ORIGINAL ARTICLE

DYNAMICS OF CHANGES IN THE MICROBIAL PICTURE OF THE ORAL CAVITY ON THE BACKGROUND OF CHRONIC OPIOID EXPOSURE IN THE EXPERIMENT

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ABSTRACT

The aim is to investigate changes in the microbiota of dental biofilm at the end of the eighth, tenth and twelfth weeks of experimental opioid exposure.

Materials and methods: The study was performed on 36 white outbred adult male rats, which were injected with the opioid analgesic nalbuphine in increasing doses (0,212 – 0,3 mg / kg) during 8, 10 and 12 weeks. Qualitative and quantitative composition of microbiota of dental biofilm was studied using statistical analysis.

Results: After eight weeks of opioid exposure, changes in microbiocenosis of dental biofilm of rats were caused by a significant increase in saprophytic and opportunistic microbiota and an appearance of pathogenic species of indicator microbiota with potential periodontopathogenic action. At the end of the tenth week, a significant increase in the quantitative indicators of certain species of opportunistic microbiota and increase in the quantitative composition of pathogenic bacteria were determined. After twelve week of opioid exposure, a significant increase in the quantitative indicators of pathogenic microbiota of dental biofilm was detected.

Conclusions: Changes in the qualitative and quantitative composition of the microbiocenosis of the dental biofilm at the end of 8, 10 and 12 weeks of opioid exposure were established, they were manifested by a significant increase in the quantitative indicators of certain species of opportunistic microorganisms and a significant increase in pathogenic microbiota in the dynamics, which led to the progression of dysbiotic changes and purulent-inflammatory process in the oral cavity of rats.

KEY WORDS: opioid exposure, rats, microbial associations, dental biofilm

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INTRODUCTION

Opioid analgesics are vital in the treatment of the pain syndrome of varying intensity, emergencies, etc. [1, 2]. However, long-term use of opioid drugs causes the body to become addicted to them, which is a significant factor in drug addiction and proves to be life-threatening. This is a relevant medical and social problem worldwide [1, 3, 4].

According to the references, in patients with drug addiction there is a directly proportional dependence of the severity of the oral mucosa lesion and periodontium on the duration of drug use and the severity of concomitant somatic and infectious diseases [5, 6, 7, 8]. In opioid-dependent individuals and in experimental animals exposed to opioids, the researchers noted significant changes in microbiocenoses, where general and local immune deficiency was manifested by increased contamination with opportunistic and pathogenic microorganisms of the oral cavity and gastrointestinal tract [9 – 15].

It should be noted that the surface structure to which microorganisms are fixed as well as the conditions of oxygen and nutrients access directly affect the composition of the microbiota, which is reflected in the detection of individual microbial communities in different eco-niches of the oral cavity, such as mucous membranes, supragingival and subgingival plaque [16]. However, in medical practice, current methods of predicting and assessing the risks of periodontitis do not always take into account the crucial role of colonization resistance of the oral mucosa and microecological changes in dental biofilm in the etiology of inflammatory periodontal diseases [17, 18]. Dental biofilms consist of complex microbial communities embedded in the polymer matrix of bacterial and salivary origin and are one of the most important mechanisms of microbiota persistence in the oral cavity [19]. Moreover, in the microbiocenosis of the oral cavity, the replacement of coccal flora takes place, which predominates in the intact periodontium with more complex and variable microbial groups, depending on the impact of various factors on the body [20 – 24]. However, in the available professional literature we have not found data on the dynamics of changes in the microbiocenosis of the oral cavity and dental biofilm on the background of long-term effect of opioid analgesics in both clinical and experimental conditions.

THE AIM

The aim is to investigate changes in the qualitative and quantitative composition of tooth surface microbiota in the gingival margin of rats at the end of the eighth, tenth and twelfth weeks of experimental opioid exposure.

MATERIALS AND METHODS

The study was performed on 36 white outbred adult male rats, aged 4.5–7.5 months, weighing 160–270 grams. Animals were divided into four groups: Group I – control rats, which were injected intramuscularly with saline during the experiment; in Group II, intramuscular injections of the opioid analgesic nalbuphine was administered in increasing doses for eight weeks: 1-2 weeks – 0.212 mg / kg, 3-4 weeks – 0.225 mg / kg, 5-6 weeks – 0.252 mg / kg, 7-8 weeks – 0.260 mg / kg; in Group III rats were administered nalbuphine in increasing doses for ten weeks: from 0.212 mg / kg to 0.283 mg / kg; in Group IV animals were administered an opioid analgesic for twelve weeks, where the initial dose was 0.212 mg / kg with a gradual increase to 0.3 mg / kg at the end of the experiment (11-12 weeks). Thus, a method of simulating chronic opioid exposure involved administering to rats an opioid analgesic with the active ingredient nalbuphine hydrochloride intramuscularly daily, once at one time span (10-11 a.m.) for 12 weeks with single doses increasing gradually every two weeks [25]. The drug nalbuphine belongs to the pharmacotherapeutic group: analgesics, opioids, morphine derivatives; pharmacodynamics: a group of opiate receptor agonists and antagonists (κ-receptor agonist and μ-receptor antagonist).

All animals were kept in a vivarium and work on keeping, care, labeling and all the 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]. The Commission on Bioethics of Danylo Halytskyi Lviv National Medical University has stated that the study meets ethical requirements in accordance with the order of the Ministry of Health of Ukraine No. 231 of 01.11.2000 (Protocol No. 10 of 24.05.2021).

In order to conduct microbiological studies, the material was taken from the tooth surface in the gingival margin of white rats, because the microbiota of the dental biofilm is the most stable and reflects the general state of the oral microbiome. The smear material was applied on a glass slide, fixed over a burner flame and Gram stained. For species and quantitative characteristics, microbiota were collected from the studied microbiotope and seeded on dense nutrient media, where, after calculation of the microorganisms' colonies, a quantitative indicator in colony-forming units (CFU) was obtained. Identification of selected cultures was performed by a set of morphotinctorial, cultural and biochemical properties. Some species were identified using standard test systems *Api system Bio Merieuox*, France. For further statistical analysis, the obtained data were tested for normality by calculating the asymmetry and excess coefficients and the results of the Shapiro-Wilk test ($p < 0.05$). The data were presented as M ± SD, where M is the mean, and SD is the standard deviation. For normal-distribution data, a bilateral t-test was used to determine the significance of the difference between the two groups of animals. To determine the significance of the difference between three or more groups of animals, ANOVA with the Tukey's post-hoc test was used for further pairwise comparison. If the distribution of data, according to the results of the test, differed from normal, then to establish the reliability of the difference between groups the nonparametric criteria were used –Mann-Whitney U test to compare two groups and Kruskel-Wallis H test for three or more independent groups followed by post-hoc analysis using Dunn's test for pairwise comparison. All statistical calculations were performed using RStudio v. 1.1.442 and R Commander v.2.4-4. Diagrams and tables were created using Microsoft Office Excel.

RESULTS

Conducted microscopic examinations from the tooth surface in the gingival margin of rats of the first control group indicated the presence of gram-positive microbiota – *Leptothrix*, which morphologically belong to the genus *Lactobacillus*. Single leukocytes and epitheliocytes were also observed in the field of view. Bacteriological studies have shown a prevalency of gram-positive microbiota, namely, α-hemolytic streptococci and non-hemolytic streptococci, coagulase-negative staphylococci and Enterococci. Gram-positive non-spore and spore bacilli and lactose-positive enterobacteria Escherichia coli were also detected on nutrient media.

After eight weeks of opioid analgesic action microscopic examination of mears from the microbiotope of the tooth surface in the gingival margin of group II of rats, the formation of extracellular structures as dental plaque due to the interaction of cariogenic species of streptococci and organic substrate was noted, namely microbial polysaccharides, salivary proteins and cellular elements. Taking into account the previous results of microbiological studies in the early stages of opioid exposure, we investigated the qualitative and quantitative composition of microorganisms – indicators of the state of the oral biocenosis, as well as bacteria with potential periodontal activity. In particular, bacterial species *Staphylococcus epidermidis* (coagulase-negative staphylococci), *Staphylococcus aureus* and *Staphylococcus intermedius* (coagulase-positive staphylococci), cariogenic species *Streptococcus mutans* (α-hemolytic streptococci), *Streptococcus pyogenes* (β-hemolytic streptococci), fungal microbiota, Enterobacteria – typical and hemolytic Escherichia coli, Klebsiella, and Pseudomonas aeruginosa were studied.

Performed analysis of bacteriological research from the tooth surface in the gingival margin of rats after 8 weeks of opioid exposure showed an increase in the quantitative composition of saprophytic microbiota, in particular, a significant increase in quantitative indicators non-hemolytic streptococci (65.22±6.72 CFU/ml), gram-positive non-spore-forming rods (26.11±4.17 CFU/ml) and gram-positive spore-forming rods (19.11 ± 4.88 CFU/ml) – 1.5,

Table I. Qualitative and quantitative composition of microbiota of the tooth surface in the gingival margin of rats after all weeks of opioid exposure (CFU/ml).

№	Bacterial groups	Control	Group II
1.	*Non-hemolytic streptococci*	44.67±3.79	65.22±6.72*
2.	*Gram-positive non-spore-forming rods*	9.33±1.53	26.11±4.17*
3.	*Gram-positive spore-forming rods*	8.33±0.58	19.11±4.88*
4.	*α-hemolytic streptococci*	66.00±2.65	45.33±6.82
5.	*Coagulase-negative staphylococci*	10.00±3.46	54.33±7.18*
6	*Enterococci*	19.67±0.58	27.44±4.45
7.	*Escherichia coli*	6.33±1.15	37.67±2.29*
8.	*Hemolytic Escherichia coli*	-	30.33±5.36
9.	*β-hemolytic streptococci*	-	38.22±4.58
10.	*Coagulase-positive staphylococci*	-	28.44±3.84
11.	*Klebsiella*	-	24.22±3.93
12.	*Yeast-like fungi*	-	1-2

Notes: data are presented in the form of M ± SD, where M is the average value, SD is the standard deviation; * $p<0.05$ – significant difference in values relative to control.

Table II. Qualitative and quantitative composition of microbiota of the tooth surface in the gingival margin of rats after ten weeks of opioid exposure (CFU/ml).

№	Bacterial groups	Control	Group III
1.	*Nonhemolytic streptococci*	43.00±6.56	60.22±7.00
2.	*Gram-positive non-spore-forming rods*	10.33±1.15	19.22±4.63*
3.	*Gram-positive spore-forming rods*	9.67±0.58	15.11±2.52
4.	*α-hemolytic streptococci*	64.33±5.13	76.33±3.32**
5.	*Coagulase-negative staphylococci*	9.67±2.89	59.22±5.24*
6.	*Enterococci*	16.33±1.15	32.22±5.74*
7.	*Escherichia coli*	6.00±0.00	58.33±6.93*
8.	*Hemolytic Escherichia coli*	-	42.56±4.48
9	*β-hemolytic streptococci*	-	51.44±6.91
10.	*Coagulase-positive staphylococci*	-	39.11±6.68**
11.	*Klebsiella*	-	22.44±4.00
12	*Bacteroids*		2.11±0.78
13.	*Pseudomonas aeruginosa*	-	3.11±1.05
14.	*Yeast-like fungi*	-	1-2
15.	*Filamentous fungi*	-	1-2

Notes: data are presented in the form of M ± SD, where M is the average value, SD is the standard deviation; * $p<0.05$ – significant difference in values relative to control, ** $p<0.05$ – significant difference in values relative to group II.

2.8 and 2.3 times respectively, compared to the similar indicators of the control group ($p<0.05$). The quantitative composition of opportunistic pathogens also was changing. In particular, the amount of α-hemolytic streptococci was decreasing to 45.33 ± 6.82 CFU/ml, and the amount of Enterococci slightly was increasing to 27.44 ± 4.45 CFU/ml, the probable cause was the "displacement" of opportunistic strains by pathogenic species of microorganisms. At the same time, a significant increase in the quantitative indicators of coagulase-negative staphylococci (54.33 ± 7.18 CFU/ml) and gram-negative Escherichia coli (37.67 ± 2.29 CFU/ml) – in 5.4 and 5.9 times respectively, compared with similar control indicators ($p<0.05$) (Table I).

Changes in the qualitative and quantitative bacterial composition of the examined oral microbiotope were conditioned by the appearance of microbial groups that were absent in control animals and colonization by pathogenic microorganisms in the late stages of opioid exposure. In particular, at the end of the eighth week of the experimental opioid effect, a significantly high level of bacterial contamination of Hemolytic Escherichia coli (30.33 ± 5.36 CFU/ml) was observed, which has pathogenic properties, indicating the development of dysbiosis in the oral cavity, in particular in supragingival biofilm. A significant number of pathogenic species of microorganisms also drew attention.

At the same time, a significantly high level of microbial colonization of *Streptococcus pyogenes*, bacterial grouping of β-hemolytic streptococci (38.22 ± 4.58 CFU/ml), and coagulase-positive staphylococci (28.44 ± 3.84 CFU/ml) was found, where the bacterial species *Staphylococcus aureus* and *Staphylococcus intermedius* were identified by cultural characteristics. Significant amounts of gram-negative Klebsiella bacteria (24.22 ± 3.93 CFU/ml) were detected as a result of the development of dysbiotic changes in the oral cavity of rats and the formation of inflammatory foci with prolonged opioid exposure. Single colonies (1-2) yeast-like fungi (*Candida albicans*) were also sown on nutrient media.

After ten weeks of opioid exposure in microscopic examination of smears from the tooth surface in the gingival margin in group III, animals showed signs characteristic of purulent-inflammatory process, namely, an increase in degeneratively altered and destroyed epitheliocytes, neutrophilic leukocytes were visualized throughout the field of view. The number of gram-positive bacteria decreased significantly, however, the accumulation of gram-negative rod-shaped microbiota in the form of continuous supragingival biofilms was detected. Capsule bacteria, probably Klebsiella, as well as bacterial species, which were classified as bacteroids by morphotinctorial properties, were observed in smears from subgingival biofilms localized in the formed periodontal pockets. Gram-negative coccal microbiota and gram-positive diplococci and staphylococci were visualized as clusters. Filamentous and yeast-like cells, changes in bacterial morphotypes were also detected. Bacteriological researches revealed changes in the quantitative composition of normobiota. In particular, at the end of the tenth week of opioid exposure, the quantitative indicators of gram-positive non-spore-forming rods increased significantly – 1.9 times compared to controls ($p < 0.05$), and increased the number of microbial associations non-hemolytic streptococci to 60.22 7.00 CFU/ml and gram-positive spore-forming rods up to 15.11 ± 2.52 CFU/ml. The quantitative composition of bacterial species of opportunistic pathogenic microorganisms of the tooth surface in the gingival margin of rats was changing significantly. In particular, there was a significant increase in the quantitative indicators of coagulase-negative staphylococci (59.22 ± 5.24 CFU/ml) – 6.1 times, Enterococci (32.22 ± 5.74 CFU/ml) – 2.0 times, and Escherichia coli – 9.7 times, compared with the corresponding indicators of the control group of rats ($p < 0.05$). The quantitative composition of different types of α-hemolytic streptococci was slightly increasing compared with controls, however, increased significantly – 1.7 times comparatively with the corresponding indicators in animals exposed to opioids for eight weeks ($p < 0.05$), it indicated the dynamic formation of the biofilm in the gingival margin of rats on the background of ten weeks of opioid exposure (Table II).

It should be noted that the natural microbiocenosis of the population of experimental animals, which was formed during their keeping in standard conditions, gradually changed due to long-term use of opioid analgesics. After ten weeks of such action, in the body of animals appeared microorganisms uncharacteristic of natural microbiocenoses, and microbiocenoses that were formed in the early stages of opioid exposure. The level of microbial colonization of pathogenic species of bacteria in the dental biofilm of white rats increased significantly. In particular, the number of opportunistic pathogens with pathogenic properties, namely Hemolytic Escherichia coli (42.56 ± 4.48 CFU/ml), significantly increased, indicating a dynamic increase in dysbiotic changes in the oral cavity of rats. This fact was confirmed by a significant increase in the quantitative composition of the pathogenic microbiota, namely the bacterial species *Streptococcus pyogenes*, the microbial association β-hemolytic streptococci to 51.44 ± 6.91 CFU/ml, as well as a significant increase in coagulase-positive staphylococci (39.11 ± 6.68 CFU/ml) – 1.4 times compared with the corresponding indicators at eight weeks of opioid exposure ($p < 0.05$). In the third group of animals there were also consistently high values of gram-negative Klebsiella, the amount of them was 22.44 ± 4.00 CFU/ml. For the first time during the experiment, the growth of a potential pathogen – Pseudomonas aeruginosa (3.11 ± 1.05 CFU/ml), periodontopathogenic microorganisms – bacteroids (2.11 ± 0.78 CFU/ml), as well as filamentous fungi – hyphomycetes (1-2 colonies) was established. As in the previous experimental group, single colonies of Yeast-like fungi were identified.

At the end of the twelfth week of opioid exposure analgesic caused pronounced changes in the microbiocenosis of the oral cavity. It should be noted that our bacterioscopic research of the tooth surface in the gingival margin of rats indicated the formation of certain stable structures at different times of opioid exposure in dynamics. Therefore, a preliminary analysis of microscopic research showed that in the early stages of the opioid analgesic exposure in the examined microbiotope of the oral cavity of rats coccal microflora was found, it was partially adsorbed in aggregates with filamentous bacteria *Leptothrix*, which are characteristic of the formation. In the long-term opioid exposure, the formation of extracellular structures was due to the interaction of microbial factors, namely, cariogenic species of streptococci (*Streptococcus mutans*) and organic substrate (microbial polysaccharides, salivary proteins, cellular elements). Accordingly, supragingival biofilm was formed. During the detailed description of the biofilm, was found that it included aggregates – plaques, which were formed with the participation of extracellular polysaccharides produced by these microorganisms. Apparently, the production of these polysaccharides increased due to the development of the inflammatory process against the background of long-term opioid exposure.

These data were confirmed by microscopic research of plaque at the end of the twelfth week of opioid exposure, which revealed supragingival biofilm in the form of solid dental plaque, which was formed by aggregates of coccal microflora and *Leptothrix*. Examination of subgingival biofilm smears revealed aggregates consisting of polymorphic gram-negative rods which according to their morphotypes belonged to bacteroids. Therefore, due to chronic opioid exposure in the microbiotope the tooth

Table III. Qualitative and quantitative composition of microbiota of the tooth surface in the gingival margin of rats after twelve weeks of opioid exposure (CFU/ml).

№	Bacterial groups	Control	Group IV
1.	*Nonhemolytic streptococci*	44.67±3.21	67.33±5.34*
2.	*Gram-positive non-spore-forming rods*	9.00±1.00	18.11±1.45*
3.	*Gram-positive spore-forming rods*	9.33±1.15	15.11±1.83
4.	*α-hemolytic streptococci*	65.00±8.89	67.00±3.39
5.	*Coagulase-negative staphylococci*	10.00±1.00	65.33±6.24*
6	*Enterococci*	18.67±3.79	29.00±5.41
7.	*Escherichia coli*	6.67±2.31	26.33±3.84*
8.	*Hemolytic Escherichia coli*	-	42.33±3.87
10.	*β-hemolytic streptococci*	-	57.44±5.70
9.	*Coagulase-positive staphylococci*	-	38.67±4.80
11.	*Klebsiella*	-	11.56±1.74
12.	*Bacteroids*	-	12.11±2.15**
13.	*Pseudomonas aeruginosa*	-	7.89±2.89**
14.	*Yeast-like fungi*	-	1-2
15	*Filamentous fungi*	-	1-2

Notes: data are presented in the form of M ± SD, where M is the average value, SD is the standard deviation; * $p<0.05$ – significant difference in values relative to control, ** $p<0,05$ – significant difference in values relative to group III.

surface in the gingival margin of rats there were found changes that contributed to the formation of stable elements of plaque similar to biofilm, with the participation of morphotypes that are inherent in periodontopathogenic and cariogenic species of microorganisms and organic substrate. Bacteriological research of the microbiotope of the oral cavity in forth group of animals showed an increase in the quantitative composition of saprophytic and oportunistic pathogens. A significant increase in quantitative indicators has been determined, namely non-hemolytic streptococci (67.33 ± 5.34 CFU/ml), gram-positive non-spore-forming rods (18.11 ± 1.45 CFU/ml), as well as coagulase-negative staphylococci (65.33 ± 6.24 CFU/ml) and Escherichia coli (26.33 ± 3.84 CFU/ml) – 1.5, 2.0, 6.5 and 3.9 times, respectively, compared with similar control ($p<0.05$). The number of gram-positive spore-forming rods in animals during this period of opioid exposure increased slightly to 15.11 ± 1.83 CFU/ml, Enterococci to 29.00 ± 5.41 CFU/ml and α-hemolytic streptococci to 67.00 ± 3.39 CFU/ml (Table III).

After 12 weeks of opioid exposure, there was an increase in the quantitative composition of opportunistic microbial species with pathogenic properties and pathogenic bacteria of the oral microbiotope of rats in dynamics. As in the previous group, a significant increase in the quantitative composition of Hemolytic Escherichia coli (42.33 ± 3.87 CFU/ml), β-hemolytic streptococci (57.44 ± 5.70 CFU/ml) and coagulase-positive staphylococci (38.67 ± 4.80 CFU/ml) was found. Other factors of virulence of microorganisms were also noted, which were caused by the appearance or increase in the level of microbial colonization by pathogenic species of microorganisms uncharacteristic of normal biocenoses.

These data were confirmed by contamination of Klebsiella (11.56 ± 1.74 CFU/ml) and filamentous fungi – hyphomycetes (1-2 colonies), as well as a significant increase in the quantitative indicators of periodontal pathogens – bacteroids (12.11 ± 2.15 CFU/ml) and Pseudomonas aeruginosa (7.89 ± 2.89 CFU/ml) – 5.7 and 2.5 times, respectively, compared with similar indicators at ten weeks of opioid exposure ($p<0.05$), which led to the complication of the dysbiotic process in the oral cavity of rats under such conditions.

DISCUSSION

According to the research results, it is obvious that the increase in the quantitative composition of opportunistic pathogens and the emergence of pathogenic microorganisms in supragingival and subgingival biofilms can be regarded as evidence of etiological factors in the development of inflammation in the rats' gums. Because of the fact that drug abuse suppresses the immune response, which increases the incidence of fungal and viral infections, dysbiotic changes contribute to caries and periodontitis, which are regarded as a result of imbalance between bacterial symbiosis and oral tissues [6, 12, 15].

Based on the data received, we can conclude that the development of dysbiotic changes in the oral cavity on the background of chronic opioid exposure involved several microbial associations of biofilm, which are not characteristic of natural microbiocenoses and which were not detected in the animal control group. The study has shown dynamic changes in the microbiome of the tooth surface in the gingival margin, the formation of stable structures based on the

interaction of microbial factor and organic substrate, and established uniformity of changes in quantitative indicators of aerobic, facultative anaerobic and anaerobic microflora.

The microbial picture of the oral cavity has significant differences in periodontal disease on the background of the narcotic or psychoactive substances effect, which can be compared with our data from microbiological studies. In particular, under the effect of methamphetamine, researchers noted a significant amount of Negativicutes, Veillonella and Selenomonadales in dental plaque, which led to the development of caries [8, 26]. In drug-addicted patients, oral microbiocenosis was characterized by the predominance of coccal *(Streptococcus spp., Staphylococcus spp.)* and anaerobic *(Neisseria spp.)* microbiota, showed a significant amount of *Candida albicans*, Escherichia coli, Klebsiella, Proteus and Pseudomonas aeruginosa, which proved the development of necrotic stomatitis [6], and the predominance of *Staphylococcus aureus* and *Candida spp.* testified to the formation of infection foci in the oral cavity [12, 27]. Morphine has also been shown to increase the susceptibility of animals to the fungal (*Candida albicans*) and gram-negative (*Klebsiella pneumonia*) microbiota in in vitro opioid mice [11]. In rodents exposed to morphine, the researchers noted an increase in the number of Firmicutes, Enterococcus faecalis, Clostridium, proinflammatory bacteria Staphylococcus, Enterococcus and Proteobacteria, and observed a significant decrease in the number of *Lactobacillus spp*. and *Bifidobacterium* [9, 10, 14].

On the background of long-term action of opioids in the microbiotope of the rats' oral cavity, the following bacterial groups have been found: streptococci, staphylococci (*Staphylococcus aureus*), Klebsiella, Escherichia coli, fungal microbiota (*Candida albicans*) as well as a decrease in the number of lactic acid bacteria. In addition, the growth of hemolytic Escherichia coli, bacteroids, Pseudomonas aeruginosa and filamentous fungi of hyphomycetes was noted, which indicated the etiological role of all isolated microbial associations in the progression of the inflammatory process in the oral cavity and periodontium of white rats. Based on the results, it can be concluded that under the conditions of long-term effect of opioid analgesics, a significant increase in the quantitative composition of aerobic microbial groups in the area of dental biofilms formation reflected the general state of oral microbiocenoses. This corresponds to the established views on the interaction between these bacterial associations, because in the microbiocenosis aerobic microorganisms that formed the surface layers of the biofilm have the ability to absorb oxygen, which in turn created the preconditions for anaerobic periodontal pathogenic microbiota.

CONCLUSIONS

After eight weeks of opioid exposure, changes in the qualitative and quantitative composition of tooth surface microbiota in the gingival margin, manifested by the formation of extracellular structures (interaction of microbial factor and organic substrate), a significant increase in saprophytic and opportunistic microbiota, as well as increasing species of indicator microbiota with potential periodontopathogenic action, which served the development of dysbiosis and the formation of inflammatory foci in the oral cavity of rats.

On the background of ten weeks of opioid exposure, degeneratively altered epitheliocytes, neutrophils and accumulations of gram-negative microbiota in the formed dental biofilms were determined. There was a significant increase in the quantitative indicators of certain species of opportunistic microbiota, a significant increase in the quantitative composition of pathogenic bacteria, which generally indicated a dynamic increase in dysbiotic changes and the development of purulenti and nflammatory process in the oral cavity of rats.

At the end of the twelfth week of opioid exposure, morphotypes of periodontopathogenic and cariogenic species of microorganisms and organic substrate were detected in the microbiotope of the tooth surface in the area of the gingival margin. A significant increase in the quantitative indicators of coagulase-negative staphylococci and Escherichia coli was found, as well as an increase in the quantitative composition of pathogenic microorganism species, which led to complications of dysbiosis and progression of purulent and inflammatory process in the oral cavity of rats against chronic opioid exposure.

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