05$ $p_1<0.05$	32.6±1.4 $p<0.05$ $p_1<0.05$

Notes: p – represents the significant differences on the investigated indexes in comparison with the intact group; p_1 – represents the significant differences on the investigated indexes in comparison with the group of rats with periodontitis (ANOVA statistical criteria).

Hydroxyapatite injections had no significant effect on the degree of atrophy of the jaws of rats of the 3rd group. This indicator remained high and corresponded to the level in animals of the second group with peroxide periodontitis ($p_1>0.05$ after 4 weeks and $p_1>0.05$ – 7 weeks after injections).

Intensification of resorption processes in the mandible bone tissue of rats with periodontitis prevented one-time subgingival administration of calcium in combination with non-stabilized hyaluronic acid to rats of the 4th group. This fact contributed to the inhibition of resorption of the alveolar process 4 weeks after the injection: the rate of atrophy corresponded to that of healthy animals ($p>0.05$ and $p_1<0.05$).

Taking the above data into consideration, the study on the 9th week of periodontitis modeling or 6 weeks after the injection of calcium and non-stabilized hyaluronic acid showed the inability of this composition to completely prevent the active resorption of the jaw bone tissue induced by the alimentary intake of lipid peroxide. It is worth noting that the index of the degree of atrophy of the alveolar process in rats of the 4th group at the final stage of the study was significantly lower than that of the rats with periodontitis by 19.6% ($p<0.05$).

At the same time it exceeded the values in intact animals by 18.7% ($p_1<0.05$), which allowed us to speak about the frequency of such injections once every 4 weeks in rats to get the best result. Nevertheless, the indicator of the degree of atrophy of the alveolar process in rats of the 4th group at the final stage of the study was significantly lower than that of the rats with periodontitis by 19.6% ($p<0.05$), while at the same time exceeding the values in intact animals by 18.7% ($p_1<0.05$). The abovementioned data allowed us to speak about the frequency of such injections to rate once in every 4 weeks in order to get the best result (fig. 1).

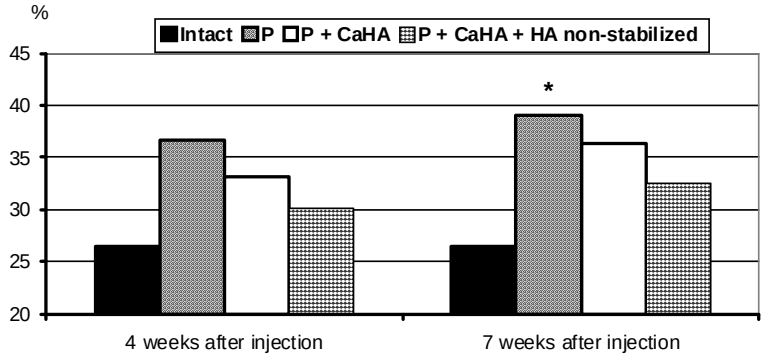

Fig. 1. The value of rats alveolar processes atrophy.

The modeling of periodontitis with the help of an alimentary excess of peroxides leads not only to atrophy of the alveolar bone, but also to a sharp decrease in the level of calcium in the bone tissue of the lower jaws of rats. Consequently, this indicator is decreased by 21.6% after 5 weeks ($p<0.02$), by 31.2% – after 7 weeks ($p<0.01$) and by 48.4% – after 9 weeks of pathology modeling ($p<0.001$, table 2). These results confirm the intensification of the resorption processes in the bone tissue of the jaws of rats, which had been fed peroxidized oil for a long time.

Table 2

Calcium content in the bone tissue of the lower jaws of rats with peroxide periodontitis after administration of calcium and unstabilized hyaluronic acid (mol/kg)

N	Groups	2 weeks after injections (5 weeks of periodontitis)	4 weeks after injections (7 weeks of periodontitis)	6 weeks after injections (9 weeks of periodontitis)
1	Intact	2.547±0.100		
2	Periodontitis	1.996±0.130 p<0.05	1.750±0.143 P<0.01	1.314±0.151 p<0.001
3	Periodontitis + hydroxyapatite	2.260±0.191 p>0.05 p_1>0.05	2.175±0.085 p<0.05 p_1<0.05	1.907±0.184 p<0.05 p_1<0.05
	Periodontitis + calcium + HA non-stabilized	2.357±0.223 p>0.05 p_1> 0.05	2.228±0.052 p<0.05 p_1<0.05	2.174±0.269 p>0.05 p_1<0.05

Notes: p – represents the significant differences on the investigated indexes in comparison with the intact group; p_1 – represents the significant differences on the investigated indexes in comparison with the group of rats with periodontitis (ANOVA statistical criteria).

Injection of hydroxyapatite to rats of group 3 somewhat inhibited the loss of calcium by the alveolar bone. 2 weeks after the injections with this medications, the calcium level corresponded to normal values, but also did not differ significantly from that of the rats with periodontitis ($p>0.05$ and $p_1>0.05$, table 2).

4 and 6 weeks after the administration of hydroxyapatite, the calcium content in the alveolar bone significantly exceeded that of the rats with periodontitis ($p_1<0.05$) but did not reach the values of intact animals ($p<0.05$, table 2).

Effective prevention of calcium loss by the jaw bone tissue was registered after subgingival calcium injections in combination with unstabilized hyaluronic acid in rats of group 4. 2 weeks after the injection, this indicator had an intermediate value between the level in healthy rats and animals with periodontitis (p>0.05 and p>0.05).

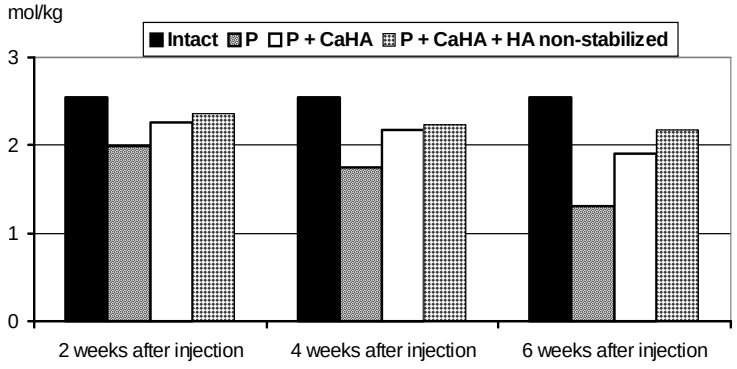

Fig. 2. The calcium content in the bone tissue of the lower jaws of rats.

It also worth mentioning that after 4 and 6 weeks after administration of the compositions of calcium with non-stabilized hyaluronic acid, the calcium content in the jaw bone tissue of rats significantly exceeded that of the rats with periodontitis (p_1<0.05) with no difference from normal values (p>0.1; fig. 2).

Biochemical study of enzymes in the jaws of experimental animals revealed metabolic disorders in the bone tissue of rats with periodontitis.

Elastase is the most active enzyme involved in the degradation of bone collagen. In addition, it is known that elastase activates procollagenase, turning it into the active form of the enzyme - collagenase, which greatly increases the intensity of destruction of bone collagen [1, 2, 4, 5]. The results of the study of the activity of elastase in the bone tissue of the jaws of rats with peroxide periodontitis are shown in the table 3.

Table 3

Effect of calcium-containing medications and non-stabilized hyaluronic acid on the activity of elastase in the bone tissue of the lower jaw of rats with peroxide periodontitis (mkkat/kg)

N	Groups	2 weeks after injections 5 weeks of periodontitis)	4 weeks after injections (7 weeks of periodontitis)	6 weeks after injections (9 weeks of periodontitis)
1	Intact	15.26±1.09		
2	Periodontitis	24.98±1.36 p<0.01	28.83±1.89 p<0.001	26.96±2.96 p<0.001
3	Periodontitis + hydroxyapatite	18.91±2.82 p>0.05 p_1> 0.05	27.20±2.89 p<0.01 p_1>0.05	20.03±1.82 p<0.05 p_1>0.05
4	Periodontitis + calcium + HA non-stabilized	16.98±2.12 p>0.05 p_1<0.05	23.28±1.31 p>0.05 p_1<0.05	18.55±1.67 p>0.05 p_1<0.01

Notes: p – represents the significant differences on the investigated indexes in comparison with the intact group; p_1 – represents the significant differences on the investigated indexes in comparison with the group of rats with periodontitis (ANOVA statistical criteria).

5 weeks after consumption of peroxidized oil by rats, elastase activity in the jaw bone tissue increased by 63.7% (p<0.01). Over the subsequent periods of observation, the activity of this destructive bone tissue enzyme increased to a greater extent - by 88.9% (p<0.001) – after 7 weeks and by 76.7% (p<0.001) – after 9 weeks of pathology modeling (table 3).

The injection of hydroxyapatite prevented an increase in the activity of elastase in the jaw bone tissue of rats with periodontitis only 2 weeks after the injection itself: this indicator corresponded to the level in healthy animals (p>0.05), but did not differ significantly from the values in rats with periodontitis (p_1>0,05). On subsequent periods of the study, the activity of elastase in the jaw bone tissue of rats that were injected with hydroxyapatite was high and corresponded to the level in animals with periodontitis (p_1>0.05), which indicates the low ability of hydroxyapatite to inhibit the destruction of bone tissue (table 3).

Calcium injections with non-stabilized hyaluronic acid had a prominent inhibitory effect on the destruction of bone collagen in the jaws of rats with periodontitis. The elastase activity in rats of the 4th group did not exceed the values in healthy animals (p>0.05) and was lower than that of rats with periodontitis (p_1<0.05). This fact indicates a more pronounced ability of the calcium composition with non-stabilized hyaluronic acid to inhibit the elastase activity, and hence the destruction of bone collagen induced by lipid peroxidation (table 3, fig. 3).

Therefore one can conclude that this research confirmed the intensification of the resorption processes in the jaw bone tissue of rats, which were fed peroxidized oil for a long time. Thus, the alimentary intake of lipid peroxides led to an increase in atrophy of the alveolar process of the lower jaw of rats and the loss of calcium in the bones of the jaws.

Our results are comparable with similar data obtained in studies [2] in which the degree of periodontal bone tissue atrophy (the lower jaw alveolar process) was investigated during the acute inflammatory process in animals modeling. The integrative indicators of periodontal atrophy in these rats (calculated by the acid/base phosphatases ratio activities and calcium concentration in the bone tissue) and the number of decay-induced lesions increased significantly (approximately per 2.5-2.7 times) and were equal to 27.9±1.0% and 5.9±0.5 (for decay rate). Therefore, one could say that, firstly, the model we used is adequate to analogous clinical condition. Secondly, we obtained the comparable data in experimental conditions of the bone tissue destruction. Finally, the subsequent results showed rat's lower jaw bone tissue functional activity normalization under the influence of calcium-containing drugs.

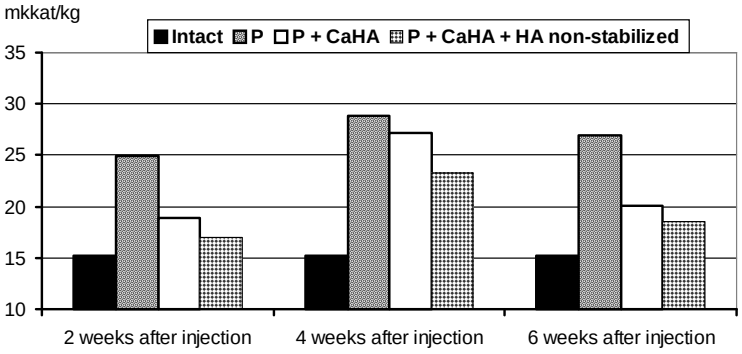

Fig. 3. The expression of elastase in the bone tissue of the lower jaws of rats.

It is also of interest that equivalent data were obtained in conditions of prednisone-induced periodontitis in rats. These data revealed the index of bone tissue mineralization in the experimental conditions was reduced on 39% and equal to 51.0±1.1%. The degree of bone tissue mineralization in such experimental conditions was equal to 4.62±0.37 g/g that was approximately 9% less pertaining with the same control index [10, 13]. Our results nevertheless showed an expressed calcium-containing drugs osteotropic efficacy if compared with the data in case of bioflavanoid quertulin administration.

Our data revealed slight effect of hydroxyapatite injections on the investigated parameters since the atrophy of the alveolar bone could not be slowed down, and the biochemical indicators, despite some improvements, did not reach normal values.

The administration of calcium in combination with unstabilized hyaluronic acid had much better effects. Despite the fact that the degree of atrophy of the alveolar process decreased only at the initial stage after injection, the calcium content was brought to almost normal values. Apparently, the calcium level was restored due to the inhibition of the activity of destructive enzymes of the jaw bone elastase under the influence of calcium in combination with unstabilized hyaluronic acid.

Calcium-containing drugs injection with aim of periodontal bone tissue mineralization increase can also have a clinical perspective in the conditions of metabolic syndrome since it has been shown the significant bone tissue reduce due to an approximately twofold increase of the connective tissue density [8]. In this case one could conclude that our experimental trials have a clinical prospect for patients with expressed osteodestruction phenomena in conditions of diabetes mellitus, obesity and in other symptom complexes combined into the metabolic syndrome concept.

Conclusions

1. Long-term alimentary intake of peroxidized oil led to the activation of resorption processes in the jaws: an increase in atrophy of the alveolar process, loss of calcium and an increase in the activity of destructive bone tissue enzymes elastase.

2. Injection of calcium hydroxyapatite to rats with periodontitis did not significantly affect the pathological processes in the jaw bone tissue.

3. According to the results of our research, the single injection of calcium with unstabilized hyaluronic acid was a much more effective method of inhibiting the atrophy of the alveolar process and normalizing the intensity of the processes of mineralization and destruction of the jaw bone of rats with periodontitis.

4. We consider it necessary to conduct additional studies to determine the frequency of subgingival calcium injections of calcium hydroxyapatite with non-stabilized hyaluronic acid.

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Реферати

ДОСЛІДЖЕННЯ ОСТЕОПЛАСТИЧНОЇ АКТИВНОСТІ ЛІКАРСЬКИХ ЗАСОБІВ, ЯКІ МІСТЯТЬ КАЛЬЦІЙ І ГІАЛУРОНОВУ КИСЛОТУ, У ЩУРІВ З ПЕРІОДОНТИТОМ

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Метою дослідження було дослідження впливу субгінгівального введення розчинів кальцію і гіалуронової кислоти на стан кісткової тканини альвеолярного відростка нижньої щелепи щурів при експериментальному періодонтиті. Проведені експериментальні дослідження підтвердили інтенсифікацію процесів резорбції в кістковій тканині щелепи щурів, яким давали в їжу перекисну олію протягом тривалого часу. Таким чином, харчове споживання перекисів ліпідів призводило до збільшення атрофії альвеолярного відростка нижньої щелепи щурів і втрати кальцію в кістках щелеп. Ін'єкції гідроксиапатиту надали невеликий вплив на досліджувані параметри, оскільки атрофію альвеолярної кістки не можна було уповільнити, а біохімічні показники, незважаючи на деякі поліпшення, що не досягли нормальних значень. Кальцій в поєднанні з нестабілізованою гіалуроновою кислотою виявився набагато більш ефективним. Автори наполягають на необхідності подальших експериментальних досліджень для визначення частоти субгінгівальних ін'єкцій гідроксиапатиту кальцію з нестабілізованою гіалуроновою кислотою.

Ключові слова: експериментальний періодонтит, кісткова тканина, кров, гіалуронова кислота, гідроксиапатит кальцію, субгінгівальние ін'єкції кальцію.
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ИССЛЕДОВАНИЕ ОСТЕОПЛАСТИЧЕСКОЙ АКТИВНОСТИ ЛЕКАРСТВЕННЫХ СРЕДСТВ, СОДЕРЖАЩИХ КАЛЬЦИЙ И ГИАЛУРОНОВУЮ КИСЛОТУ, У КРЫС С ПЕРИОДОНТИТОМ

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Целью работы было изучение влияния субгингивального введения растворов кальция и гиалуроновой кислоты на состояние костной ткани альвеолярного отростка нижней челюсти крыс при экспериментальном периодонтите. Проведенные экспериментальные исследования подтвердили интенсификацию процессов резорбции в костной ткани челюсти крыс, которым давали в пищу перекисное масло в течение длительного времени. Таким образом, пищевое потребление перекисей липидов приводило к увеличению атрофии альвеолярного отростка нижней челюсти крыс и потере кальция в костях челюстей. Инъекции гидроксиапатита оказали небольшое влияние на исследуемые параметры, поскольку атрофию альвеолярной кости нельзя было замедлить, а биохимические показатели, несмотря на незначительные улучшения, не достигли нормальных значений. Кальций в сочетании с нестабилизированной гиалуроновой кислотой оказался гораздо более эффективным. Авторы настаивают на необходимости дальнейших экспериментальных испытаний для определения частоты субгингивальных инъекций гидроксиапатита кальция с нестабилизированной гиалуроновой кислотой.

Ключевые слова: экспериментальный периодонтит, костная ткань, кровь, гиалуроновая кислота, гидроксиапатит кальция, субгингивальные инъекции кальция.
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