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THE COMPOSITION OF THE MICROBIOTES OF THE NECK PART OF THE TEETH IN THE AREA OF THE GUM EDGE OF RATS AT THE END OF THE SECOND, FOURTH AND SIXTH WEEKS OF OPIOID INFLUENCE

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ABSTRACT

The aim of the study was to investigate the microbiota in the cervical part of the teeth in the gingival margin of the oral cavity of rats at the end of the second, fourth and sixth weeks of the experimental action of the opioid analgesic nalbuphine. Under the action of opioids for six weeks, changes in the microbiocenosis in the studied subbiotope of the oral cavity of animals were found, which manifested themselves in the early stages by the appearance of gram-negative anaerobes, gradual increase in opportunistic pathogens, formation of dental biofilm experiment.

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The results of the article correspond to the research plan of Lviv National Medical University named after Danylo Halytsky and are part of the research topic of the Department of Normal Anatomy "Morpho-functional features of organs in the pre- and postnatal periods of ontogenesis, under the influence of opioids, food additives, reconstructive surgery and obesity", state registration number 0120U002129.

In modern medical practice to achieve the desired analgesic effect there is a need for gradual and long-term use of opioid analgesics [1, 7, 10, 11]. Along with achieving a stable analgesic effect, the duration of opioid use should be as short as possible to avoid addiction and the risk of overdose [6, 7, 10]. It is important that with long-term action of opioids there are metabolic disorders and severe irreversible changes in the body, develop significant lesions of the oral cavity [3, 8, 12]. Against the background of abrupt changes in the microbiocenoses of the oral cavity of addicts, carious cavities are formed and the diffuse spread of the acute course of the inflammatory process is characteristic [2]. It should be noted that the role of bacterial plaque in initiating gingivitis is not in doubt, and this was first demonstrated in experimental studies of gingivitis in the 1960s [9]. In modern professional research, one of the central places is occupied by dental plaque, which is a cluster of microorganisms in the form of a film and is one of the most complex associations of bacterial flora [4, 5].

To prevent the development of periodontal disease and the occurrence of infectious foci in the oral cavity, which are caused by bacterial biofilm formed under opioid exposure, it is important to determine the species composition of this microbial group in the experiment, which is important for

understanding the etiology of oral inflammation. further implementation of the obtained data in clinical practice.

The aim of the study was to investigate the state of the microbiome in the cervical part of the teeth in the area of the gingival margin of the oral cavity at the end of the second, fourth and sixth weeks of the experimental action of the opioid analgesic nalbuphine.

Materials and methods. The study was performed on outbred white rats - males of reproductive age (46), with an average body weight of 180 g. The animals were divided into four groups. The first group - intact rats (10). In the second group, the animals were administered the intramuscular opioid analgesic Nalbuphine daily at a dose of 0.212 mg / kg for 2 weeks. The third group - the introduction of nalbuphine for four weeks in a dosage of 0.212 to 0.225 mg / kg. In the fourth group, opioid analgesic injections were given for six weeks in increasing doses ranging from 0.212 to 0.252 mg / kg.

All rats were kept in a vivarium and work on keeping, care, labeling and all other manipulations were carried out in compliance with the provisions of the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes [Strasbourg, 1985]. To conduct microbiological studies, the microflora was taken from the cervical part of the teeth in the gingival margin of the oral cavity of the experimental animals.

Bacterial groups of dental biofilm, which is formed in this area are the most stable and reflect the state of the biocenosis of the oral cavity as a whole. In bacterioscopic examinations, the material in the form of cultures of smears from the studied subbiotope was applied to a glass slide, fixed over the flame of the burner and stained by the Gram method. Bacteriological studies performed quantitative characterization of microbial groups. Accordingly, the intake of material in the amount of 0.02 ml. was carried out using a calibrated loop and transferred to a test tube with isotonic solution (1 ml), repeating this procedure 5 times. 0.2 ml of material was taken from the test tube and plated on plates with a dense nutrient medium (blood agar, meat-peptone agar, Endo medium, etc.). Simultaneously, sowing was carried out with a loop on dense media. In 0.2 ml. saline contained microbes, which were introduced in one loop - 0.02 ml. After 24-48 years. counted the number of colonies in colony-forming units (CFU) of each bacterial species.

The obtained data, for further statistical analysis, were tested for normality by calculating the coefficients of asymmetry and excess and by calculating the Shapiro-Wilk test (significance level $p < 0.05$). Data were presented as $M \pm SD$, where M is the mean, SD is the standard deviation. Non-parametric criteria were used to establish the significance of the difference in the quantitative parameters of microorganisms between the groups - Mann-Whitney U-test to compare the two groups and Kraskel-Wallis H-test for three or more independent groups. All statistical calculations were performed using RStudio v. 1.1.442 and R Commander v.2.4-4.

Research results. In the study of the microbiome in the cervical part of the teeth in the gingival margin of rats after two weeks of action of the opioid analgesic nalbuphine, we found changes in the species and quantitative composition of bacterial groups. According to the results of a statistical study, the significance of the difference for all microbial groups at different terms of opioid exposure during the experiment was proved ($p < 0.0001$).

Bacterioscopic examinations of smears in the field of view showed small accumulations (up to 10) of cocci gram-positive bacteria, as well as gram-positive rods, which by morpholotinctrial properties belonged to the genera Leptothrix and Lactobacillus. Gram-negative rods, which were morphologically similar to enterobacteria, were isolated. The smears also showed polymorphic gram-negative anaerobic rods, probable periodontal pathogens - bacteroids. Leukocytes and epitheliocytes with signs of adsorption on their surfaces of microorganisms were also visualized in small quantities.

Bacteriological studies after two weeks of action of the opioid analgesic revealed changes in the quantitative composition of the microflora of the studied biotope of the oral cavity of white rats. Thus, the number of non-hemolytic streptococci at the beginning of the experiment was 45.11 ± 5.16 CFU / 0.02 ml., And after two weeks of opioid action - 35.44 ± 5.39 CFU / 0.02 ml., Ie decreased 1.3 times compared with the intact group of animals. However, the quantitative indicators of gram-positive non-spore rods, compared with intact animals decreased by 3.0 times - 3.00 ± 0.87 CFU / 0.02 ml., And the number of gram-positive spores sticks by 2.7 times, with an index of 3.33 ± 1.00 CFU / 0.02 ml.

Bacteriological studies also revealed changes in the quantitative composition of opportunistic pathogens. Thus, the number of α-hemolytic streptococci (Fig. 4.4.1.2.) Did not change significantly - at the beginning of the experiment their number was 65.44 ± 4.36 CFU / 0.02 ml., And at the end of the second week of opioid action - 70.78 ± 8.35 CFU / 0.02 ml.

However, consistently high rates of bacterial species of α-hemolytic streptococci at this time of the experiment indicated a dynamic formation of structures of the supragingival biofilm. Quantitative indicators of other microbial groups of opportunistic pathogenic microflora had more pronounced changes. Thus, the number of coagulase-negative staphylococci increased 2.0 times compared to similar indicators of the intact group of animals and amounted to 20.78 ± 4.35 CFU / 0.02 ml. At the same time, the quantitative composition of enterococci, in comparison with intact animals decreased by 3.0 times, with an index of 6.11 ± 1.62 CFU / 0.02 ml., And the number of Escherichia coli colonies of the bacterial group of lactose-positive enterobacteria decreased 1.7 times - up to 4.11 ± 0.78 CFU \ 0.02 ml. The appearance of single colonies of fungal microflora was observed on nutrient media, which were represented by Candida albicans species (Table 1).

Table 1. Species and quantitative composition of the microflora of the cervical part of the teeth in the gingival margin of rats at the end of the second, fourth and sixth weeks of opioid exposure

№	Bacterial groups	Intact animals	In 2 weeks	In 4 weeks	In 6 weeks
1.	Non-hemolytic streptococci	45.11±5.16	35.44±5.39	15.22±3.90	60.67±5.55
2.	Gram-positive non-spore rods	9.00±1.22	3.00±0.87	4.33±0.71	18.00±2.45
3.	Gram-positive spores rods	9.00±1.12	3.33±1.00	5.11±0.78	19.00±2.65
4.	α-hemolytic streptococci	65.44±4.36	70.78±8.35	75.67±10.75	40.56±6.02
5.	Coagulase-negative staphylococci	10.33±3.67	20.78±4.35	35.44±4.69	35.78±4.66
6.	Enterococci	18.33±2.96	6.11±1.62	9.33±2.00	30.22±6.40
7.	Escherichia coli	7.00±1.50	4.11±0.78	10.33±2.50	28.11±2.71
8.	Hemolytic Escherichia coli	-	-	10.22±2.91	20.33±5.00
9.	β-hemolytic streptococci	-	-	14.22±1.72	27.11±5.93
10.	Coagulase Positive staphylococci	-	-	-	16.11±2.03
11.	Klebsiella	-	-	-	12.00±1.50
12.	Fungal microflora	-	1-2	1-2	1-2

At the end of the fourth week of the opioid analgesic, there was an activation of opportunistic pathogens. Bacterioscopic examinations of smears from the cervical part of the teeth in the gingival margin of the oral cavity of rats showed the formation of extracellular structures in the form of a biofilm based on filamentous bacteria leptotrix with organized gram-positive coccal microflora, the so-called "kukura". For a more detailed description of the detected changes, the species composition of microorganisms of this subbiotope was investigated, identifying bacterial isolates by a complex of morpho-tinctorial and cultural properties and counting the number of colonies of each species during primary cultures.

At bacteriological researches it was established that the quantitative structure of indicators of normal microflora changed. Thus, the number of non-hemolytic streptococci decreased to 15.22 ± 3.90 CFU / 0.02 ml, ie 3.0 times compared with the intact group of animals. As in the previous period, there was a tendency to reduce the quantitative composition of gram-positive rods, compared with intact animals. In particular, the number of gram-positive non-spore rods decreased 2.0 times to 4.33 ± 0.71 CFU / 0.02 ml, and the number of gram-positive spore rods decreased 1.8 times to 5.11 ± 0.78 CFU / 0.02 ml.

However, in contrast to the saprophytic microflora, there was an increase in the quantitative indicators of opportunistic bacterial species. These data show that the isolated microorganisms were dominated by coagulase-negative staphylococci, which do not produce the enzyme plasmacoagulase and are part of common biocenoses, in particular, bacterial species Staphylococcus edermidis, Staphylococcus saprophyticus increased by 35 to 35, and others 4.69 CFU / 0.02 ml. An increase in the number of α - hemolytic streptococci to 75.67 ± 10.75 CFU / 0.02 ml was also noted, in particular, in most animals - Streptococcus mutans, which is part of the biofilm with potential odontopathogenic properties. The quantitative composition of enterococci compared to the intact group of animals decreased 2.0 times - up to 9.33 ± 2.00 CFU / 0.02 ml., And the number of Escherichia coli increased 1.5 times -10.33 ± 2.50 CFU / 0.02 ml. It should be noted that at this time of the experiment colonies of hemolytic Escherichia coli were sown in the amount of 10.22 ± 2.91 CFU / 0.02 ml. / 0.02 ml., Which were absent in the previous stages of the study. At bacteriological researches single colonies (1 - 2) of Candida albicans of fungal microflora were also observed (tab. 1).

At the end of the sixth week of opioid exposure, dynamic changes in the qualitative and quantitative composition of the microbiota of the studied subbiotope of the oral cavity of animals were

noted. Bacterioscopic examination of smears in the cervical part of the teeth in the gingival margin of white rats indicated a predominance of gram-positive microflora, the presence of cellular elements and changes in bacterial morphotypes. Accumulations of gram-negative coccal microflora and gram-positive diplococci were detected on smear preparations. In bacteriological studies it was found that changes in the microbiocenoses of the studied subbiotope tended to increase the number of most microbial groups, and the species composition of the microflora was supplemented by opportunistic and pathogenic microorganisms.

In the study of saprophytic microflora, changes were observed, which were manifested by an increase in the quantitative composition in comparison with the intact group of animals and a significant increase in their number compared to the previous terms of the experiment. In particular, the quantitative composition of non-hemolytic streptococci increased 1.3 times to 60.67 ± 5.55 CFU / 0.02 ml, and gram-positive rods 2.0 times compared with the intact group of animals, where the number of gram-positive non-spore rods was 18.00 ± 2.45 CFU / 0.02 ml., And gram-positive spores – 19.00 ± 2.65 CFU / 0.02 ml.

Bacteriological studies also revealed changes in the quantitative composition of opportunistic pathogens. The number of α-hemolytic streptococci decreased to 40.56 ± 6.02 CFU / 0.02 ml, where most rats were inoculated with the cariogenic species of Streptococcus mutans. There was a tendency to increase the number of colonies of coagulase-negative staphylococci - up to 35.78 ± 4.66 CFU / 0.02 ml, which was 3.5 times more compared to the intact group of animals. The number of enterococci increased 1.6 times - up to 30.22 ± 6.40 CFU / 0.02 ml. and 4.0 times Escherichia coli, which was 28.11 ± 2.71 CFU / 0.02 ml. The number of colonies of hemolytic Escherichia coli, which was not sown in intact animals, was - 20.33 ± 5.00 CFU / 0.02 ml.

And the pathogenic species Streptococcus pyogenes bacterial group β - hemolytic streptococci - 27.11 ± 5.93 CFU / 0.02 ml. was twice as much as in the previous study period. It should be noted that in addition to a significant increase in the quantitative indicators of Streptococcus pyogenes, for the first time in our studies we also noted the appearance of other pathogenic species, namely Staphylococcus aureus and Staphylococcus intermedius bacterial group of coagulase-positive staphylococci, the number of which was 16.11±2.03 CFU / 0.02 ml, where Staphylococcus intermedius predominated, the ecological niche for which is the animal body. In addition, sown gram-negative Klebsiella in the amount of 12.00 ± 1.50 CFU / 0.02 ml., Which were absent in the previous stages of the study, their appearance indicated dysbiosis of the oral cavity. At this time of the experiment were also sown single colonies of Candida albicans fungal microflora (Table 1).

Conclusions.

1. Conducted microbiological studies with two weeks of opioid exposure revealed a decrease in the quantitative composition of microorganisms of most bacterial species, as well as the appearance of gram-negative anaerobes - bacteroids, which indicated a violation of microbiocenosis in the cervical part of the teeth in the gingival margin subbiotope.

2. Changes in the microbiocenosis of the cervical part of the teeth in the gingival margin of rats after four weeks of opioid action were manifested by the formation of dental supragingival biofilm, as well as an increase in the quantitative composition of opportunistic pathogens with a predominance of Streptococcus mutans. to consider these bacterial species as etiological agents of development of inflammatory changes in the studied area of the oral cavity of animals.

3. At the end of the sixth week of opioid exposure there were significant changes in the microbiocenosis of the studied biotope, which was manifested by the formation of supragingival dental biofilm, activation of opportunistic microflora, indicating the development and slow progression of dysbiosis in the cervical part of the teeth and quantitative characteristics of pathogenic species of microorganisms - probable etiological agents of purulent-inflammatory processes.

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