ORIGINAL ARTICLE

CONTENTS

Research of oral cavity microflora virulence factors on the background of experimental opioid exposure

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ABSTRACT

Aim: to investigate the factors of bacterial virulence of oral microbiotopes against the background of long-term experimental action of an opioid analgesic.
Materials and Methods: The study was performed on 72 white outbred rats, which were injected "Nalbuphine" with a gradual increase in dose for 12 weeks. The microbial composition and virulence factors of bacteria in the microbiotopes of the oral cavity were studied.
Results: At the end of the 4th week of opioid exposure, the appearance of hemolytic escherichia was noted. At the end of the 8th week the appearance of capsular bacteria Klebsiella was registered, as a virulence factor. The production of staphylococcal virulence factors – lecithinase, was noted. At the end of the 12th week of opioid use, the appearance of phycomycete fungi was noted, Gram-negative diplococci, Klebsiella, Hemolytic Escherichia coli and Pseudomonas aureginosa. These changes indicated the development of dysbiotic changes, as well as the gradual formation of foci of the inflammatory process in the oral cavity.
Conclusions: In the short-term stages of opioid exposure, changes in biocenoses were manifested in an increase in the contamination of certain types of microorganisms. In the long-term effects of the opioid, the activation of bacterial elements of the microbiocenosis was revealed, which were characterized by increased expression of virulence factors. At the later stages of opioid exposure, the predominance of gram-negative microflora and the activation of cytotoxic action against the background of suppressed activity of the body's protective *response* were established.

KEY WORDS: experiment, oral cavity, microbiotopes, opioid, rats, microorganisms, virulence factors

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INTRODUCTION

Opioid analgesics are indispensable in clinical practice and remain the basis of pharmacotherapy of visceral and somatic pain, which today has no adequate alternative in modern medicine [1-3]. However, uncontrolled or long-term use by patients of opioid drugs in chronic pain causes intoxication of the body, it is a determining risk of tolerance to the opioid effect, the development of withdrawal symptoms and high mortality from overdose [1, 4, 5].

Clinical studies have shown that the use of drugs (including opioids) changes the diversity and composition of the microbiota of the oral cavity and intestines, causing side effects [6]. Analysis of the literature has shown that addicted patients are hypersensitive to bacterial and viral infections, significantly increasing contamination by pathogenic and opportunistic microbiota [7-9]. At the same time, opioids cause the development of cellular hypoxia, which leads to disruption of oxygen-dependent phagocytosis processes [10].

In drug addicts, the processes of suppression of the body's immune status progress against the background of severe dysbacteriosis in the oral cavity, and the severity of these changes directly depends on the duration of drug use [11-13]. The microbial spectrum in the oral mucosa of addicted patients is reflected in the qualitative diversity, which is dominated by coccal, anaerobic and fungal microflora [14, 15]. Since the interaction of macrophages of the gingival mucosa with the microbiota leads to excessive production of cytokines and toxic substances that cause destruction of periodontal tissues, it is important early diagnosis of dysbiosis in the oral cavity in inflammatory processes [16-21]. According to the professional literature, the leading role in the development of the inflammatory process in the oral cavity belongs to enzymes, exo- and endotoxins that form the immune re-

sponse [22-24]. The effect of endotoxins depends on their concentration, in particular, their toxicity is manifested in much higher concentrations than exotoxins, and in small doses they are able to activate phagocytosis and other protective reactions of the body [24, 25].

According to modern views, an important role in the development of the immune response and immunopathological reactions belongs to the recognition process of conservative molecules of microorganisms "patterns" by TLRs of immunocompetent cells. Conservative bacterial patterns include structures such as peptidoglycan and cell wall lipopolysaccharides, capsule polysaccharides, etc. [26]. TLRs belong to the oldest evolutionary system for recognizing "aliens", which is common to all multicellular organisms, including plants and animals, and is important in the interaction of gram-negative bacteria with TLRs in the pathogenesis of oral diseases [27]. In addition, periodontopathogenic microorganisms of the oral cavity, which appear when the imbalance between the autochthonous and allochthonous microbiota in the area of formed periodontal pockets are quite diverse, which directly depends on the nature and course of the disease [17, 21, 28-31], since the periodontopathogenic microbiota has a pronounced virulence, mechanisms of adhesion to periodontal tissues and aggressive destructive action [24, 32-34]. It should be noted that due to the adhesion of bacteria, biofilms are formed and the production of virulence factors is significantly increased. The pathogenetic role of film formation has been established for oral cavity microorganisms in periodontal lesions and the development of dental caries [35]. These research data indicate that the study of microbiota in the gingival sulcus is important for predicting the inflammatory process, because normally the level of microbial colonization in this microbiotope of the oral cavity is insignificant, it is dominated by facultative gram-positive bacteria [29, 36].

To establish the role of microorganisms as etiopathogenetic factors in the development of inflammatory processes, it is necessary to determine their etiology in experimental animal models [37, 38]. The above literature data indicate the need for research of virulence factors of microorganisms in the study of the pathogenesis of oral cavity diseases. However, in the available professional sources we have not found data on the features of the microbiocenosis and the study of virulence factors of oral bacteria at different terms of opioid exposure in the dynamics.

AIM

The aim is to investigate the factors of bacterial virulence of oral microbiotopes against the background of long-term experimental action of an opioid analgesic.

MATERIALS AND METHODS

The study was performed on 72 white outbred adult male rats, weighing 160 – 270 grams, aged 4.5 – 7.5 months. In the experiment, the animals were divided into two groups. Group I – control rats (18), which were injected intramuscularly with saline during the experiment. Group II – rats (54) at different terms of the experimental action of the opioid analgesic "Nalbuphine" with the active substance nalbuphine hydrochloride (dilution of 1 ml of nalbuphine in 50 ml of 0.9% NaCl solution). The drug was administered intramuscularly, daily, once, for the same period of time for 12 weeks, with a gradual increase in dose every 2 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, 9-10 weeks – 0.283 mg / kg, 11-12 weeks – 0.3 mg / kg.

The animals were quarantined in the vivarium, on a standard diet in separate cages. Before the experiment, a thorough examination of the animals was performed, paying attention to the condition of the oral cavity in the area of gums and teeth, focusing on the color, humidity and absence of damage to the mucous membrane. The Commission on Bioethics of Danylo Halytsky Lviv National Medical University established that the conducted research meets ethical requirements according with the provisions to the European Convention "for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes" [Strasbourg, 1985], to the order of the Ministry of Health of Ukraine № 231 of 01.11.2000 (Protocol № 5 jf 24.05.2021).

Bacterioscopic and bacteriological methods were performed to study the species microbial composition and virulence factors of bacteria of oral microbiotopes – tooth surface in the gingival margin and gingival furrows of rats after 2, 4, 6, 8, 10 and 12 weks of experiment, as well as in control animals. During bacterioscopic examinations, smears were prepared from colonies typical of morphology and stained by the Gram method. Evaluation of prokaryotic gram-positive and gram-negative microflora was performed on the morphology of cells, the mutual arrangement of cells, the presence of extracellular structures with the participation of different morphotypes of bacteria as elements of the biofilm. Quantitative relationships of the main elements in the smear, the presence of yeast like cells, as well as cellular elements of the tissues of the oral cavity were taken into account.

Simultaneously, sowing was carried out directly on dense media with a loop. Conventional special, differential diagnostic and selective media were used: meat-peptone agar, blood agar, Endo medium, Saburo medium, yolk-salt agar, mannitol salt agar (MSA), as well as tubes with rabbit plasma. Herewith the production

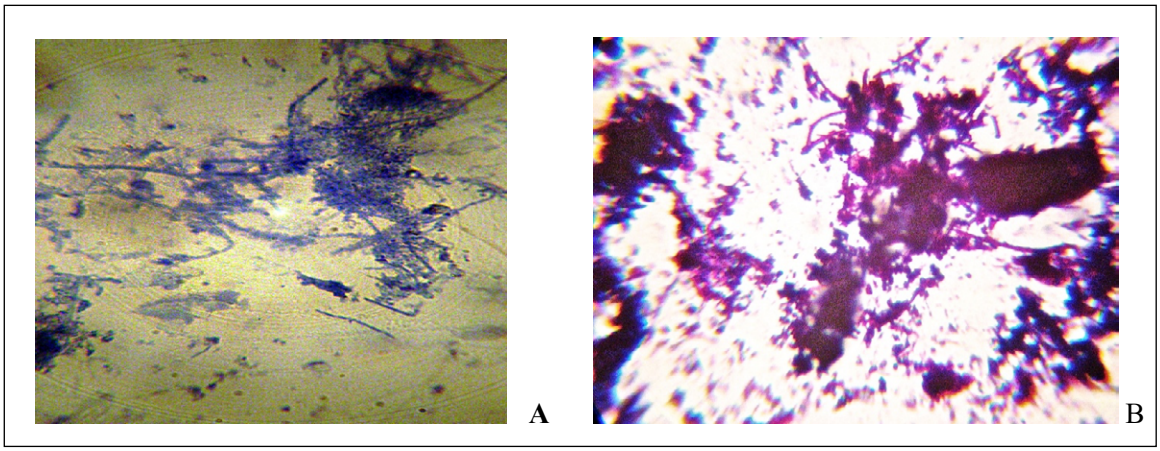

Fig. 1. Morphology of bacteria in the supragingival biofilm of rats after 2 weeks (A. Staphylococci and *Leptothrix* filamentous bacteria. 90 x objective 8 x eyepiece.) and 4 weeks (B. Accumulation of gram-positive and gram-negative cocci and their adsorption on *Leptothrix*. 90 x objective 8 x eyepiece.) of opioid exposure

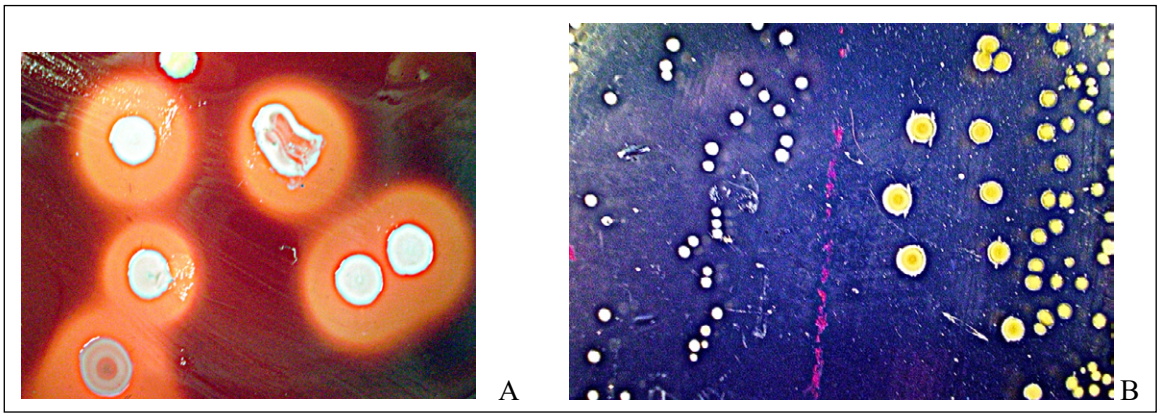

Fig. 2. Bacteriological studies of the microbiota of the supragingival biofilm of rats after 6 weeks (A. 1 – *Staphylococcus aureus* with zones of hemolysis. Sowing was carried out on blood agar. Magnification 1x8.) and 8 weeks (B. *Staphylococcus intermedius* and *Staphylococcus aureus* with lecithinase activity. Sowing was carried out on *salt* egg y*olk agar*. Magnification 1x4) of opioid exposure

of hemolysins, plasma coagulase and lecithinase was determined. Identification of isolated cultures was performed by a complex of morphotinctorial, cultural and biochemical properties. Individual species were identified using standard test systems «Apisystem Bio Merieuox», France.

Cultivation was carried out under normal atmospheric conditions, as well as under elevated CO2 levels. Cultures were cultivated in a thermostat at t 37^{0}C, for differentiation of enterococci at t 10^0 C, at which the growth of other species is delayed. Identification of streptococci was carried out by a complex of tests, namely: α-hemolysis – α-hemolytic streptococci or β-hemolysis – β-hemolytic streptococci, sensitivity to optochin (opt+) and by a complex of biochemical properties – hydrolysis of arginine (arg+), decomposition of esculin (esk+) and mannitol (man+), Foges-Proscauer reaction. As virulence factors of staphylococci, the production of α-hemolysin, plasma coagulase and lecithinase was recorded. In streptococci, the type of hemolysins was determined (α-hemolysis or β-hemolysis, capsule formation). Hemolysins of *Escherichia*, pyocyanine production of *Pseudomonas aureginosa*, capsule formation in *Klebsiella* were also detected. In order to identify periodontopathogenic anaerobic microorganisms, the material was sown immediately after being taken into Petri dishes on special media for bacteroids – Schedler's agar with erythrocytes, growth factors and an antibiotic (gentamicin). Petri dishes were placed in a Bio Merieux microanaerostat with bags to create the gas composition necessary for anaerobes and cultivated for 48 h at t 37^0 C.

RESULTS

During bacterioscopic research of the tooth surface in the gingival margin of the I (control) group of rats, the same type of microflora was noted. In general, coccal gram-positive microbiota and gram-positive

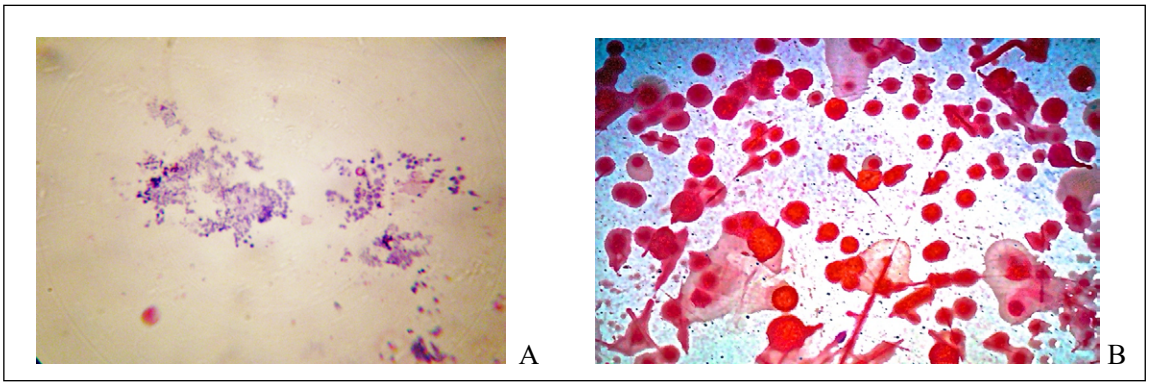

Fig. 3. Morphology of bacteria in the subgingival biofilm of rats after 10 weeks (A. Aggregates of gram-negative polymorphic rods–bacteroids. 90 x objective 8 x eyepiece.) and 12 weeks (B. Adsorption of gram-negative rods on the surface of epitheliocytes, autophagocytosis of erythrocytes. 90 x objective 8 x eyepiece.) of opioid exposure

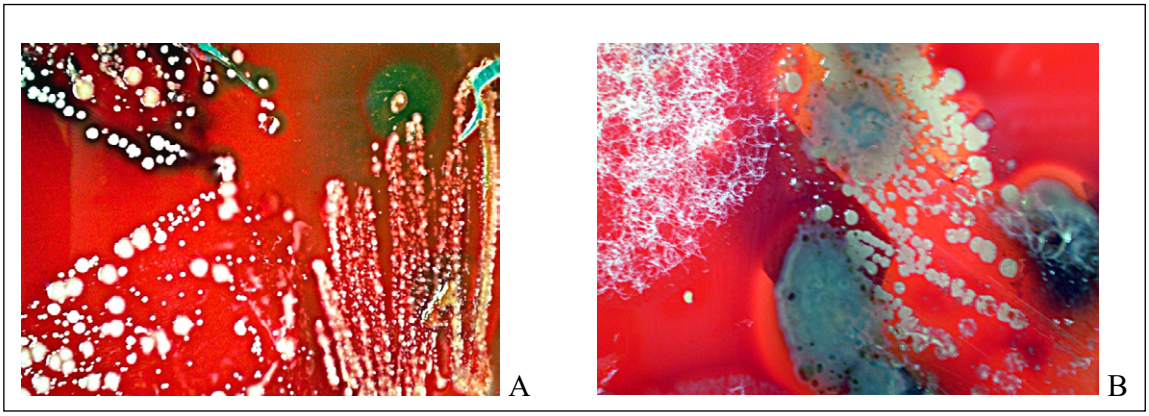

Fig. 4. Bacteriological studies of the subgingival biofilm microbiota of rats after 10 weeks (A. β-*hemolytic streptococci*, *Hemolytic Escherichia* coli, growth of *Pseudomonas aureginosa* colonies. Sowing was carried out *on blood agar.* Magnification 1 x 4.) and 12 weeks (B. Colonies of *Pseudomonas aureginosa*, fungal microflora. Sowing was carried out *on blood agar.* Magnification 1 x 4.) of opioid exposure

microorganisms – long filamentous gram-positive *Leptothrix* bacteria were detected, lactobacilli of the genus *Lactobacillus* are also identified by morphological features in smears. In the field of view, the cellular elements were single epitheliocytes and leukocytes. Bacteriological analysis of the microbiota of control animals indicated the dominance of gram-positive species, among which nonhemolytic streptococci and α-hemolytic streptococci, coagulase-negative staphylococci, Enterococci, gram-positive non-spore-forming rods and gram-positive spore-forming rods were distinguished by biochemical and morphotinctorial properties. Single colonies of gram-negative microorganisms – lactose-positive enterobacteria, which were identified as *Escherichia coli*, were detected in cultures on Endo medium.

In Group II animals, during the short-term periods of opioid exposure (2–4 weeks), bacterioscopic examinations of smears of the tooth surface in the gingival margin of rats revealed clusters of coccal gram-positive bacteria and gram-negative rods, which were morphologically similar to enterobacteria. The formation of supracellular structures in the form of a biofilm was noted, the basis of which was the filamentous *Leptothrix* bacteria with gram-positive coccal microflora organized around them, the so-called "corn cobs" were formed (Fig. 1).

At the end of the 4th week of opioid exposure, bacterioscopic studies revealed supracellular formations in the form of a biofilm with the participation of both gram-positive and gram-negative bacteria. During bacteriological studies, the appearance of hemolytic *Escherichia* coli on nutrient media was noted, the pathogenic properties of which are determined by the ability to dissolve erythrocytes due to the secretion of special enzymes – hemolysins, which was determined by the formation of a zone of hemolysis around the colony when the bacteria were seeded on blood agar. The appearance of *Escherichia* coli with hemolytic properties, which were absent both in animals of the control group and in rats in the end of the 2th week of opioid exposure, indicated the development of dysbiotic changes in the studied microbiotope and the oral cavity in general.

In the long term (6–8 weeks), the formation of supracellular structures as a continuous supragingival biofilm was noted as a result of the interaction of a microbial factor (cariogenic species of streptococci) and an organic substrate, namely, microbial polysaccharides, saliva proteins, and cellular elements. Bacteriological studies revealed an increase in the contamination of α-hemolytic streptococci, as well as β-hemolytic streptococci and staphylococci – producers of plasma coagulase. The appearance of capsular bacteria *Klebsiella* was registered, they were characterized by pronounced capsule formation, as a virulence factor that ensures adhesion and antiphagocytic action of bacteria, which also indicated the development of dysbiosis in the oral cavity.

The appearance of coagulase-positive staphylococci – bacterial causative agents of purulent-inflammatory processes – drew attention, as they are characterized by the production of toxins with hemolytic and necrotic effects. The hemolytic properties (the ability to cause hemolysis of rabbit erythrocytes) of *Staphylococcus aureus* were studied to detect α-toxin, which is characterized by dermonecrotic and lethal effects. Lecithinase activity and α-hemolysin production were detected in the selected isolates of *Staphylococcus aureus*.

In particular, colonies of staphylococci with large zones of hemolysis of rabbit erythrocytes were determined on nutrient media, as one of the signs of the virulence of these microorganisms. The production of one of the virulence factors of staphylococci – lecithinase, which destroys the lecithin of cell membranes, causing cell lysis – was also noted. In particular, colonies of two species of the bacterial group of coagulase-positive staphylococci – *Staphylococcus aureus* and *Staphylococcus intermedius* were visualized. A zone of lecithinase activity was observed around the colonies of *Staphylococcus aureus*, however, such a zone was not formed around the colonies of *Staphylococcus intermedius*, which indicated the absence of lecithinase (Fig. 2). It should be noted that hemolysins are one of the most important virulence factors of the aerobic microbiota of the oral cavity. *Staphylococcus produces* several types of hemolysins, among which α-hemolysin, which has cardiotoxic and dermonecrotic effects, is important. In addition, *staphylococci lecithinase*, as a membrane toxin, has a cytotoxic effect on leukocytes.

At the later stages of opioid exposure (10–12 weeks), microscopic examination revealed changes in the morphological types of the constituent supracellular structures (dental plaques). A significant decrease in the relative number of gram-positive coccal microflora and the appearance of phycomyces fungi was noted. Gram-negative polymorphic microflora formed supracellular structures with the participation of capsular bacteria. In smears from subgingival biofilms in the area of ingival furrows (formation of periodontal pockets), signs of tissue damage were noted – multinucleated epithelial cells, signs of autophagocytosis of erythrocytes (Fig. 3). Among the cells of lymphogenic or hematogenous origin in the gingival fluid, lymphocytes predominated, granulocytes were not visualized, and no signs of bacterial phagocytosis were found. Instead, the adsorption of polymorphic gram-negative rods in the form of small granular structures was found on epitheliocytes. These data indicated the absorption and intracellular placement of microorganisms – bacteroids, which are capable of adsorbing on cells and causing a cytotoxic effect due to the action of the pathogenicity enzyme of these bacteria – phospholipase. The absence of an inflammatory reaction, in particular, the absence of neutrophils and phagocytosis (Fig. 3), also indicated a cytotoxic effect.

At the end of 10 and 12 weeks of opioid exposure, bacteriological studies of the microbiota of supragingival and subgingival biofilms revealed Gram-negative diplococci, as well as *Klebsiella*, hemolytic *Escherichia coli* and *Pseudomonas aureginosa*. Bacteroids were isolated under anaerobic conditions. Virulence factors were found in most bacterial isolates, namely, α-hemolysin staphylococci, which caused hemolysis of rabbit erythrocytes at 37^0C, as well as lecithinase. Coagulase-positive staphylococci belonged to two species – *Staphylococcus aureus* and *Staphylococcus intermedius*.

Hemolysis and a high density of microbial colonization of *Streptococcus pyogenes*, a bacterial group of β-hemolytic streptococci (Fig. 4), which produces hemolysin and protein M, which is associated with antiphagocytic and cytotoxic effects on neutrophils, were noted on blood agar. It should be noted that the hemolysins of streptococci are an important taxonomic feature, in particular, the group of α-hemolytic species, among which are identified cariogenic and opportunistic species that cause systemic diseases during immunosuppression. The ability to cause β-hemolysis (O- and S-hemolysins) is a species characteristic of *Streptococcus pyogenes*, and they also have a cytotoxic effect. At the same time, the production of virulence factors significantly increases during the formation of supracellular structures of microorganisms – biofilm, which plays a key role in the pathogenesis of periodontal diseases.

Colonies of *Pseudomonas aureginosa*, which produced the blue-green pigment pyocyanin, were also detected in the *long-term* stages of opioid exposure (Fig. 4). These changes indicated the development of dysbiotic changes, as well as the gradual formation of foci of the inflammatory process in the oral cavity as a

result of the expression of virulence factors by bacteria of various species, primarily staphylococci of the species *Staphylococcus aureus* and *Staphylococcus intermedius*, as well as β-hemolytic streptococci.

Thus, against the background of long-term opioid exposure, the development of dysbiotic changes in the microbiocenoses of the studied biotope was revealed, namely the predominance of gram-negative bacteria, the appearance of microorganisms not characteristic of this biotope – *Klebsiella, Pseudomonas aeruginosa* and phycomyces fungi. In the majority of selected isolates, the expression of virulence factors characteristic of certain species was found. The absence of cellular elements characteristic of purulent-inflammatory processes was noted, and signs of cytotoxic action on epitheliocytes and blood cells were also noted.

DISCUSSION

So, the action of opioids in the oral cavity is determined both by their presence in the blood and by their sufficiently high content in the oral fluid and saliva, so the tissues of the gums and periodontium can be considered as "shock tissues" in relation to the action of the opioid. With long-term opioid exposure, systemic changes in the body occur at the cellular level, in particular, disruption of redox processes, which leads to tissue hypoxia [10]. At the same time, mechanisms of immune protection dependent on tissue respiration, in particular phagocytosis, may be suppressed. As a result of the reduction of natural protective mechanisms, there is a shift in the balance between microbial populations in microbiotopes. This balance can be disturbed by the activation of opportunistic microflora, infection by allochthonous microflora, as well as due to an increase in virulence caused by genotypic and phenotypic mechanisms [30]. There is a shift in the balance and the replacement of some microbial populations by others. Therefore, in the oral cavity there is a high risk of the formation of foci of chronic microbial infection, which later become a source of constant pathogenic contamination and sensitization of the alimentary canal and the body as a whole [19].

According to the literature, the role of microbiota in the development of periodontitis is not fully understood, although some "pathogens" individually or in groups can play an important role in the development of the inflammatory process in the oral cavity [17, 19, 29, 36, 38]. Also important is the formation of compound dental biofilms of different composition of bacteria embedded in the matrix of polymers of microbial and salivary origin, which are recognized as a virulence factor of many infectious and inflammatory diseases of the oral cavity [22, 28, 32, 33, 36] and cause destruction epithelial connection in the area of the gingival sulcus, ensuring the spread of infection in the underlying tissue [17, 34]. Whereas the level of microbial colonization of the oral cavity depends on the duration of drug use, the heterogeneity of microbiocenosis in both qualitative and quantitative composition dominates in opioid-dependent individuals [12, 13]. It is reported that periodontopathogenic bacteria support the dysfunction of the epithelial barrier in the gums by producing several virulence factors [34]. In addition, due to the spread of plaque microflora in the subgingival space, bacterial toxins stimulate the epithelium to produce biologically active mediators with the subsequent involvement of various types of cells in the process. Exo- and endotoxins of microorganisms directly and indirectly cause a number of pathological changes, in particular, toxins from periodontal pockets *penetrate into the bloodstream in significant quantities and without obstacles*, i.e. the state of bacteremia becomes permanent [21, 23, 34].

As a result of the conducted microbiological research, we evaluated the effect of individual bacterial isolates based on the detection of their virulence factors, in particular, cytotoxins. It can be asserted that initial inflammatory changes within the gingival sulcus caused by changes in the microbiocenosis were detected already in the short-term stages of opioid exposure. Thus, during the microscopic examination, acid-producing bacteria – *Leptothrix* with gram-positive coccal microflora organized in relation to them were detected in the short-term stages of opioid exposure. The formation of supragingival biofilm (cariogenic species of streptococci and organic substrate) was determined in the long-term stages opioid exposure.

At the later stages of opioid exposure, the activation of gram-negative opportunistic microflora (*Klebsiella, Escherichia, Pseudomonas, Bacteroids*) led to the development of necrotic changes as a result of the interaction of conservative lipopolysaccharide structures (patterns) with the TLRs of macrophages that penetrated the periodontal pockets at the earlier stages of the development of the inflammatory process caused by gampositive cocci producing exotoxins (α-hemolysins and lecithinase of staphylococci, β-hemolysins of streptococci, phospholipase of *Bacteroides*). This is evidenced by the adsorption of polymorphic gram-negative microflora (bacteroids) on epitheliocytes, autophagocytosis of erythrocytes, and the appearance of multinucleated cells as a result of impaired division processes. In addition, during the destruction of bacterial cells, lipopolysaccharides of gram-negative microflora act as endotoxins, causing both a general

toxic and a local effect on tissues and serve as a key factor in the formation of foci of infection [22].

In the *experimental conditions with the constant action of opioids* disrupt redox processes and cause suppression of cellular protective reactions, in particular, phagocytosis. This process contributes to increased colonization of periodontal tissues and the mucous membrane of the oral cavity, in particular, by phenotypically virulent variants of *Staphylococcus aureus* with a high degree of tropism to the epitheliocytes of the gingival mucosa, which is an extremely negative prognosis [25].

Thus, when comparing the experimental results obtained by us with data from the literature, it can be assumed that with the constant and long-term action of opioid analgesics, dysbiotic changes in the microbiota of the oral cavity develop consistently. In the short-term stages of opioid exposure, these changes were manifested by the activation of autochthonous microflora, which was evidenced by an increase in the density of the colonization of certain types of microorganisms and the formation of supracellular microbial structures as components of the biofilm. Under the conditions of further long-term action of the opioid analgesic, microorganisms that are not characteristic of the natural microbiota of the oral cavity of rats, which are characterized by virulence factors, mainly coccal microflora and hemolytic gram-negative bacteria were detected. In the long-term stages of opioid exposure, the microflora of the studied microbiotope changed towards the predominance of gram-negative aerobic and anaerobic microflora, and necrotic-inflammatory processes developed in periodontal tissues against the background of signs of inhibition of the body's *protective response*.

CONCLUSIONS

1. Depending on the duration of the experimental action of the opioid in the microbiotopes of the oral cavity (the surface of the teeth in the area of the gingival margin, the gingival groove), changes in the species composition of the biocenosis and the expression of virulence factors by microorganisms were detected.
2. In the short-term stages of opioid exposure, changes in biocenoses were manifested in an increase in the contamination of certain types of microorganisms and the formation of supracellular structures with a predominance of gram-positive bacteria.
3. In the long-term effects of the opioid analgesic, the activation of bacterial elements of the microbiocenosis was revealed, which were characterized by increased expression of virulence factors – hemolysins, coagulase, lecithinase, and cytotoxins.
4. At the later stages of opioid exposure, the predominance of gram-negative microflora and the activation of cytotoxic action against the background of suppressed activity of the body's protective *response* were established, which waere manifested in the development of necrotic-inflammatory changes in the tissues of the studied biotope.

REFERENCES

1. Cohen B, Ruth LJ, Preuss CV. Opioid Analgesics. 2021 Oct 18. In: StatPearls. Treasure Island (FL): StatPearls Publishing; 2022.
2. Dalal Sh, Bruera E. Pain Management for Patients With Advanced Cancer in the Opioid Epidemic Era. American Society of Clinical Oncology Educational Book. 2019;39:24–35. doi: 10.1200/EDBK_100020. DOI
3. Garg RK, Fulton-Kehoe D, Franklin GM. Patterns of opioid use and risk of opioid overdose death among Medicaid patients. Medical Care. 2017;55(7):661–668. doi:10.1097/MLR.0000000000000738. DOI
4. Béliveau A, Castilloux AM, Tannenbaum C, Vincent Ph. Predictors of long-term use of prescription opioids in the community-dwelling population of adults without a cancer diagnosis: a retrospective cohort study. CMAJ OPEN. 2021;9(1):96–106. doi:10.9778/cmajo.20200076. DOI
5. Taqi MM, Faisal M, Zaman H. OPRM1 A118G Polymorphisms and Its Role in Opioid Addiction: Implication on Severity and Treatment Approaches. Pharmgenomics Pers Med. 2019;12:361–368. doi: 10.2147/PGPM.S198654. DOI
6. DeClercq V, Nearing JT, Langille MGI. Investigation of the impact of commonly used medications on the oral microbiome of individuals living without major chronic conditions. PLoS One. 2021;16(12):e0261032. doi: 10.1371/journal.pone.0261032. DOI
7. Kosciolek T, Victor TA, Kuplicki R et al. Individuals with substance use disorders have a distinct oral microbiome pattern. Brain Behav Immun Health. 2021;15:100271. doi:10.1016/j.bbih.2021.100271. DOI
8. Mukherjee A, Dye BA, Clague J et al. Methamphetamine use and oral health-related quality of life. Qual Life Res. 2018;27(12):3179–3190. doi:10.1007/s11136-018-1957-6. DOI
9. Simpson S, Mclellan R, Wellmeyer E et al. Drugs and Bugs: The Gut-Brain Axis and Substance Use Disorders. J Neuroimmune Pharmacol. 2022;(1-2):33-61. doi:10.1007/s11481-021-10022-7. DOI

10. Imtiaz MS, Bandoian CV, Santoro TJ. Hypoxia driven opioid targeted automated device for overdose rescue. Sci Rep. 2021;11:24513. doi: 10.1038/s41598-021-04094-x. DOI
11. Eisenstein TK. The Role of Opioid Receptors in Immune System Function. Front Immunol. 2019;10:2904. doi: 10.3389/fimmu.2019.02904. DOI
12. Hartnett KP, Jackson KA, Felsen C et al. Bacterial and Fungal Infections in Persons Who Inject Drugs – Western New York, 2017. MMWR Morb Mortal Wkly Rep. 2019;68(26):583–586. doi: 10.15585/mmwr.mm6826a2. DOI
13. Yang Y, Yu X, Yang X et al. Oral Microbiota Profile of Individuals Who Abuse Methamphetamine. Front Cell Infect Microbiol. 2021; 11:706961. doi: 10.3389/fcimb.2021.706961. DOI
14. Jenkins WD, Beach LB, Rodriguez C, Choat L. How the evolving epidemics of opioid misuse and HIV infection may be changing the risk of oral sexually transmitted infection risk through microbiome modulation. Crit Rev Microbiol. 2020;46(1):49–60. doi: 10.1080/1040841X.2020.1716683. DOI
15. Petrushanko TO, Boichenko OM, Popovych IYu, Gryshchenko VV. Chastota rotovoyi porozhnyny u narkozalezhnykh patsiyentiv, [The rate of Oral Cavity in the Drug-Depedent Patients]. Svit medytsyny ta biolohiyi. 2021;3(77):148–151. doi: 10.26724/2079-8334-2021-3-77-148-151. (Ukrainian) DOI
16. Celik D, Kantarci A. Vascular Changes and Hypoxia in Periodontal Disease as a Link to Systemic Complications. Pathogens. 2021;10(10):1280. doi: 10.3390/pathogens10101280. DOI
17. Dahlen G, Fejerskov O, Mangi F. Current concepts and an alternative perspective on periodontal disease. MC Oral Health. 2020;20(1):235. doi: 10.1186/s12903-020-01221-4. DOI
18. Fik VB, Fedechko YM, Paltov YeV. The composition of the microbiotes of the neck part of the teeth in the gum edge of rats at the end of the second, fourth and sixth weeks of opioid influence. World science. 2020;9(61):1–5. doi: 10.31435/rsglobal_ws/30122020/7338. DOI
19. Khmil OV, Khmil DO, Kaskova LF et al. Mikrobiotsenoz ta stan mistsevoho imunitetu slyzovoyi obolonky rotovoyi porozhnyny ditey ta pidlitkiv iz zakhvoryuvannyamy pechinky. [Microbiocenosis and the state of local mucosal immunity of the oral cavity of children and adolescents with liver diseas. Ukrayins'kyy zhurnal medytsyny, biolohiyi ta sportu. 2021;5(33):316–321. doi: 10.26693/jmbs06.05.316. (Ukrainian) DOI
20. Nisha S, Samyuktha GS, Shashikumar P, Chandra S. Periodontal disease – Historical and contemporary hypothesis: A review. SRM Journal of Research in Dental Sciences. 2017;8(3):121–125. doi: 10.4103/srmjrds.srmjrds_15_17. DOI
21. Peng X, Cheng L, You Y et al. Oral microbiota in human systematic diseases. Int J Oral Sci. 2022;14(1). doi:10.1038/s41368-022-00163-7. DOI
22. Marcano R, Rojo MÁ, Cordoba-Diaz D, Garrosa M. Pathological and Therapeutic Approach to Endotoxin-Secreting Bacteria Involved in Periodontal Disease. Toxins (Basel). 2021;13(8):533. doi: 10.3390/toxins13080533. DOI
23. Minasyan H. Sepsis: mechanisms of bacterial injury to the patient. Scand J Trauma Resusc Emerg Med. 2019;27(1):19. doi:10.1186/s13049-019-0596-4. DOI
24. Sylenko HM, Skrypnykov PM, Sylenko YI, Pisarenko OA. Features of development of generalized periodontitis in persons with secretory immunoglobulin a deficiency and its treatment (literature review). Wiad Lek. 2021;74(6):1510–1514. doi: 10.36740/WLek202106141. DOI
25. Zghair M, Hussain MS, Sahib AA. Studying Some Factors affecting in Gingivitis caused by Staphylococcus aureus. International Journal of Pharmaceutical Quality Assurance. 2019;10(2):280–284. doi:10.25258/ijpqa.10.2.11. DOI
26. Kantsler V, Ontañón-McDonald E, Kuey C et al. Pattern Engineering of Living Bacterial Colonies Using Meniscus-Driven Fluidic Channels. ACS Synth Biol. 2020;9(6):1277–1283. doi:10.1021/acssynbio.0c00146. DOI
27. Burcham ZM, Garneau NL, Comstock SS et al. Patterns of Oral Microbiota Diversity in Adults and Children: A Crowdsourced Population Study. Sci Rep, 2020;10:2133 doi:10.1038/s41598-020-59016-0. DOI
28. Graves DT, Correa JD, Silva TA. The Oral Microbiota Is Modified by Systemic Diseases. J. Dent. 2019;98:148–156. doi:10.1177/0022034518805739. DOI
29. Lenartova M, Tesinska B, Janatova T et al. The Oral Microbiome in Periodontal Health. Front Cell Infect Microbiol. 2021;11:629723. doi: 10.3389/fcimb.2021.629723. DOI
30. Preda M, Mihai MM, Popa LI et al. Phenotypic and genotypic virulence features of staphylococcal strains isolated from difficult-to-treat skin and soft tissue infections. PLoS One. 2021;16(2):e0246478. doi: 10.1371/journal.pone.0246478. DOI
31. Sichkoriz HA. Zminy mikrobiotsenozu porozhnyny rota pry zakhvoryuvannyakh parodontu na tli khronichnoho hepatytu S. [Changes in the microbiocenosis of the oral cavity in periodontal disease on the background of chronic hepatitis C]. Visnyk problem biolohiyi ta medytsyny. 2018;2(4):348–352. doi: 10.29254 / 2077-4214-2018-4-2-147-348-352. (Ukrainian) DOI
32. Paul O, Arora P, Mayer M, Chatterjee S. Inflammation in Periodontal Disease: Possible Link to Vascular Disease. Front Physiol. 2021;11:609614. doi:10.3389/fphys.2020.609614. DOI

33. Sedghi LM, Bacino M, Kapila YL. Periodontal Disease: The Good, The Bad, and The Unknown. Front Cell Infect Microbiol. 2021;11:766944. doi: 10.3389/fcimb.2021. DOI
34. Şenel S. An Overview of Physical, Microbiological and Immune Barriers of Oral Mucosa. Int J Mol Sci. 2021;22(15):7821. doi: 10.3390/ijms22157821. DOI
35. Berger D, Rakhamimova A, Pollack A, Loewy Z. Oral Biofilms: Development, Control, and Analysis. High Throughput. 2018;7(3):24. doi: 10.3390/ht7030024. DOI
36. Willis JR, Gabaldón T. The Human Oral Microbiome in Health and Disease: From Sequences to Ecosystems. Microorganisms. 2020;8(2):308. doi: 10.3390/microorganisms8020308. DOI
37. Hillmann ET, Lu H, Yao T, Nakatsu CH. Microbial Ecology along the Gastrointestinal Tract. 2017;32(4):300–313. doi: 10.1264/jsme2.ME17017. DOI
38. Walkenhorst MS, Reyes L, Perez G et al. A Uniquely Altered Oral Microbiome Composition Was Observed in Pregnant Rats With Porphyromonas gingivalis Induced Periodontal Disease. Front Cell Infect Microbiol. 2020;10:92. doi: 10.3389/fcimb.2020.00092. DOI

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CONFLICT OF INTEREST
The Authors declare no conflict of interest

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