The Status of Local Immunity and Biocenosis of the Oral Cavity After the Removal of Wisdom Teeth Against the Background of Phage Therapy

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Abstract

This article examines the qualitative and quantitative composition of the oral microbiota after the impacted lower wisdom teeth removal. A significant bacterial growth was noted, which is manifested by a shift in the equilibrium of the oral biocenosis towards the pathogenic flora with a predominance of facultative anaerobes. The dynamics of changes in microflora against the background of antibacterial treatment and phage therapy is described. Multidirectional changes in the indices of local immunity were also established against the background of antibacterial treatment and phage therapy, which indicated a decrease in the activity of inflammation due to the elimination of pathogens. The obtained data confirm the clinical, bacteriological and immunological effectiveness of oral phage therapy for inflammatory diseases in dental practice and can serve as the basis for the development of a new algorithm for the use of bacteriophages in the treatment of purulent-inflammatory diseases of the oral cavity.

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Introduction

The urgency of the problem of studying purulent-inflammatory diseases of the oral cavity is due to a significant increase in the number of these patients and changes in the clinical course of diseases of the maxillofacial region. [1,2]The presence in the oral cavity of over 500 species of microorganisms, the possibility of their combined action, the variability of the composition of the saprophytic microbiota present significant difficulties in assessing the role of bacteria as etiological and pathogenetic factors in the development and progression of inflammatory diseases of the oral cavity[1,2].

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In the oral fluid, filamentous and various obligate-anaerobic species of bacteria predominate: fusobacteria, leptotrichia, actinomycetes and spirochetes. There are also protozoa, yeast-like fungi and mycoplasmas. And the dynamic balance between the human body and the infectious focus is disturbed most often after tooth extraction, which is often accompanied by transient bacteremia.

The leading place in the complex treatment of purulent-inflammatory diseases of the oral cavity is still occupied by the local or general use of antibacterial drugs. However, at present, more and more cases of the development of resistance of microorganisms to commonly used antibiotics are noted, which in turn necessitates the use of new ones and, as a rule, with an increased level of side effects. There is also an increase in the number of patients with drug intolerance, as well as the development of side effects and complications such as allergic manifestations and dysbiotic

changes in the oral cavity. In addition, the appointment of antibiotic therapy, even taking into account the sensitivity of the microflora, increasingly does not bring the expected effect. this, in turn, is due to the peculiarities of the connection between the immune, nervous and hematopoietic systems, which regulate the body's defense reactions[3,4].

Recently, in medicine, more and more attention has been paid to the search for means, the action of which can selectively suppress the multiplication of pathogens inherent in a specific inflammatory disease. Bacteriophages belong to this kind of natural agents capable of selectively affecting microorganisms. Of particular interest is the polyvalent pyobacteriophage Sextafag®, the effectiveness of which has been confirmed by the results of a number of studies. Sextaphage® is a mixture of Staphylococcusspp., Streptococcusspp., Escherichiacoli, Proteusspp., Pseudomonas aeruginosa and Klebsiella pneumonia phagolysates[5,6].

In the literature, there is a small number of works devoted to the study of changes in the local immunity of the oral cavity during phage therapy of inflammatory diseases. In comparison with all other known antibacterial drugs, bacterial phages have the following advantages:

- do not suppress the growth of representatives of human normal flora;
- lyse antibiotic-resistant pathogenic microorganisms;
- do not have a negative effect on eukaryotic cells;
- quickly penetrate into the focus of infection;
- are capable of self-reproduction and self-regulation;
-safe and have no contraindications for use;
- fully compatible with any medicinal products;
- stimulate specific and non-specific immunity.

The activity of drugs against various clinically significant strains of bacteria ranges from 70% to 100%. Bacteriophages are effective even when all other conservative treatments have been tried and failed.

The use of a combination of bacteriophages is not accompanied by their interaction with each other and does not lead to a change in the schemes of their use. Within the existing set of therapeutic bacteriophages, there are a number of well-proven drugs - coliprotein bacteriophage, polyvalent pyobacteriophage (Sextaphage), and intestinal bacteriophage. On the other hand, bacteria do not have common mechanisms of resistance to antibiotics and phages, therefore, they can be used both in the case of resistance of the pathogen to one of the drugs, and in the combination "antibiotic + bacteriophage". This combination is especially effective for the destruction of microbial biofilms.
7In patients with pyoinflammatory diseases, within an hour, phages enter the bloodstream, after 1–1.5 hours they are detected from exudates and from the surface of wounds, and after 2 hours - from the cerebrospinal fluid7.
Thus, the clinical application of these drugs in the context of therapy of purulent-inflammatory diseases of the oral cavity requires the identification of sensitive types of microorganisms.

To study the dynamics of biocenosis and indicators of local immunity of the oral cavity after the removal of impacted wisdom teeth against the background of phage therapy.

Materials and methods

The selection of clinical material was carried out on the basis of the Dental Polyclinic of the Volgograd State Medical University in the course of a prospective comparative randomized study with a crossover (crossover) design. At this stage, 20 patients aged 18 to 35 years with retention and dystopia of third molars without concomitant diseases were examined. Of these, there were 9 men and 11 women aged 20 years - 4, 21 years old - 4, 22 - 3, 23 years old - 1, 26 years old - 5, 28 years old - 3. In 12 patients, wisdom teeth occupied a medial-oblique position, 5 has a horizontal position and 3 has a vertical position.

All patients underwent atypical removal of the impacted third molar with trepanation of the cortical plate with a bur. Patients of the first group were prescribed antibacterial therapy (amoxicillin with clavulanic acid 500 + 125 mg 3 times a day for 5 days), desensitizing therapy (chloropyramine 25 mg once a day for 3-5 days), anti-inflammatory therapy (nimesulide 100 mg 1-2 times per day 3 days), antiseptic local treatment (oral baths with a solution of 0.05% chlorhexidine digluconate).

Patients of the second group were prescribed Sextafag inside 10 ml (after ingestion of a solution of baking soda) 2 times a day for 5-7 days), desensitizing therapy (chloropyramine 25

mg once a day for 3-5 days), anti-inflammatory therapy (nimesulide 100 mg 1 -2 times a day for 3 days), antiseptic local treatment (oral baths with Sextafag solution).

Laboratory studies were performed at the Department of Clinical Laboratory Diagnostics of the Volgograd State Medical University. The material for the study was gingival fluid, from which individual representatives of the microbiota were isolated by the culture method. The microbiota was studied by the method of Haenel (1979) modified by S.K. Kanareikina et al. (1985); - cultivation of anaerobes was carried out in a Gas Cancer microanerostat (OXOID, England). The isolated microorganisms were identified on the basis of morphological, cultural, biochemical, and antigenic characteristics in accordance with the classification of Burgey (1980).

The content of individual interleukins in gingival fluid was determined by the method of enzyme-linked immunosorbent assay (ELISA) using a kit "Cusabio Biotech Co., Ltd" (China), according to the manufacturer's instructions. The liquid of the gingival pockets obtained from the patients was taken 1.0 ml in a plastic tube with a volume of 2.0 ml and 1.0 ml of phosphate buffer (pH 7.4) and 0.1 ml of a non-polar detergent (Tween-60) were added, carefully shaken and kept for 1 hour at a temperature of 20-30 ° C. Then the tube with the sample was centrifuged in a CM-50 Elmi centrifuge (Latvia), at a speed of 3000 rpm for 3-5 min. 0.1 ml of a light homogeneous supernatant was taken with a doser and placed in a well of a plate for subsequent ELISA.

Descriptive and analytical statistics were used for statistical processing of the results using GraphPad Prism software. The data on the graphs are presented as arithmetic mean and standard deviation. The statistical significance of the differences was assessed by the method of two-way analysis of variance with the Newman-Keuls post-hawk test.

Results

In the course of the study, it was found that in the composition of the oral microbiota in patients with impacted third lower molars during therapy with Amoxiclav and Sextafag, there are statistically significant intergroup differences. The sextaphage is definitely effective against Staphylococcus aureus spp., Streptococcus pyogenes spp., Streptococcus haemolyticus spp., Pseudomonas spp., Streptococcus salivarius spp. As can be seen from table 1, sextaphage had a weak effect on Waylonell, as it is not the target microorganism for him. We also noted that when using the bacteriophage, no growth of candida was observed in patients.

Microorganism	Antibiotic therapy	Pyobacteriophage
Streptococcus mutans spp.	2,49 ± 0,73	2,52 ± 0,46
Streptococcus salivarius spp.	4,82 ± 0,73	3,42 ± 0,48*
Streptococcus oralis spp.	4,44 ± 0,89	4,49 ± 0,49
Staphylococcus aureus spp.	2,73 ± 0,61	1,12 ± 0,16*
Streptococcus pyogenes spp.	2,39 ± 0,51	1,00 ± 0,71*
Streptococcus haemolyticus spp.	2,48 ± 0,35	1,62 ± 0,26*
Pseudomonas spp.	2,54 ± 0,08	0,60 ± 0,89*
Candida spp.	2,54 ± 0,12	0,40 ± 0,55*
Veillonella spp.	2,47 ± 0,04	3,84 ± 0,85*

Table 1. The state of the oral cavity microbiota in persons with retention and dystopia of the third molars against the background of the use of pyobacteriophage on the 10th day.
Note: data are presented as mean log10 (CFU / ml) ± standard deviation (n = 10); * - p <0.001 in intergroup comparison.

Discussion

When assessing the content of anti-inflammatory cytokines, carried out by ELISA, a statistically significant decrease in the concentration of IL-1β was revealed both against the background of antibiotic therapy and against the background of the use of bacteriophage, while there were no statistically significant intergroup differences in the value of this indicator (p> 0.05). During the course of therapy, the IL-10 content increased statistically significantly (p <0.001), and with the use of bacteriophage, the increase in this indicator was more pronounced than against the background of antibiotic therapy. The concentration of TNFα did not change significantly against the background of antibiotic therapy, while by the 10th day of phage therapy, this indicator decreased by more than 2 times (p <0.001 when compared with the initial value of the indicator).

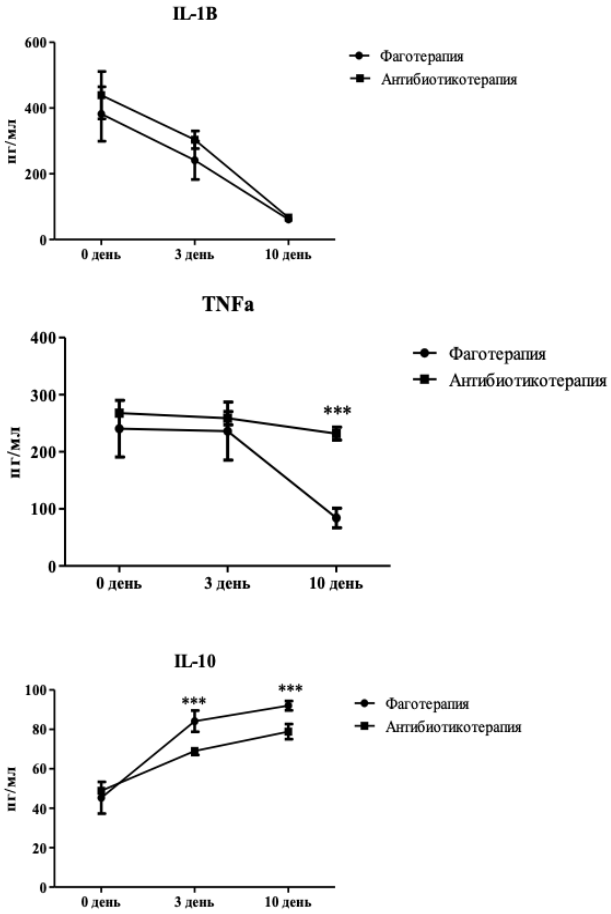

Figure 1. Dynamics of the level of IL-1β, IL-10, TNFα in gingival fluid in persons with retention and dystopia of third molars.
Note: the analysis was performed using the Mann-Whitney U-test; * - p <0.05; *** - p <0.001.

A decrease in the concentration of pro-inflammatory cytokines (IL-1β and TNFα) and an increase in the concentration of anti-inflammatory cytokine (IL-10) indicate a change in the immune reactivity in the area of inflammation and a decrease in the intensity of the latter, which was apparently due to the elimination of pathogenic microorganisms. Thus, the determination of the level of anti-inflammatory cytokines in the gingival fluid is an indicator of the effectiveness of therapy in persons with odontogenic inflammatory diseases.

The IgA level did not change significantly, however, on the 3rd day of phage therapy, this indicator increased statistically significantly (p <0.05 when compared with the indicator recorded against the background of antibiotic therapy). The IgG level, as follows from the graph, did not change significantly. An increase in the level of immunoglobulins of the main classes IgA, IgG in the gingival fluid against the background of odontogenic inflammatory diseases is a consequence of their local synthesis in the inflamed gums, as well as extravasation from the affected periodontal tissues into the gingival fluid [8]. IgG, which is responsible for the activation of the complement system, binds to some surface antigens cells, thereby making these cells more accessible for phagocytosis. As can be seen from the graph, the I gM level decreased both during phage therapy and during the use of amoxicillin with clavulanic acid, while there were no intergroup differences, which demonstrates a decline in the acute period of inflammation.

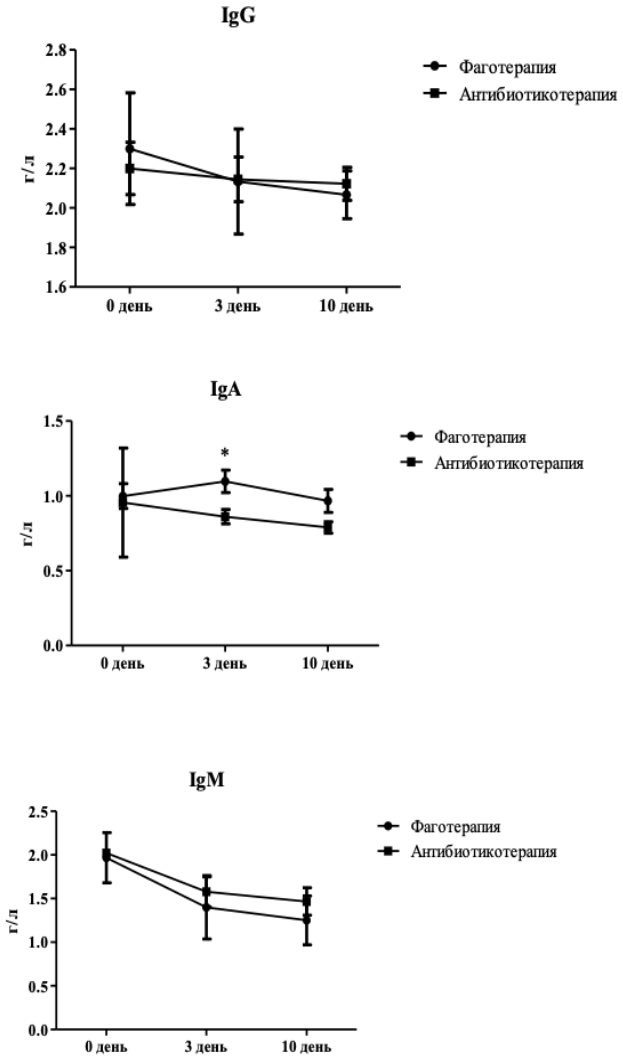

Figure 2. Content of IgG, IgA, IgM of gingival fluid in persons with retention and dystopia of third molars.
Note: the analysis was performed using the Mann-Whitney U-test; * - p <0.05.

Conclusions

Thus, the study of the microbiota and local immunity of the oral cavity in the context of modern biomedicine creates the prerequisites and opens up prospects for the personalized selection of drug therapy and early prognosis of diseases and the development of their complications.

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Declaration of Interest

The authors report no conflicts of interest pertaining to any of the products or companies discussed in this article.

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