

ANÁLISE DA EFICÁCIA DA IMUNOTERAPIA USANDO UM COMPLEXO AUTOLÓGICO DE IMUNOPEPTÍDEOS NO TRATAMENTO CIRÚRGICO DA PERIODONTITE**ANALYSIS OF THE EFFECTIVENESS OF IMMUNOTHERAPY USING AN AUTOLOGOUS COMPLEX OF IMMUNOPEPTIDES IN THE SURGICAL TREATMENT OF PERIODONTITIS****АНАЛИЗ ЭФФЕКТИВНОСТИ ПРИМЕНЕНИЯ ИММУНОТЕРАПИИ С ИСПОЛЬЗОВАНИЕМ АУТОЛОГИЧНОГО КОМПЛЕКСА ИММУНОПЕПТИДОВ ПРИ ХИРУРГИЧЕСКОМ ЛЕЧЕНИИ ПАРОДОНТИТА**

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RESUMO

A doença periodontal na população adulta é um dos problemas mais urgentes da odontologia em todo o mundo. Graças à introdução de modernas tecnologias na prática, foi possível identificar os principais mecanismos de desenvolvimento desta doença nos níveis molecular e genético. A interação de patógenos periodontais com fatores imunes da proteção antimicrobiana do corpo é a base da inflamação do tecido periodontal e leva ainda à destruição do osso alveolar. As células epiteliais da mucosa gengival desempenham um papel crucial contra bactérias patogênicas periodontais. Os fatores de imunidade inata desempenham um papel não apenas na proteção antimicrobiana, mas também apóiam as condições necessárias para a cura e regeneração dos tecidos periodontais. Portanto, várias abordagens terapêuticas que afetam os fatores de imunidade inata são consideradas efetivas e promissoras. O exame clínico e a determinação dos fatores de imunidade inata foram realizados em 115 pacientes. Indivíduos saudáveis compunham um grupo de 30 pessoas. Pacientes com periodontite crônica generalizada com diferentes graus de gravidade eram 85 pessoas. Cada paciente foi submetido à ortopantomografia no ortofantomógrafo Orthophosis XG-DS/Ceph (SIRONA Dental System GmbH, Alemanha) para avaliar o estado do tecido ósseo dos maxilares (o grau de destruição da camada cortical, o grau de reabsorção do interalveolar). Radiografias intraorais direcionadas foram usadas para avaliar o estado do tecido ósseo e a qualidade do tratamento cirúrgico. Os métodos imunológicos de pesquisa foram realizados em várias etapas. Os achados deste artigo afirmam que o uso de imunopeptídeos complexos autólogos no tratamento cirúrgico da periodontite reduz o tempo de obtenção de um efeito terapêutico 2 vezes, resultando no alívio rápido dos sintomas de inflamação e na aceleração dos processos reparativos.

Palavras-chave: *imunidade, periodontite, imunopeptídeos, retalhos, tecido ósseo.*

ABSTRACT

Periodontal disease in the adult population is one of the most pressing problems of dentistry around the world. Thanks to the introduction of modern technologies in practice, it was possible to identify the main mechanisms of the development of this disease at the molecular and genetic level. The interaction of periodontal pathogens with immune factors of antimicrobial protection of the body is the basis of periodontal tissue inflammation and further leads to the destruction of the alveolar bone. Epithelial cells of the gum mucosa play a crucial role against periodontal pathogenic bacteria. Factors of innate immunity play a role not only in antimicrobial protection, but they also support the conditions necessary for the healing and regeneration of periodontal tissues. Therefore, various therapeutic approaches that affect the factors of innate immunity are considered as effective and promising. Clinical examination and determination of factors of innate immunity were performed in 115 patients. Healthy individuals made up a group of 30 people. Patients with chronic generalized periodontitis with varying degrees of severity were 85 people. Each patient underwent orthopantomography on the orthopantomograph Orthophosis XG DS/Ceph (SIRONA Dental System GmbH, Germany) in order to assess the state of the bone tissue of the jaws (the degree of destruction of the cortical layer, the degree of resorption of the

interalveolar partitions). Targeted intraoral radiographs were used to assess the state of bone tissue and the quality of surgical treatment. Immunological methods of research were carried out in several stages. The findings of this article make the claim that the use of autologous complex immunopeptides in the surgical treatment of periodontitis reduces the time of achieving a therapeutic effect 2 times, resulting in the rapid relief of the symptoms of inflammation and acceleration of reparative processes.

Keywords: *immunity, periodontitis, immunopeptides, patchwork, bone tissue.*

АННОТАЦИЯ

Заболевания пародонта среди взрослого населения представляют одну из актуальных проблем стоматологии во всем мире. Благодаря внедрению в практику современных технологий, удалось выявить основные механизмы развития данного заболевания на молекулярно-генетическом уровне. Взаимодействие пародонтопатогенов с иммунными факторами противомикробной защиты организма лежит в основе воспаления тканей пародонта и в дальнейшем приводит к деструкции альвеолярной кости. Эпителиальные клетки слизистой оболочки десны играют решающую роль в отношении пародонтопатогенных бактерий. Факторы врожденного иммунитета играют роль не только в противомикробной защите, они также поддерживают условия, необходимые для заживления и регенерации тканей пародонта. Поэтому различные лечебные подходы, влияющие на факторы врождённого иммунитета, рассматриваются как эффективные и перспективные. Клиническое обследование и определение факторов врождённого иммунитета было проведено у 115 пациентов. Здоровые лица составили группу из 30-и человек. Пациенты с хроническим генерализованным пародонтитом с разной степенью тяжести составили 85 человек. Каждому пациенту проводили ортопантомографию на ортопантомографе Orthophosis XG DS/Ceph (SIRONA Dental System GmbH, Германия) с целью оценки состояния костной ткани челюстей (степень деструкции кортикального слоя, степень резорбции межальвеолярных перегородок). С целью оценки состояния костной ткани и качества хирургического лечения использовали прицельные внутриротовые рентгенограммы. Иммунологические методы исследования проводили в несколько этапов. Результаты исследования данной статьи позволяют сделать утверждение, что применение аутологичного комплекса иммунопептидов при хирургическом лечении пародонтита сокращает сроки достижения лечебного эффекта в 2 раза, что выражается в быстром снятии симптомов воспаления и ускорении репаративных процессов.

Ключевые слова: *иммунитет, пародонтит, иммунопептиды, лоскутные операции, костная ткань.*

1. INTRODUCTION

Periodontal diseases in the adult population are one of the most pressing problems of dentistry in the world. Thanks to the introduction of modern technologies in practice, it was possible to identify the main mechanisms of development of this disease at the molecular genetic level [Balykin, 2016; Zorina *et al.*, 2016, 2017; Mamedov *et al.*, 2019; Utyuzh *et al.*, 2019].

Epithelial cells of the gingival mucosa together with humoral and cellular factors of saliva and gingival fluid from the first line of protection of the body from infection and play a crucial role against periodontal pathogenic bacteria [Platonova *et al.*, 2018; Sevbitov *et al.*, 2018; Mitin *et al.*, 2019; Sevbitov *et al.*, 2020; Timoshin *et al.*, 2018, 2019]. The epithelium of the gingival mucosa expresses Toll-like receptors (TLR) that recognize the lipopolysaccharides of anaerobic bacteria and contribute to the production of proinflammatory cytokines, chemokines, and other mediators that regulate inflammation [Sevbitov *et al.*, 2019, 2020;

Bostanci *et al.*, 2012; Kolodkina *et al.*, 2018; Kuznetsova *et al.*, 2018]. Along with cytokines, the epithelium of the gingival mucosa produces antimicrobial peptides β -defensins (hBD), which have a wide range of antimicrobial activity, causing the death of microorganisms due to violation of the integrity of their membranes [Belibasakis *et al.*, 2015; Cochran, 2008; Cortés-Vieyra *et al.*, 2016; Turgaeva *et al.*, 2020].

Factors of innate immunity play a role not only in antimicrobial protection, but they also support the conditions necessary for healing and regeneration of periodontal tissues. Therefore, various therapeutic approaches that affect the factors of innate immunity are considered as effective and promising [Ding *et al.*, 2014; Dommisch *et al.*, 2015; Garlet *et al.*, 2010; Giannobile *et al.*, 2015; Groeger *et al.*, 2015; Voloshina *et al.*, 2018].

At the present stage of periodontitis, treatment attempts are being made to restore all periodontal structures simultaneously, and methods of tissue

engineering and directed tissue regeneration are actively implemented [Hans *et al.*, 2011; Katz *et al.*, 1989; Mudda *et al.*, 2011; Sevbitov *et al.*, 2018, 2019, 2020].

One of the ways to stimulate reparative tissue regeneration is the possibility of influencing cell proliferation and differentiation by a composition of cytokines and growth factors synthesized by autologous cells of the immune system [Reddi, 2001; Shuai *et al.*, 2018; Shue *et al.*, 2012; Song *et al.*, 2016; Enina *et al.*, 2019; Ergesheva *et al.*, 2018; Mironov *et al.*, 2020].

The purpose of our study: to evaluate the effectiveness of immunotherapy using an autologous complex of immunopeptides in the surgical treatment of periodontitis.

2. MATERIALS AND METHODS

Clinical examination and determination of factors of innate immunity were carried out in 115 patients. Healthy individuals made up a group of 30 people. Patients with chronic generalized periodontitis with varying degrees of severity were 85 people. With a mild degree - 25 patients, with an average - 40 patients, with a severe-20 patients. Patients with moderate periodontitis (40 people) received conservative treatment, and 1 month after it, surgical treatment was performed in the form of flap operations. The monitoring of long-term results after surgical treatment was carried out within 1, 3, and 6 months.

The gender distribution of patients was as follows: 73 women and 42 men. The age of the surveyed was from 35 to 65 years. The median age was 52 years (Table 1).

Table 1. Age distribution of patients

Groups surveyed	Age of persons examined (years)			
	n	35-45	46-55	56-65
Healthy faces	30	12	9	9
ChP (complex) patients	85	22	40	23
Total	115	34	49	32

The group of people surveyed was

dominated by women, and they were 1.7 times more than men (Table 2).

During the examination of patients, General clinical methods were used (survey, examination, determination of clinical indices), x-ray methods, and filling out medical documentation (Appendix 1).

Table 2. Gender distribution of patients

Groups surveyed	n	Men	Women
Healthy faces	30	12	18
ChP (complex) patients	85	30	55
Total	115	42	73

Each patient underwent orthopantomography using an orthopantomograph Orthophosis XG DS/Ceph (SIRONA Dental System GmbH, Germany) to assess the condition of the jaw bone (the degree of destruction of the cortical layer, the degree of resorption of the interalveolar septa). In order to assess the state of bone tissue and the quality of surgical treatment, targeted intraoral radiographs were used.

Immunological research methods were carried out in several stages (Figure 6). The first stage involved determining factors of innate immunity in healthy individuals (30 patients) and patients with ChP (complex) with varying degrees of severity (85 patients) before treatment.

At the second stage, in patients with moderate ChP (complex) (40 patients), the factors of innate immunity were determined before and after conservative treatment before they underwent surgical treatment.

The third, fourth, and fifth stages included determining factors of innate immunity in patients with moderate ChP (complex) after surgical treatment within 1, 3, and 6 months.

To determine the expression of the TLR4 and hBD-3 gene in the gum epithelium, a scrape was obtained in the area of the attached gum using disposable sterile probes, which placed an Eppendorf test tube for 2-3 seconds with 0.5 ml of saline solution and frozen at a temperature of -20 °C until the time of the study (Figure. 1). Biological material was collected before treatment, after conservative treatment, before flap operations,

and then 1, 3, and 6 months after surgical treatment.

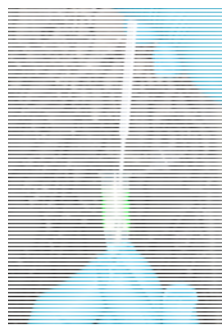


Figure 1. Material intake in the area of the gingival margin during periodontitis (a) and placing the biological material in an Eppendorf tube (b)

Of the samples has been isolated RNA using a kit of reagents "Ampli PRIME RIBO-Sorb" To determine the level of expression of the studied genes, cDNA was obtained using a reverse transcription reaction. At the next stage of the study, a real-time polymerase chain reaction (PCR-RV) was performed on the DT-96 device with the resulting reaction OT cDNA. The reaction was performed using "A set of reagents for PCR-RV in the presence of the intercalating dye SYBR Green I".

To determine cytokines, the contents of periodontal pockets (PC) were collected using sterile paper strips made of filter paper, measuring 0.3 by 0.5 mm, for 30 seconds, then placed in an Eppendorf tube with 100 microl of saline solution and frozen at a temperature of -20 °C until the study was completed (Figure. 2).



Figure 2. Gingival fluid intake from a pocket for cytokine detection

The study of the cytokine spectrum in the gingival fluid included determining the level of cytokines - interleukin-6 (IL-6) and transforming growth factor - TGF β 1. We used commercial sets of TGF β 1 "eBioscience" (USA) for setting the solid-

phase enzyme immunoassay (ELISA) according to the recommended methods. This immunological method for determining various compounds in biological fluids is based on the antigen-antibody reaction [Harrington, L. *et al.*, 2005].

Complex treatment consisted of successive stages and included the following measures: pre-treatment stage (thorough professional, hygienic treatment of the oral cavity), conservative treatment (local antibacterial therapy), surgical treatment (conducting flap operations using the Widman-Neumann method). In total, flap operations were performed in 40 patients with moderate ChP (complex) in the area of 165 teeth. Dynamic follow-up was performed 1-3-6 months after surgical treatment.

On the eve of the flap operations, patients were collected 10 ml of venous blood and immediately transported to the laboratory to obtain an autologous complex of immunopeptides (AKI). Then, according to the technology developed at the Department of Immunology, 5-7 ml of AKI containing cytokines and antimicrobial peptides (TNF α , TGF-1, α -defensin-HNP1-3) were obtained from leukocyte cultures of patients. In parallel, AKI was obtained from white blood cell cultures of 30 healthy donors.

The course of the operation was as follows: under infiltration anesthesia Sol. Ultracaini 2%-5.0 exfoliated the Muco-periosteal flap, removed dental deposits, granulations, polished the bone edge with a drill, de-epithelization of the gingival edge, the wound surface was irrigated with AKI solution from a syringe in an amount of 1.0-1.5 ml.

The bone defect was filled with osteoplastic material, which was also impregnated with AKI, then sutured in each interdental space.

3. RESULTS AND DISCUSSION:

In the postoperative period after flap surgery, patients of the main group with the use of AKI on the 1st day showed no pain symptom, and the mucous membrane was pink. On day 2-3, there was no inflammatory reaction (edema, hyperemia), the interdental papillae were in the epithelization stage, and the sutures were preserved. By day 6-7, there was complete epithelization of the mucosa. The gums were tightly attached to the necks of the teeth, had a pink color, and there was a need to remove the sutures, they were removed completely. Without this method of treatment in the comparison group,

all processes of healing and complete epithelization of the mucosa took place on the 9th-10th day. The removal of stitches was on 13-14 days.

Table 3. Clinical indicators of the effectiveness of the use of an autologous complex of immunopeptides in the surgical treatment of periodontitis

Signs	Without AKI	Local AKI
	n=20	n=20
1. Disappearance of signs of inflammation (edema, hyperemia)	7,0±0,3 day.	3,0±0,1 day.*
2. Removal of stitches full	13,0±1,0 day.	7,0±0,1 day.*
3. Complete tissue repair	14,0±1,0 day.	7,0±1,0 day.*

* - reliability of differences in indicators compared to the control, $p < 0,01$.

For 12 months. after flap operations (period of observation), patients in the main group and in the comparison group were noted: the absence of inflammatory phenomena, the absence of dental pockets, compaction of the gingival margin, and the lack of tooth mobility.

After 12 months. on the x-ray in the area of the operated teeth, the bone density was determined, and the cortical plate was clearly expressed in both groups compared. There were no differences in bone x-rays in these groups (Figure 4,5).



Figure 4. Radiograph of patient A. before surgical treatment in the field of teeth 15.16.17. and 45.46.47. and after surgical treatment with AKI in the field of teeth 15.16.17. (a) after 1 year and 45.46.47. after 1.5 years (b)



Figure 5. Radiograph of patient L. before surgical treatment without the use of AKI in the field of teeth 25.26.27. and 34.35.36. (a) and 6th (b) after surgical treatment without AKI in the field of teeth 25.26.27. after 1 year and 34.35.36. after 1.5 years

Thus, after surgical treatment with the use of AKI, pain relief was observed more pronounced in terms of time, the disappearance of inflammatory phenomena (edema, hyperemia), and the onset of epithelization of the wound. All these phenomena occurred 2-3 times faster than in the comparison group. Treatment with AKI was easily tolerated by patients. It allowed limiting the treatment of the wound with antiseptics and taking antibiotics after surgical treatment. Suppuration or other complications were not observed in any case.

The advantages of surgical treatment with the use of AKI can be attributed to its harmlessness in respect of the hepatitis b virus and HIV.

X-ray analysis was performed to determine the density of bone tissue in the area of the operated teeth, as well as the clear severity of the cortical plate in both groups compared. There were no differences in bone radiographs in these groups.

4. CONCLUSIONS:

In patients with ChP (complex), the expression of the TLR4 gene in the gum epithelial cells was increased by 12 times compared to healthy individuals. In severe ChP (complex), the TLR4 gene expression level was highest, 5 times higher than in mild and 3 times higher than in average. The expression of the hBD-3 gene in the gum epithelium in patients with ChP (complex) is increased by 5 times compared to healthy individuals. In mild ChP (complex), the expression of the hBD-3 gene in the gum epithelial cells exceeded the indicators in moderate and severe cases by 2 times. The use of an autologous complex of immunopeptides in the surgical treatment of periodontitis reduced the time to achieve the therapeutic effect by 2 times, which was expressed in the rapid removal of symptoms

of inflammation and acceleration of reparative processes.

5. ETHICS APPROVAL:

The study conforms to strobe guidelines. all procedures performed in studies involving human participants were in accordance with the ethical standards of the Sechenov University ethics committee and with the 1964 Helsinki declaration and its later amendments. All human subjects' rights have been protected by the Sechenov University ethics committee, and written informed consent was obtained from all subjects who participated in the study.

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Appendix 1. Oral Health Impact Profile (OHIP-14) (Slade G.D., Spencer A.J., 1994)

Question	Very often	Rarely	Usually	Almost never	Never
	5	4	3	2	1
1. Have you had trouble pronouncing any words because of problems with your teeth, mouth or dentures?					
2. Have you felt that your sense of taste has worsened because of problems with your teeth, mouth or dentures?					
3. Have you had painful aching in your mouth?					
4. Have you found it uncomfortable to eat any foods because of problems with your teeth, mouth or dentures?					
5. Have you been self conscious because of your teeth, mouth or dentures?					
6. Have you felt tense because of problems with your teeth, mouth or dentures?					
7. Has your diet been unsatisfactory because of problems with your teeth, mouth or dentures?					
8. Have you had to interrupt meals because of problems with your teeth, mouth or dentures?					
9. Have you found it difficult to relax because of problems with your teeth, mouth or dentures?					
10. Have you been a bit embarrassed because of problems with your teeth, mouth or dentures?					
11. Have you been a bit irritable with other people because of problems with your teeth, mouth or dentures?					
12. Have you had difficulty doing your usual jobs because of problems with your teeth, mouth or dentures?					
13. Have you felt that life in general was less satisfying because of problems with your teeth, mouth or dentures?					
14. Have you been totally unable to function because of problems with your teeth, mouth or dentures?					