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MEDICINE AND PHISIOLOGY

EFFICIENCY OF USING RECOMBINANT MORPHOGENETIC PROTEIN IN PATIENTS WITH AGGRESSIVE (RAPIDLY -PROGRESSING) GENERALIZED PERIODONTIS

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Annotation. *Currently, the inclusion in the complex therapy of generalized periodontitis of drugs that significantly affect the processes of physiological remodeling and bone regeneration is becoming more common.*

Given the relevance of the search for drugs to restore periodontal bone structures, the aim of our study was to increase the effectiveness of standard therapy for aggressive (rapidly progressing) generalized periodontitis by additionally incorporating the morphogenetic bone protein rhbmp-2 into the generally accepted treatment complex.

We observed a contingent of patients in the amount of 61 people, with a diagnosis of aggressive (rapidly progressing) generalized periodontitis, which were divided into the main - 30 people, and the comparison group - 31 people. we used standard clinical, paraclinical and laboratory research methods, supplemented by dental volumetric tomography. In the main group, patients in addition to the standard treatment regimen (comparison group) included the recombinant morphogenetic protein rhbmp-2.

The results of a clinical examination conducted after 6-12 months revealed the absence of inflammatory phenomena in periodontium in 90% of the main group, and only 77.4% of patients in the comparison group. Measurements of bone density on the hounsfield scale (hu) in the same period showed a 2-fold increase in the density of periodontal bone structures, with a comparison group. it follows that the inclusion in the standard regimen of complex treatment for patients with rapidly progressing generalized periodontitis of the osteoinductive drug rhbmp-2 allows for long-term clinical and radiological remission, and creates conditions for the subsequent restoration of the density of periodontal bone structures.

Keywords: *Periodontitis, Aggressive (Rapidly Progressive) Periodontitis, Rhbmp-2, Recombinant Morphogenetic Protein, Physiological Bone Remodeling, Bone Tissue Regeneration, Treatment Of Periodontitis.*

Introduction. *The problem of aggressive (rapidly progressive) generalized periodontitis treatment currently is the one of the most actual and complicated in modern dentistry. Disease in a short period of time leads to the destruction of bone structures in the periodontal complex, which becomes the leading reason for the significant loss of*

intact teeth of young and adult people. [6, 10, 11]

Currently, the increasing prevalence in the complex treatment of aggressive generalized periodontitis is receiving medications that significantly affect the processes of bone remodeling, capable of inhibiting bone resorption and increasing the process of bone regeneration. [2, 7, 9]

In the literature, there are a few details about the positive therapeutic effect of the application of osteoinductive agents in the treatment of generalized periodontitis, regardless of its severity and symptoms. Recommended of using Etidronic acid (Xidiphon), Alfacalcidol, ossein-hydroxyapatite compound (Osteogenon), calcium gluconate and vitamin D3 (Calcium-D3 Nycomed Forte, Vitacalcin), nanodiscrete mechanically activated amorphous calcium salt of gluconic acid, Strontium ranelate, Selenium and other's. [1, 4, 8]

Attempts to improve and include numerous of osteoinductive agents in the complex therapy of aggressive generalized periodontitis indicate that the problem of optimization mineralization processes in osteoporosis area and recover bone structures in the periodontal complex, extremely relevant and not fully resolved. Nowadays we need to search for new approaches and osteotropic drugs that improve the effectiveness of traditional methods and normalize bone metabolism. [3, 5]

In this regard, a number of authors in the last decade have been paying attention to new osteoinductive drugs that can actively influence bone regeneration. These includes recombinant bone morphogenetic protein BMP-2, BMP-7, BMP-15, and others who come in TGF- β superfamily of growth factors. [14, 15]

Using recombinant human bone morphogenetic protein (rhBMP-2) is due to the fact that it's "natural" for the body, contained in bone tissue, cartilage and connective tissue where it controls and determines the intensity of physiological remodeling and regeneration of bone tissue. [12, 13]

In literature there are a few data of the effective using of recombinant human bone morphogenetic proteins in the complex treatment of aggressive generalized periodontitis. The largest number of publications is devoted to the use of rhBMP-2 to activate the reparative function of osteogenesis in cases fractures of tubular and cancellous bone tissues. [12, 13, 14, 15]

All of the above has determined the purpose of this study and its relevance. Objective: increasing the efficiency of standard therapy aggressive generalized periodontitis by additionally including the morphogenetic bone protein rhBMP-2 in the conventional medical complex.

Material and research methods. We observed 61 patients with a diagnosis of aggressive (rapidly progressive) generalized periodontitis of I-II severity aged from 35 to 50 years. Among of them was 41 (67.2%) women and 20 (32.8%) men. The average age was $47.6 \pm 2,4$ years.

For conduct a comparative analysis of the functional state of the bone tissue was formed control group of 20 gender matched and age volunteers people, without dental diseases, which could have an effect on bone remodeling.

All subjects were included in the study only after signing an informed consent to carry out the planned clinical, laboratory and therapeutic measures.

Diagnosis verification of aggressive generalized (rapidly progressive) periodontitis was carried out on the basis of the analysis and results of clinical and radiological examinations and dynamic observations in accordance with the criteria of classifications inflammatory periodontal diseases (Mashchenko I.S., 2003; Danilevsky N.F., Borisenko A.V., 1999).

All patients underwent a full clinical examination, including the assembling of complaints, anamnestic information, and the determination of the objective state of periodontal status using gingival and periodontal indexes.

The oral hygiene index was studied according to the method of Green VC Vermilion, 1964. The intensity and prevalence of the inflammatory process in the gingival tissue was assessed by the severity of bleeding (Muhlemann H, Cowell J., 1975) and taking into account the values of the papillary-marginal alveolar test (Parma, 1960) The severity of inflammatory-destructive changes in periodontal complex was established using the periodontal index (PI) proposed by Russel AL (1961).

A comprehensive assessment of the prevalence and severity of destructive processes in the bone structures in the periodontium complex was determined based on the results of "Florida Probe system" and with X-ray examination using orthopantomography and computer tomography.

Orthopantomography was performed on the Planmeca PRO ONE device (Finland), dental digital volumetric computer tomography on the Planmeca PRO MAX 3D device.

Using computer tomography, we determined changes in bone density in lesions of Hounsfield index where values <300 units regarded as the presence of an active osteoporotic process in the bone tissue of the alveolar processes.

State of remodeling - the intensity of the processes of bone resorption and bone formation was established on the content of bone turnover markers. Markers of bone formation include Osteocalcin, the bone fraction of alkaline phosphatase, and markers of the resorptive process are the C-terminal telopeptides of type I collagen (β -Cross Laps) and the bone isoform of acid phosphatase.

The finding of the β -Cross Laps was carried out by the method of enzyme-linked immunosorbent assay using the Metra Bar EIE Kit from Quidel Corp. and "B. Cross Laps ELISA" from Nordic Bioscience Diagnostic A/S, respectively. The determination and calculation procedures were carried out in accordance with the attached instructions. The Cross Laps method is based on the use of antibodies against a synthetic octapeptide, which is identical to the C-telopeptide of type I collagen that is formed during collagen degradation under the influence of cathepsin K of osteoclasts and matrix metalloproteinase.

To study Osteocalcin (OC), an Chemiluminescent immunoassay (CLIA) was used using an automatic electro chemiluminescence immunochemical analyzer "ELEESVS-2010" (Roche Diagnostics GmbH).

In accordance with the purpose of this work, the observed patients were divided into two groups: the main group - 30 people, and a comparison group - 31 people.

At the main group in the standard (protocol) of treatment was included combination of proteolytic enzymes Wobenzym®, and rhBMP-2 that was used to increase bone tissue regeneration. The rhBMP-2 drug in the generally accepted dosage was injected twice into the sub periosteum space of the alveolar bone with an interval of 7-8 days.

In the comparison group, a similar treatment was used with the same regimen for the entire complex of therapeutic measures, but without rhBMP-2 and Wobenzym® prescription.

At the initial stage patients were trained in the rules of controlled oral hygiene. Then professional hygienic interventions were performed aimed at eliminating local traumatic factors (treatment of carious cavities, elimination of inlay and prosthetics defects, restoration of interdental contacts, correction of traumatic occlusion, removal of dental plaque).

According to the indications, curettage of periodontal pockets was performed, surgical interventions - gingivotomy or flap surgery, depending on the depth of periodontal pockets and the length of the defect in bone structures.

As the local antibacterial therapy using preparations containing chlorhexidine (0.05% chlorhexidine bigluconate, gel paste "Metrogil-Dent" or "Parahelium"), which amplifies the purpose inwardly Amoksiklava antibiotic (500 mg. 2 times a day, 5-7 days).

According to the generally accepted protocol, immunocorrective and antioxidant agents were also included in the treatment regimen for aggressive (rapidly progressive) generalized periodontitis into comparison group (Polyoxidonium, 1 tablet per day, Mexidol, 2 tablets per day, lasting 10-12 days).

Mathematical data processing was carried out on a personal computer using the Statistica 6.1(serial number AGAR909E415822FA) software package and Microsoft Excel 2010 (license number 02260-018-0000106-48794). The program provided for the calculation of the average numerical characteristics of the clinical and laboratory indicators of certain series and the standard error of the mean: the assessment of the significance of different mean indicators in the named samples using the Student's t-test. Average values were expressed as $M \pm m$, where M is the average value of the indicator, m is the standard deviation. Differences between the compared indicators were taken as significant when the significance level was reached $p < 0.05$.

Research results and discussion. Based on the analysis of all clinical, paraclinical, radiological and laboratory studies carried out before treatment, it was found that the formed groups were identical and can be considered comparable. Thus, the purity of the revealed clinical symptoms and objective signs characterizing the activity of the inflammatory process in the gingival tissue turned out to be equivalent in patients of the main and compared groups. In addition, against the background of the same hygienic state of the oral cavity, recorded by the OHI-S hygiene index, the initial changes in the bleeding indices and PMA of both groups did not have significant differences (2.56 ± 0.2 points and 85.8 ± 2.9 point in patients of the main group versus 2.49 ± 0.2 points and 84.2 ± 3.0 points; $p > 0.05$).

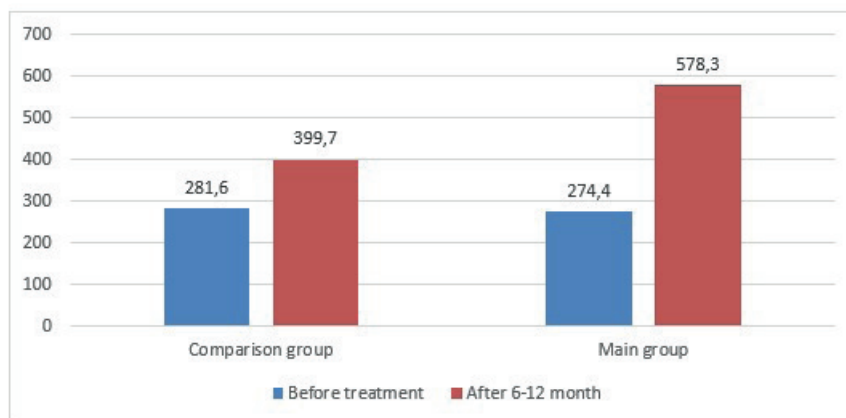
On admission to the clinic, values of the periodontal index (PI) in the primary study

averaged 5.3 ± 0.3 point's patients of the main group and 4.9 ± 0.3 points in the comparison group. These data showed that the severity of inflammatory and destructive process in patient's analyzed groups was identical. These conclusions were confirmed by the data obtained in the study of the state of the periodontal tissues with the Florida Probe system. The depth of periodontal pockets (in mm) patients of the analyzed groups were approximately equal and did not have a statistically significant difference ($p > 0.05$).

In studying the initial indicators of bone remodeling founded that patients of the main group and patients of comparison group value resorption and bone formation markers were not significantly different among themselves. Revealed increase in average concentration of C-terminal telopeptide of collagen type I (β Cross-Laps) in serum (or an average of 3.84 ± 0.3 pg/ml versus 3.81 ± 0.3 pg/ml) was significantly different from levels of healthy individuals (1.13 ± 0.3 pg/ml; $p < 0.05$). The fact of a pronounced increase in this marker of resorption can serve as one of the explanations for the rapid rate of the destructive process in the bone structures of the periodontal complex. Moreover, in patients of both groups, along with this, there was a significant decrease in the average values of osteocalcin (on average 22.9 ± 1.1 pg/ml), which indicated a decrease in the functional capacity of cells of the osteoblastic series against that.

The results of clinical examination conducted after 6-12 months of treatment revealed no inflammatory process in periodontal complex in 90% patients of the main group and in 77.4% of patient's comparison group noted the absence of bleeding and soreness of the gums, termination exudation in the periodontal pockets, improved tooth statics. The presence of clinical and radiological remission was confirmed by the achieved normalization of gingival and periodontal indices and an increase in the bone tissue density of the alveolar processes, revealed by computed volumetric radiographic tomography.

The measurements of tissue density according to the Hounsfield scale (HU) 6-12 months after treatment revealed the advantages of the developed complex used in patients of the main group over the protocol generally accepted method used in patients in the comparison group. The HU index bone tissue density of the alveolar processes in the named period of the study increased more than 2 times (on average up to 578.3 ± 10.6 units) in patients of the main group. At the same time, its increase in the comparison group was significantly lower (on average, up to 399.7 ± 12.4 units), which indicated incomplete elimination of the osteoporotic process and less significant restoration of the bone structures of the periodontal complex patient's receiving standard therapy. Besides dynamic determination changes of Hounsfield index (HU) showed that the inclusion in the standard therapy recombinant morphogenetic protein (rhBMP-2) promoted patients of the main group and more complete recovery expressed bone metabolism processes in a short time. (Schedule 1)



Schedule 1 HU index between main and comparing group before treatment and after 6-12 month

Immediately after treatment in patients of the main group, significant decreases in the levels of the C-terminal telopeptide of type I collagen (β -Cross Laps) and an increase in osteocalcin in the blood serum were revealed (Table 1). In most cases (90%) upon completion of treatment, patients of the group recorded by reconstitution of bone metabolism, and in this point 54.8% of patients in Group comparisons indicated only a slight reduction in bone resorption marker and a tendency to improve bone formation.

Table 1

Dynamics of bone remodeling markers in patients with rapidly progressing generalized periodontitis treated with conventional protocol therapy (comparison group) and receiving the developed treatment complex (main group)

Indicators of bone remodeling	Study groups					
	Comparison group (n=31)			Main group (n=30)		
	Before treatment	After 1 month	6 months after treatment	Before treatment	After 1 month	6 months after treatment
β - CL (pg/ml)	3.84±0.3	3.18±0.4*	2.8 ±0.3*	3.82±0.3	1.22±0.2***	1.59±0.2***
Osteocalcin (OC) (pg/ml)	11.4±0.3	14.8±0.4*	13.9±0.4*	10.9± 0,3	19.1±0.4***	18.4±0.4***

Note: * $p < 0.05$ - a significant difference with the indicators before treatment.

** $p < 0.05$ - significant difference between the groups of the studied.

The final analysis of the results of the study of bone remodeling processes showed that group of patients with aggressive (rapidly progressive) generalized periodontitis who received Wobenzym and rhBMP-2, average values of the β -CL resorption marker after treatment decreased by 3.2 times, osteocalcin increased by 1.7 times and had a significant difference in the levels of the group of patients treated with the improved

traditional method ($p < 0.05$). Patient's comparison group indicators of bone remodeling after treatment average changed as follows: serum concentration resorption marker β -CL decreased in 1.2 times and bone formation markers osteocalcin in 1.3 times (accordingly to 3.18 ± 0.4 pg/ml and 14.8 ± 0.4 pg/ml versus 1.22 ± 0.3 pg/ml and 19.1 ± 0.4 pg/ml; $p < 0.05$).

The analysis of the state of bone metabolism function after 6 months and more showed that the levels of markers of the bone resorption and bone formation in patients of the main group in this period of time are not subjected to negative dynamics, and did not differ significantly from those after treatment and almost matched healthy individuals. In the patients of the comparison group during this observation period, no significant ($p > 0.05$) further decrease in the concentration of the β -Cross Laps resorption marker and the content of osteocalcin in the blood serum was found, which illustrated the persistence of bone remodeling dysfunction in the subjects.

Thus, using developed method of treating patients with aggressive (rapidly progressive) generalized periodontitis showed high clinical, radiological and laboratory efficacy, exceeding in all clinical and laboratory parameters the therapeutic effect obtained in patients treated with the protocol conventional complex therapy.

Conflict of interest. The authors claim no conflict of interest.

Conclusions. In 100% of patients suffering from aggressive (rapidly progressive) generalized periodontitis, the method of digital volumetric dental tomography revealed regular changes in the density of periodontal bone tissue: the Hounsfield index (HU) is recorded below 300 units in most cases (96.6%) and indicate the presence of osteoporotic effect in the alveolar bone.

1. Inclusion in the protocol (standard) scheme of complex treatment of patients with aggressive (rapidly progressive) generalized periodontitis, osteoinductive human recombinant morphogenetic bone protein (rhBMP-2), allows to achieve long-term clinical and radiological remission in a greater number of persons (by 34.5%).

2. The developed method for the treatment of rapidly progressive generalized periodontitis has a targeted normalizing effect on the process of bone remodeling: it creates conditions for the subsequent restoration of the density of bone structures of the periodontium and an increase in the therapeutic effect.

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