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Research Article

Pharmacological correction of periodontitis using synthetic analogues of indolicidin

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Abstract

Introduction: The leading role of pathogenic microorganisms in the pathogenesis of periodontitis is beyond doubt. However, the use of antibiotics for periodontitis is associated with a number of problems. Indolicidins have a unique anti-microbial effect. The relevance of the search for new drugs for the treatment of acute periodontitis based on the natural indolicidin peptide becomes obvious.

Materials and methods: The investigation was performed on 320 Wistar male rats, using synthetic analogues of natural indolicidin: No. 7 and No. 8, which were administered intraperitoneally at a dose of 500 μg/kg in a volume of 0.2 ml once a day for 7 days. Periodontitis was simulated in animals according to the method proposed by Volozhin A.I. and Vinogradova S.I.

Results and discussion: The correcting effect of indolicidin analogues on the periodontitis course, was manifested by a decrease in edema and in the relative area of cell infiltrates, a significant increase in the relative area of normal tissue, and a correction of the periodontal composition. The use of indolicidin analogues led to an increase in the functional activity of neutrophils and macrophages, acute phase proteins concentration, a correction of pro-oxidant-antioxidant balance and production of vasoactive substances. The effect of indolicidin analogues was higher than that of lincomycin. The greater effectiveness of peptide No.8 compared to that of No.7 was established.

Conclusion: The investigation opens up the prospects of the synthesis of new antimicrobial drugs on the basis of the synthetic analogues of indolicidin.

Keywords

periodontitis, indolicidin analogues, antimicrobial drugs, neutrophils, macrophages.

Introduction

The most important periodontal disease is periodontitis – inflammation of periodontal tissues. Its prevalence among the adult working-age population reaches 75% (Tsepov et al. 2014). In this case, periodontitis leads to the loss of a large number of intact teeth, as well as the emergence of foci of chronic infection due to the formation of gingival and periodontal pockets, which contributes to the formation of somatic pathology. Several theories have been proposed to explain the occurrence and development of periodontitis, but the leading role of pathogenic micr-

oorganisms in the pathogenesis of this disease is beyond doubt (Guyodo et al. 2012, Komman 2008, Promsudthi et al. 2014, Samuels et al. 1993). However, the use of antibiotics in periodontitis is associated with a number of problems: the low sensitivity of microbes to antibiotics, since most pathogens are related to gram-negative flora, the formation of resistance of microorganisms to drug exposure, and the development of allergic reactions to antibiotics.

In this regard, the search for new methods of treating and preventing periodontitis has particular relevance. In recent years, the new class of microbicides – antimicrobial peptides, including indolicidins, has become of interest to researchers (Artamonov et al. 2014, Brogden and Brogden 2011, Dixon et al. 2009, Dosler and Karaaslan 2014, de la Fuente-Núñez and Hancock 2015, Ghaffar et al. 2015, Gordon et al. 2005, Reffuveille et al. 2014). A unique effect of antimicrobial peptides is characterized by their selective action to microorganisms, as indolicidin cationic proteins have a high affinity to bacterial membranes containing a large amount of negatively charged molecules of lipopolysaccharides. The mechanism of the bactericidal action of antimicrobial peptides is associated with a rapid increase in membrane permeability of bacterial cells, which leads to osmotic destruction (Orlov et al. 2002, Nan et al. 2009, Yeaman and Yount 2003, Zhu et al. 2006).

Antimicrobial peptides are not accumulated in the organism because they are destroyed by proteases and inactivated by plasma proteins, wherein bacterial resilience to indolicidins does not develop (Artamonov et al. 2014, Gordon et al. 2005). By now synthetic analogs of indolicidin with a strong bactericidal effect, not causing hemolysis, have been synthesized (Ando et al. 2010, Artamonov et al. 2014, Guani-Guerra et al. 2010, Jindal et al. 2015, Recdal et al. 2012, Torcato et al. 2013, Yu et al. 2015). It has been established that synthetic indolicidins manifest an immunomodulatory effect (Artamonov et al. 2009, Bowdish et al. 2005, Chang et al. 2013, Kovacs-Nolan et al. 2009, Sur et al. 2015), affect the pro-oxidant-antioxidant balance, and stimulate the regeneration of burn wounds (Lazarenko et al. 2017).

In connection with the foregoing, the relevance of the search for new drugs for the treatment of acute periodontitis based on the natural peptide indolicidin becomes obvious.

The objective of the investigation is to improve the efficacy of the pharmacological correction of acute periodontitis through the use of synthetic analogues of indolicidin.

Materials and methods

In the study, 320 male Wistar rats weighing 180–250 g, obtained from Stolbovaya nursery (Russian Academy of Medical Sciences). The investigations were conducted in compliance with the provisions concerning animals set out in the European Community Directives (86/609 EC) and the Rules of Good Laboratory Practice in the Russian Federation (Order No.199n of the Ministry of Public Healthcare of the Russian Federation dated 01.04.2016). The execution of experiments was approved by the Regional Ethics Committee (REC) (minutes No. 3 dated 10.27.2015).

In the study, synthetic analogues of natural indolicidin No.7 (H-Ile-Leu-Pro-Trp-Lys-Lys-Pro-Trp-Lys-Pro-Trp-Arg-Arg-NH_2) and No. 8 (H-Ile-Lys-Pro-Trp-Lys-Trp-Pro-Trp-Lys-Pro-Trp-Arg-Arg-NH_2) (NPF Verta Ltd, St. Petersburg, Russia). Like the natural peptide, synthetic analogues have a high antibacterial activity, while they do not show a hemolytic effect inherent in natural indolicidin (Artamonov et al. 2009). The peptides were pre-dissolved in saline and injected intraperitoneally at a dose of 500 μg/kg in a volume of 0.2 ml once a day for 7 days, starting from the day the ligature was removed. The rats of the control group were similarly injected with saline.

Lincomycin was used as a reference drug, which was injected intramuscularly as a 30% solution at a dose 0.5 g/kg for 7 days of the experiment in a volume of 0.2 ml.

Periodontitis was modeled according to the method proposed by A.I.Volozhin and S.I. Vinogradova (1990). Animals were anesthetized by intraperitoneal administration of chloral hydrate at a dose of 0.015 ml/kg bw. Local anesthesia in the area of the lower incisors was performed with a 2% solution of novocaine. Sutures were inserted in a figure-eight pattern on the lower jaw incisors, followed by placing the surture thread in the gingival crevice and fixing it with additional knots. The thread was left for 14 days and then removed. The animals were sacrificed with an overdose of ether on days 7, 14 and 21 after the removal of the surture thread.

The color, the presence of the swelling of the mucous membrane of the gums, the development of hypertrophy of the interdental papillae, the appearance of periodontal pockets, the sulcus bleeding index (SBI) were evaluated in the experimental animals on the 7^{th}, 14^{th}, and 21^{st} days after removing the surture thread; besides Schiller-Pisarev test was made and the amount (weight) of gingival fluid (AGF) was determined (Leontyev and Galenko-Yaroshevsky 2013).

SBI was determined as follows: careful probing of the sulcus from the vestibular and lingual sides was carried out, using a bulbous bougie. The tip of the probe without pressure is placed against the wall of the groove and slowly moved from the medial to the distal side of the tooth. The periodontal condition was assessed according to the following scale: 0 – no bleeding, 1 – bleeding occurs within 30–60 seconds after probing, 2 – bleeding occurs within 0–30 seconds after probing.

Schiller-Pisarev test was performed as follows: the gingival margin was treated with Lugol's solution. In the inflammation, glycogen is accumulated in the gum, getting stained brown with Lugol's solution. Traditionally, the color of the papilla was estimated as 1 point (P), the color of the gingival margin (M) was 2 points, the color of the alveolar gum (A) was 3 points, and the lack of color was 0 point.

AGF according to Brill N. and Crasse B. (1961) was measured as follows: a strip of filter paper was weighed, the pointed end of the filter paper strip was inserted into the opening of the gingival crevice, without reaching the

bottom in order to avoid mechanical stimulation of the tissue, which may lead to an increase in the release of gingival fluid. The soaking time of the strip – 3 minutes. The strip of filter paper was measured again on a torsion balance. Three strips of paper were used for each rat, the average value was entered into the protocol.

Phagocytic activity of peripheral blood neutrophils was assessed according to the standard technique in the modification of the authors of the present paper, for which the bacterial suspension of Staphylococcus aureus (10^9 microbial cells/ml) was added to the tube with 0.5 ml of heparinized blood at the rate of 10 microbes per 1 neutrophil. The mixture was incubated, smears were prepared and stained according to Romanovsky-Giemsa method. The following indices of the phagocytic activity of neutrophils were determined in the microscopy of the smears: phagocytic index (PI) – the number of actively phagocytic neutrophils out of 100 cells; phagocytic number (PN) – the average number of microbial bodies captured by 1 phagocytic neutrophil; and the opsonic phagocytic index (OFI) – the average number of microbial bodies absorbed per 100 neutrophils.

The literature data show that the adhesive ability of macrophages is an integrative index of their functional activity (Andre et al. 1990). The adhesive properties of macrophages were investigated by the non-dynamic method, which is based on the ability of cells to attach to a clean glass surface (Frimel 1987). Sterile medium 199 (10 ml) was injected into the abdominal cavity, and 3–5 minutes later, the medium was withdrawn. The resulting suspension was incubated for one hour in a thermostat at 37°C. After incubation, non-adhered macrophages were removed from the suspension. Smears of the initial suspension of macrophages and suspension of non-adhered cells were prepared. The resulting smears, as well as the adhered cells in a Petri dish, were stained according to the Romanovsky-Giemsa method. In the smears, the percentage of macrophages of the total number of cells in the smear and the relative number of macrophages adhering to the glass in the macrophage population were determined.

After euthanasia, tissues were withdrawn from the animals for examination. Preliminarily separated gingival tissue on the upper and lower half jaws weighing 100 mg were homogenized in 1 ml of 0.025 M Tris – HCl buffer (pH 7.4). The condition of the connective tissue matrix (CTM) was assessed by the level of free and bound oxyproline (OP) and glycosaminoglycans (GAG) in the gum tissue (Sharayev 1981, Sharayev et al. 1987).

The content of OP and GAG in the connective tissue matrix (CTM) of the periodontium was determined. The concentration of free and bound OP was determined by a modified method based on the oxidation of OP by chloramine B and the condensation of its oxidation products by paradimethylaminobenzaldehyde. OP content was expressed in mmol/g of tissue. The content of GAG was determined by modification of the method of quantitative analysis using trichloroacetic acid and a known carbonyl reaction. GAG content was expressed in mg/g of tissue.

The condition of pro-oxidant-antioxidant balance was evaluated by the content of lipid peroxidation products (LPO) in the blood plasma or gum tissue homogenate: acylhydroperoxides (AHP) and malonic dialdehyde (MDA), as well as the activity of enzymes of the antioxidant system: superoxide dismutase (SOD) and catalase, which were evaluated by traditional methods.

The level of endothelin-1 in blood plasma was determined by ELISA, using Biomedica kits (Medizinprodukte GmbH Co KG (Austria)) and expressed in fmol/ml.

The stable metabolites of nitric oxide (NO_2/NO_3) (SMNO) in blood were determined spectrophotometrically by their excretion (ENO_x) (Golikov and Nikolayeva 2004). The content of C-reactive protein (CRP) was evaluated by an immunoturbidimetricassay, using Analyticon reagents (Germany) on a Vikalab Flexor E. automatic biochemical analyzer.

The concentration of alpha$_1$-antitrypsin was determined by an immunoturbidimetric assay, using Sentinel reagents (Italy) on a BTS 330 automatic biochemical analyzer (Spain). The measurement was performed at a wavelength of 340 nm, using multipoint calibration.

The concentration of ceruloplasmin was determined by an immunoturbidimetric assay, using Sentinel reagents (Italy) on a BTS 330 automatic biochemical analyzer (Spain). The measurement was carried out at a wavelength of 520 nm, using multipoint calibration.

After tissue isolation, a fragment of the mandible of the rats was fixed in 10% neutral formalin, followed by decalcification. After washing in running water for 24 hours, the tissues were dehydrated in alcohols of increasing concentration; then the material was passed through alcohol-chloroform, chloroform, chloroform-paraffin and embedded in paraffin. Paraffin blocks were used to make sections of 5–6 um thick, which were stained with hematoxylin-eosin or according to van Gieson. The sections were microscoped layer-by-layer. A histological analysis of tissue structure was carried out. A microscopic examination of sections of a mandible fragment stained with hematoxylin and eosin was performed, and the average thickness of the gingival epithelial layer, the relative area of the vascular bed in the reticular gingiva, the relative area of infiltrates in the periodontal tissue, the relative area of fibrous connective tissue and the relative area of normal tissue were determined, using Avtandilov grid and a micrometric ruler.

The results of the studies were statistically processed. The arithmetic mean values and their standard errors were calculated. The significance of differences in mean values was assessed by Student's t. A correlation analysis was carried out, with the calculation of the strength and directionality of correlations.

Results and discussion

On days 7–21 of the experiment, the mucous membrane of the gums is dark red in color, hyperemic and swollen in the animals with simulated periodontitis. Several erosions

Table 1. Effect of Iindolicidin Analogues No.7 and No.8 on the Change of Periodontal Indices in Acute Periodontitis (M±m, n=8).

Group	Indicator			
	Period of experiment, day	Sulcus Bleeding Index, points	Schiller-Pisarev test, points	Amount of gingival fluid, mcg
Intact		0.25±0.16	0.25±0.16	22.4±1.5
Control (periodontitis + 0.9% NaCl solution)	7	1.75±0.16^{xxx}	2.88±0.13^{xxx}	81.5±3.5^{xxx}
	14	2.00±0.00^{xxx}	3.00±0.00^{xxx}	96.2±3.2^{xxx}
	21	1.63±0.18^{xxx}	2.50±0.19^{xxx}	84.0±3.6^{xxx}
Injection of indolicidin No.7	7	1.88±0.13	2.88±0.13	90.8±3.1^{1}
	14	1.63±0.18	2.75±0.16	81.9±3.3*
	21	0.63±0.18**	1.38±0.18***	45.4±2.9***
Injection of indolicidin No.8	7	1.88±0.13	2.88±0.13	91.5±4.0^{1}
	14	1.75±0.16	2.75±0.16	82.3±3.3*
	21	0.38±0.18***	1.25±0.16***	43.1±2.6***
Injection of lincomycin	7	1.63±0.18	2.50±0.19	77.4±4.0
	14	1.50±0.19***	2.63±0.18	75.9± 2.7***
	21	0.38±0.18***	1.25±0.16***	41.5±3.4***

Note: ^{x} –p<0.05, ^{xx} – p<0.01, ^{xxx}– p<0.001 compared to intact animals; * – p<0.05, ** – p<0.01, *** – p<0.001 compared to control animals; ^{1} – p<0.05, ^{11} – p<0.01, ^{111} – p<0.001 compared to animals treated with a solution of lincomycin.

were observed on the mucous. The teeth had plaque. The interdental papillae were enlarged. The gums above the incisors were spindle-shaped. Periodontal connections in the area of incisors were broken, and periodontal pockets were formed, releasing purulent material. SBI increased 6.5–8.0 times (p<0.001), Schiller-Pisarev test was positive in all the animals, and its index increased 10–12 times (p<0.001) (Table 1). AGF increased throughout the experiment 3.64–4.29 times (p<0.001).

The use of analogues of indolicidin No.7 and № 8 did not affect the studied parameters (SBI, Schiller-Pisarev test, AGF) on the 7th days after removing the suture thread. On the 14th day of the experiment, AGF decreased statistically significantly: by 14.9% in rats treated with indolicidin No.7, and by 14.4% – with the administration of indolicidin No.8 (p<0.05). Twenty-one days after the removal of the suture thread, there was a drop in all the studied parameters characterizing the severity of inflammatory processes in the gum tissue in rats treated with indolicidin analogues No.7 and No.8: SBI –2.59 and 4.29 times (p<0.001); Schiller-Pisarev test –1.81 and 2.0 times (p<0.001); AGF – by 46.0% and 48.7% (p<0.001), respectively.

The use of lincomycin in the form of a 30% solution at a dose of 0.5 g/kg led to a statistically significant decrease in SBI on the 14th and 21st days of the experiment, Schiller-Pisarev test – on the 21st day and AGF – on the 14th–21st days after the completion of periodontitis modeling (p<0.001).

On the sections of the periodontal tissue on the 7th–21st days after the completion of periodontitis modeling, it was established that inflammatory changes developed in all the gum tissues and decreases during the experiment. The thickening of the epithelial layer was established due to the swelling (Table 2). There were signs of acanthosis of the epithelium, while the acanthotic processes were thickened and elongated, penetrating into the lamina propria of the mucous membrane. There was diapedesis of neutrophils and their accumulations in the epithelial layer with the formation of purulence foci. Alterative changes were also manifested by dystrophic changes of epithelium cells in the form of hydropic and, less often, balloon dystrophy.

Lamina propria of the gums is thickened due to swelling. The relative area of the vascular bed is reduced compared to the intact group, with some of the vessels expanded. Inflammatory infiltrates, consisting mainly of neutrophils, occupy a significant area of the lamina propria. Fibrous connective tissue is formed at the site of cellular infiltrates. Normal tissue in the gum is insignificant. The periosteum is swollen, and the vessels are sclerotic.

In the groups of animals treated with analogues of indolicidin or lincomycin, the morphological picture did not differ significantly on the 7th day, but as early as on the 14th day after removal of the suture thread, the epithelium thickness was less than that in the control group by 10.4% (p<0.05), with the administration of indolicidin No.7 and – by 8.8% when using indolicidin No.8 (p<0.05). There was a decrease in the relative area of cellular infiltrates by 22.3% (p<0.01) and by 19.7% (p<0.01) with the use of indolicidins No.7 and No.8, respectively. There was a significant increase in the relative area of normal tissue compared with the control group: 2.1 times with the injection of indolicidins No.7 and No.8 (p<0.001). No differences were established in the following indicators: the relative area of the vascular bed and the relative area of fibrous connective tissue between animals of the control group and rats receiving peptides.

In the group treated with lincomycin at a dose of 0.5 mg/kg, there was a statistically significant decrease in the relative area of cell infiltrates (by 17.5%, p<0.05) and an increase in the relative area of normal tissue by 51.7% (p<0.01). There were no statistically significant differences between the following indicators: the thickness of the epithelial layer of the gums, the relative area of the vascular bed and the relative area of the unformed fibrous connective tissue between the animals of the control group and those who received lincomycin.

Table 2. Effect of Indolicidin Analogues No.7 and No. 8 on the Development of Regenerative Processes in the Gingival Tissue in Wistar Rats with Acute Periodontitis (M±m, n=8).

Group	Indicator					
	Period of experiment, day	Tickness of gingival epithelial layer, mcm	Relative area of vascular bed in gum tissue,%	Relative area of cellular infiltrates in gum tissue,%	Relative area of fibrous connective tissue in gums,%	Relative area of normal tissue,%
Control	7	316.6±7.3	6.1±0.3	46,9±2.0	28.9±2.4	4.6±0.4
	14	280.6±7.0	6.4±0.4	35.5±1.7	44.3±1.7	8.9±0.5
	21	226.0±3.3	8.9±0.3	24.9±1.3	37.9±1.5	25.4±1.2
Injection of indolicidin No.7	7	312.4±5.1	6.4±0.3	42.4±1.8	32.1±2.3	5.0±0.3
	14	251.4±5.8*	7.5±0.3	27.6±0.9**	41.1±2.5	18.3±1.2*** ^{xx}
	21	172.4±4.2***	8.5±0.3	18.8±0.8**	30.0±1.3**	37.6±1.3** ^{xx}
Injection of indolicidin No.8	7	305.1±5.4	6.1±0.3	40.1±2.7	35.1±1.8	5.1±0.3
	14	255.8±4.3*	7.3±0.4	28.5±1.2**	39.3±1.2*	18.8±1.0*** ^{xx}
	21	176.3±3.5***	8.8±0.4	18.0±1.1**	29.8±1.5**	36.3±1.5*** ^{x}
Injection of lincomycin	7	306.4±5.1	6.3±0.3	43.0±2.1	31.1±1.9	4.3±0.3
	14	264.6±4.4*	7.1±0.3	29.3±1.6	40.3±1.0	13.5±0.8**
	21	206.0±5.1**	8.6±0.4	19.9±1.0**	31.8±1.0**	30.9±1.0**

Note: ^{x} – $p<0.05$, ^{xx} – $p<0.01$, ^{xxx} – $p<0.001$ compared to intact animals; * – $p<0.05$, ** – $p<0.01$, *** – $p<0.001$ compared to control animals; ^{1}– $p<0.05$, ^{11} – $p<0.01$, ^{111} – $p<0.001$ compared to animals treated with a solution of lincomycin.

The administration of indolicidins No.7 and No.8 to animals with acute periodontitis abrogated epithelial edema on the 21st day – its thickness did not differ significantly from the corresponding index in intact rats ($p>0.05$). The relative area of cellular infiltrates in these groups was significantly less: by 24.5% and 27.7%, respectively, when administering indolicidins No.7 and No 8, compared to the control group ($p<0.01$). More than one-third of the section was occupied by normal tissue in the groups treated with indolicidin analogues No.7 and No.8,, which was 48.0% and 42.9%, respectively, compared to the control group ($p<0.001$). The relative area of the unformed fibrous connective tissue was by 20.8% and 20.9% lower, respectively, when administering analogues No.7 and No.8 ($p<0.01$) than in rats of the control group.

In the rats that were treated with lincomycin, there was a decrease in the relative area of cellular infiltrates by 20.1% ($p<0.01$) and the relative area of unformed fibrous connective tissue by 18.7% ($p<0.01$). At the same time, the relative volume of normal tissue in this group was by 21.7% higher than that in the animals of the control group ($p<0.01$). No differences were established between the animals of the control group and those who were treated with lincomycin in the following indicators: the relative area of the vascular bed and the thickness of the epithelial layer of the gums.

Thus, the results suggest that indolicidins No.7 and No.8 have a corrective effect on the development of acute periodontitis, which is manifested by a decrease in the swelling, a reduction of the relative area of cellular infiltrates and a significant increase in the relative area of normal tissue. No significant differences were found between the effects of the indolicidin analogues under study. Comparison of the effects of peptides and lincomycin made it possible to establish that the corrective effect of the peptides was more pronounced than the effect of lincomycin. This was manifested by an increase in the relative area of normal tissue on days 14 and 21 of the experiment. So, the increase was by 35.6% and 39.3% ($p<0.01$) on day 14, and 21.7% ($p<0.01$) and 17.5% ($p<0.05$) on day 21, when using indolicidins No.7 and No.8, respectively ($p<0.01$). This indicates that the indolicidin analogues have a pronounced stimulating effect on the development of regenerative processes in the gum.

On the 7th day, there was a 16.2% decrease in the relative number of macrophages in peritoneal lavage in the rats with acute periodontitis ($p<0.001$), but there was an increase in their adhesive ability: the percentage of adhered macrophages increased by 9.6% ($p<0.05$) (Table 3). A significant decrease in both the relative number of macrophages and the percentage of adhered cells was noted in the control group on the 14th day after removal of the suture thread: Both indices decreased by 34.4% and 23.5% compared to the previous period ($p<0.001$). On the 21st day, the studied parameters increased: by 32.6% and 29.1%, respectively, compared to the previous period. At the same time, the relative number of macrophages in the peritoneal lavage remained significantly lower ($p<0.001$), and the percentage of adhered cells did not significantly differ from the similar indicators in the intact animals.

The use of indolicidin analogues in animals with acute periodontitis did not significantly affect the relative number of macrophages in the peritoneal lavage and the percentage of adhered macrophages on the 7th day after removing the suture thread compared to the control group.

On the 14th day, both analogues had a stimulating effect on both the relative amount of macrophages in the peritoneal (indolicidin No.7 – by 24.0% and indolicidin No.8 – by 20.0%, $p<0.001$), and on their adhesive ability: an increase of the percentage of adhered cells was 21.7% and 17.1% when using indolicidin No.7 and indolicidin No.8, respectively, compared to the control group ($p<0.05$).

On the 21st day of the experiment, only indolicidin No.7 exerted a stimulating effect on the number and functional

Table 3. Effect of Indolicidin Analogues No.7 and No.8 on the Adhesive Ability of Macrophages in the Rats with Acute Priodontitis (M±m, n=8).

Group	Index		
	Period of experiment, day	Relative amount of peritoneal macrophages in the lavage,%	Relative amount of adhered peritoneal macrophages,%
Intact		95.4±0.8	65.5±2.1
Control	7	79.9±2.2***	71.8±1.3*
	14	52.4±2.0***	54.9±2.8**
	21	69.5±2.4***	70.9±2.0
Injection of indolicidin No.7	7	76.0±2.3	70.8±1.6
	14	65.0 ± 2.1^{xxx}	66.8 ± 2.4^{x}
	21	78.0 ± 2.0^{x}	79.3 ± 1.8^{x}
Injection of indolicidin No.8	7	77.5±2.5	70.3±2.1
	14	62.9 ± 2.1^{xxx}	64.3 ± 2.1^{x}
	21	75.3±2.0	76.1±2.0
Injection of lincomycin	7	73.8±2.1	68.9±2.0
	14	54.9±2.5	58.4±2.7
	21	76.0±2.2	72.5±1.5

Note: ^{x} – p<0.05, ^{xx} – p<0.01, ^{xxx} – p<0.001 compared to intact animals; * – p<0.05, ** – p<0.01, *** – p<0.001 compared to control animals; ^{1} – p<0.05, ^{11} – p<0.01, ^{111} – p<0.001 compared to animals treated with a solution of lincomycin.

Table 4. Effect of Indolicidin Analogues No.7 and No.8 on the Phagocytic Activity of Peripheral Blood Neutrophils in Rats with Acute Periodontitis (M±m, n=8).

Group	Index			
	Period of experiment, day	Phagocytic index, n	Phagocytic number, n	Opsonic-phagocytic index, n
Intact		45.1±2.3	1.63±0.2	75.5±10.9
Control	7	27.0±1.4***	1.50±0.19	43.1±6.2*
	14	31.5±1.4**	2.13±0.30	69.6±11.8
	21	46.0±1.7	2.38±0.18*	110.9±12.0
Injection of indolicidin No.7	7	39.9 ± 1.6^{xxx}	3.00 ± 0.33^{xx}	117.9 ± 16.4^{xx}
	14	47.3 ± 1.4^{xxx}	3.13 ± 0.30^{xx}	150.0 ± 17.6^{xx}
	21	69.0 ± 1.3^{xxx}	3.38 ± 0.26^{x}	217.3 ± 23.1^{xx}
Injection of indolicidin No.8	7	38.6 ± 1.7^{xxx}	2.63 ± 0.26^{xx}	104.4 ± 15.2^{xx}
	14	47.1 ± 1.5^{xxx}	2.75 ± 0.31^{x}	132.5 ± 18.9^{xx}
	21	67.6 ± 1.4^{xxx}	2.88±0.30	196.8 ± 23.3^{x}
Injection of lincomycin	7	31.4±1.5	2.00±0.27	65.1±11.0
	14	39.8±1.5**	2.38±0.18	95.8±10.3
	21	58.9±1.6**	2.63±0.26	157.1±19.7

Note: ^{x} – p<0.05, ^{xx} – p<0.01, ^{xxx} – p<0.001 compared to intact animals; * – p<0.05, ** – p<0.01, *** – p<0.001 compared to control animals; ^{1} – p<0.05, ^{11} – p<0.01, ^{111} – p<0.001 compared to animals treated with a solution of lincomycin.

activity of macrophages: the relative number of cells exceeded the same indicator in the control group by 12.2% (p<0.05), and the percentage of adhered cells – by 11.8 % (p<0.05). The use of lincomycin did not affect the functional activity of macrophages at any period of observation.

In animals with simulated acute periodontitis, a decrease in the phagocytic activity of peripheral blood neutrophils is observed, which is manifested by a decrease in PI by 40.1% (p<0.001) and OPI by 42.9% (p<0.05) on the 7th day after removing the suture thread compared to the intact rats (Table 4). In this case, PN did not change significantly.

On the 14th day, PI still remained significantly, by 30.2%, (p<0.01), lower than that in the group of intact animals. However, there was a significant increase in PN (by 42.0%) compared to the previous observation period. At the same time, neither PN, not OPI significantly differed from the corresponding parameters in intact rats.

A statistically significant increase in PN in animals of the control group was observed on the 21st day of the experiment (by 46.0%, p<0.05). No significant differences were found between the values of PI and OPI in control and intact rats during this period.

The use of the studied indolicidin analogues had a stimulating effect on the phagocytic activity of neutrophils during all periods of observation. So, on the 7th day after removal of the suture thread, an increase in PI was 47.8% (p<0.001), PN – 100.0% (p<0.01), OPI – 366.6% (p<0.01) when using indolicidin No.7 and 43.0%, 75.3% and 142.2%, respectively, when using indolicidin No.8 (p<0.01–0.001).

On the 14th day of the experiment, an increase in PN was 49.5%, in PI – 46.9%, in OPI – 155.5% ($p<0.01$–0.001) in rats treated with indolicidin No.7. The stimulating effect of indolicidin No.8 was slightly lower: PN – 49.5%, PI – 29.1%, and OPI – 90.4% ($p<0.01$–0.001).

The stimulating effect of indolicidin No.7 on the phagocytic activity of neutrophils was also more pronounced on the 21st day 21 the removal of the suture thread. An increase in PI was 50.0%, the PN – 42.0%, OPI – 95.9% ($p<0.05$–0.001) with the administration of indolicidin No.7, and 47.0% ($p<0.001$), 21.0% ($p>0.05$) and 77.5% ($p<0.05$), respectively, with the administration of indolicidin No.8.

The use of lincomycin did not significantly affect the phagocytic activity of neutrophils on the 7th day after the removal of the suture thread. On the 14th and 21st days, an increase in PI in rats treated with lincomycin was 26.3% and 28.0% ($p<0.01$). The remaining indicators did not significantly differ from the corresponding values in control animals.

Thus, a decrease was established in the functional activity of neutrophils and macrophages in animals with experimental periodontitis on the 7th and 14th day of the experiment, which was manifested by a decrease in PI and OPI. The change in the functional activity of macrophages in terms of their adhesive ability was biphasic: an increase of the 7th day and a decrease of the 14th day.

Synthetic analogues of indolicidin No.7 and No.8 had a stimulating effect on the functional activity of phagocytes. This effect seems to be due both to the direct action of peptides on phagocytic cells, and indirectly through a change in the production of cytokines.

Simulation of acute periodontitis led to a statistically significant decrease in the content of free and bound OP, GAG in CTM throughout the experiment. So, on the 7th day, there was a decrease in the level of free OP by 21.9% ($p<0.01$), bound OP – by 23.8% ($p<0.01$), GAG – by 36.8% ($p<0.01$) in rats with periodontitis compared to intact animals (Table 5). On the 14th day, the process of reducing the studied components of CTM of periodont continued: the content of free OP decreased by 22.0%, bound OP decreased by 16.7%, GAG by 7.0%, compared to the previous period. However, these indicators were significantly lower compared to similar indices in intact rats ($p<0.001$). Twenty-one days after simulating periodontitis, the rats of the control group showed an increase in the content of the investigated CTM indices: free OP – by 20.5%, bound OP – by 22.5%, GAG – by 27.5%, compared to the previous period of the experiment (14th day). However, all of these indices still remained statistically significantly lower than those of intact animals ($p<0.01$–0.001).

The destruction of the periodontal CTM is associated primarily with the activation of LPO. An increase in LPO was manifested by the accumulation of final and intermediate products: MDA and AHP, as well as by a decrease in the activity of the antioxidant enzyme catalase. It was shown that on the 7th day of simulating periodontitis, the concentration of MDA in the periodontium increased by 92.7% ($p<0.001$) and AHP –2.63 times ($p<0.001$), compared to the intact group. At the same time, a 13.7% decrease of catalase activity in periodontal tissue was established ($p<0.05$). On the 14th day, the content of MDA and AHP decreased slightly: by 15.0% and 17.2%, respectively, compared to the previous observation period. During this period, catalase activity continued to decline: by 11.6%. All of these indicators remained significantly lower than the corresponding indices in rats of the intact group ($p<0.01$–0.001). Twenty-one days after simulating periodontitis, the concentrations of MDA and AHP was still significantly higher than in the intact group: by 37.0% and 56.8%, respectively ($p<0.01$). Catalase activity, in contrast, increased compared to the previous observation period by 12.6% and remained lower than that of intact rats ($p<0.05$).

The investigated analogues of indolicidin No.7 and No.8 did not have a significant effect on the content of free and bound OP, GAG in periodontium of rats with periodontitis compared to animals of the control group on days 7 and 14 of the experiment ($p>0.05$). After 7 days of simulating periodontitis, an increase in the concentrations of MDA and AHP in periodontal tissue was found in animals that had been administered indolicidin analogues compared to the control group ($p<0.05$–0.01). Thus, the content of MDA increased by 9.8% ($p<0.05$), and AHP – by 20.1% ($p<0.01$) with the administration of indolicidin No.7, and with administration of indolicidin No.8 – by 14.7 % and 28.4%, respectively ($p<0.01$). Only on the 14th day, an increase in catalase activity was established compared to the control group (by 41.6%, $p<0.001$). On the 21st day, the corrective effect of indolicidin analogues No.7 and No.8 on the development of periodontitis appeared. Thus, the administration of indolicidin No.7 led to an increase in the content of free and bound OP, GAG in the periodontium compared to the control group by 17.0%, 16.3% and 21.6%, respectively ($p<0.05$). A similar dynamics was observed when injecting indolicidin No.8: the concentration of free and bound OP, GAG rose by 25.5%, 22.4%, and 25.5%, respectively. However, the content of free OP remained significantly lower than in rats of the intact group ($p<0.05$) only in the group treated with indolicidin No.7. During this period, the concentrations of MDA and AHP in the periodontium of rats that were treated with the investigated indolicidin analogues were significantly lower than those in control animals ($p<0.05$–0.01). So, the content of MDA decreased by 17.3%, and AHP – by 26.3% when injecting indolicidin No.7; when administering indolicidin No.8: MDA – by 14.3% and AHP – by 21.3%. Catalase activity significantly differed from that in the control group.

In the group receiving lincomycin, the activation of LPO in the simulated periodontitis was significantly lower than in animals of the control group. Thus, the concentration of MDA was by 18.8% lower, and AHP – by 23.1% lower than in the rats of the control group ($p<0.01$) on the 7th day after the periodontitis simulation. Also in

Table 5. Effect of Indolicidin analogues No.7 and No.8 on the composition of the connective tissue matrix of periodontium in the rats with periodontitis (M±m, n=8).

Group	Index						
	Period of experiment, day	Content of free OP in gum, mmol/g	Content of bound OP in gum, mmol/g	GAG content in gum, mg/g	Content of MDA in gum, μmol/g	Content of AHP in gum, cu	Catalase activity in gum, mcat/g
Intact		6.4±0.2	6.3±0.3	6.8±0.4	13.8±0.5	5.1±0.4	24.9±0.6
Control	7	5.0±0.3^{xx}	4.8±0.3^{xx}	4.3±0.3^{xx}	26.6±0.7^{xxx}	13.4±0.7^{xxx}	21.5±0.9^{x}
	14	3.9±0.2^{xxx}	4.0±0.1^{xxx}	4.0±0.2^{xxx}	22.6±0.7^{xxx}	11.1±0.5^{xxx}	19.0±0.8^{xx}
	21	4.7±0.2^{xxx}	4.9±0.2^{xx}	5.1±0.2^{xx}	18.9±1.0^{xx}	8.0±0.4^{xx}	21.4±0.6^{x}
Injection of indolicidin No.7	7	4.8±0.2^{xxx}	4.3±0.3^{xxx}	4.4±0.2^{xxx}	29.2±0.6*^{xxx111}	16.1±0.5*^{xxx111}	22.0±0.6
	14	4.1±0.2^{xxx}	3.9±0.2^{xxx}	4.3±0.1^{xxx}	24.0±0.6^{xxx}	12.4±0.6^{xxx}	26.9±0.5***^{111}
	21	5.5±0.2*^{x}	5.7±0.2*	6.2±0.2*	14.7±0.5**^{1}	5.9±0.2**^{11}	23.3±0.6^{1}
Injection of indolicidin No.8	7	4.6±0.2^{xxx}	4.5±0.2^{xxx}	4.5±0.2^{xxx}	30.5±0.6**^{xxx111}	17.2±0.6**^{xxx11}	20.6±0.7
	14	4.0±0.1^{xxx}	4.2±0.1^{xxx}	4.1±0.2^{xxx}	23.8±0.6^{xxx11}	11.9±0.6^{xxx1}	24.4±0.8**^{1}
	21	5.9±0.2**^{1}	6.0±0.2**^{1}	6.4±0.2**^{1}	15.3±0.9*	6.3±0.4*^{1}	22.3±0.7
Injection of linkomycin	7	4.7±0.3^{xx}	4.6±0.2^{xxx}	4.5±0.2^{xxx}	21.6±0.7**^{xxx}	10.3±0.5**^{xxx}	22.9±0.8
	14	4.1±0.1^{xxx}	4.1±0.1^{xxx}	4.2±0.1^{xxx}	19.5±0.8*^{xxx}	10.1±0.5^{xxx}	19.9±0.8^{xx}
	21	5.2±0.2^{xx}	5.3±0.2^{x}	5.6±0.2^{x}	16.5±0.6^{xx}	7.8±0.4^{xx}	20.8±0.6^{xx}

Note: ^{x} – p<0.05, ^{xx} – p<0.01, ^{xxx} – p<0.001 compared to intact animals; * – p<0.05, ** – p<0.01, *** – p<0.001 compared to control animals; ^{1} – p<0.05, ^{11} – p<0.01, ^{111} – p<0.001 compared to animals treated with a solution of lincomycin.

rats treated with lincomycin, the concentration of MDA and AHP was lower than in the animals treated with indolicidins (p<0.001). The same trend was maintained on the 14th and 21st day: both the MDA concentration, and AHP concentration in rats treated with lincomycin were significantly lower than those in the control animals and rats treated with indolicidin (p<0.05-p<0.01). Catalase activity was higher in the group to which the investigated peptides were administered, compared to the rats treated with lincomycin (p<0.05–0.001). The activity of the enzyme when administering lincomycin did not differ significantly from the corresponding parameters in the control group (p>0.05). Only on the 21st day, the corrective effect ofindolicidin No.8 on the composition of CTM was manifested: the contents of free and bound OP, GAG were significantly higher than those in the group treated with lincomycin.

Thus, the obtained results indicate the correcting effect of the synthetic analogues of indolicidin No.7 and No.8 on the composition of periodontal CTM, which is associated primarily with a decrease in LPO.

In rats with acute periodontitis, there was an increase in the content of LPO products in blood plasma 7 days after the removal of the suture thread from the lower incisors. So, MDA concentration increased 2.3 times, and of AHP – 2.1 times (p<0.001). A decrease in SOD activity during this period was established by 24.7% (p<0.001), and catalase – by 21.4% (p<0.01) (Table 6). On the 14th day, the concentration of MDA and AHP decreased compared to the previous observation period, but still remained significantly higher compared to the intact animals by 70.0% and 2.1 times, respectively (p<0.001). SOD activity in this period was lower by 24.8% (p<0.001), and catalase – by 33.1% (p<0.001). On the 21st day, the content of LPO products was higher than that in the control group: MDA – by 29.3% (p<0.05) and AHP – by 61.7% (p<0.001). The activity of SOD and catalase still remained below the corresponding values in the intact group by 13.2% (p<0.05) and 20.2% (p<0.01).

On the 7th day, the content of LPO products was significantly higher in rats with acute periodontitis which had received analogues of indolicidin No.7 and No.8 than in rats of the control group: MDA – by 9.1% (p<0.05) and AHP – by 13,6% (p<0.05) when administering indolicidin No.7, MDA – by 11.9% (p<0.05) and AHP – by 18.4% (p<0.05). SOD activity in the animals treated with the investigated peptides was significantly higher compared to the control group: when administering peptide No.7 – by 16.2% (p<0.05) and when administering peptide No.8 – by 14.5% (p<0.05). Catalase activity did not significantly differ in rats of the control group and in rats treated with indolicidin analogues.

On the 14th and 21st days, there were no significant differences between the animals of the control group and the rats treated with the investigated peptides. On the contrary, the administration of indolicidin analogues stimulated the activity of antioxidant enzymes in these periods of investigation. So, the injection of indolicidin No.7 increased the investigated indices on the 14th day: SOD activity – by 34.1% (p<0.001) and catalase activity – by 36.0% (p<0.001), on the 21st day: SOD activity – by 40.5% (p<0.001), catalase activity – by 30.2% (p<0.001) higher than those in animals of the control group. When administering indolicidin No.8, on the 14th day, SOD activity increased by 30.2% (p<0.001), and catalase activity increased by 31.4% (p<0.01). On the 21st day, SOD activity was 43.3% higher (p<0.001), and catalase activity – by 40.5% (p<0.001) in rats treated with peptide No.8 compared to the control group.

The administration of lincomycin resulted in a decrease in the content of LPO products on the 7th and 14th days of the experiment. So, on the 7th day, MDA concen-

Table 6. The effect of Indolicidin Aanalogues No.7 and No.8 on the Content of Lipid Peroxidation Metabolites and the Activity of Antioxidant Enzymes in Plasma after Simulating Acute Periodontitis (M±m, n=8).

Group	Index				
	Period of experiment, day	Content of MDA in plasma, mcmol/l (M±m)	Content of AHP in plasma, units/l, (M±m)	SOD activity in plasma units/l (M±m)	Plasma catalase activity, mcat/ml, (M±m)
Intact		15.0±0.3	6.0±0.2	24.2±0.9	25.7±0.9
Control	7	39.4±1.1***	14.7±0.6***	17.9±0.6***	20.2±0.7**
	14	25.5±0.8***	12.6±0.6***	18.2±0.5***	17.2±0.8***
	21	19.4±0.7*	9.7±0.6***	21.0±0.9*	20.5±0.9**
Injection of indolicidin No.7	7	43.0±1.0^{x}	16.7±0.6^{x}	20.8±0.6^{x}	21.0±0.7
	14	27.7±0.7	13.9±0.7	24.4±0.8^{xxx}	23.4±0.6^{xxx}
	21	19.2±0.8	9.3±0.6	29.5±1.1^{xxx}	26.7±0.8^{xxx}
Injection of indolicidin No.8	7	44.1±1.0^{x}	17.4±0.6^{x}	20.5±0.4^{x}	20.4±0.6
	14	26.7±0.7	14.4±0.7	23.7±0.8^{xxx}	22.6±0.7^{xxx}
	21	18.1±0.8	8.6±0.4	30.1±1.1^{xxx}	28.8±1.1^{xxx}
The injection of lincomycin	7	26.4±0.7	13.2±0.4	18.7±0.6	19.6±0.5
	14	21.2±0.6	10.8±0.4	22.8±0.8	24.2±0.7
	21	17.4±0.9	7.5±0.4	22.0±0.8	23.0±1.0

Note: ^{x} – p<0.05, ^{xx} – p<0.01, ^{xxx} – p<0.001 compared to intact animals; * – p<0.05, ** – p<0.01, *** – p<0.001 compared to control animals; ^{1} – p<0.05, ^{11} – p<0.01, ^{111} – p<0.001 compared to animals treated with a solution of lincomycin.

Table 7. Effect of Indolicidin Analogues No.7 and No.8 on the Content of Acute Phase Proteins in the Plasma after Simulating Acute Periodontitis (M±m, n=8).

Group	Index			
	Periods of experiment, day	Content of C-reactive protein in plasma, g/l	Content of cerulloplasmin in blood plasma, g/l	Content of alpha$_1$-antitrypsin in blood plasma, g/l
Intact		0.05±0.01	0.06±0.003	0.34±0.03
Control	7	0.25±0.02^{xxx}	0.24±0.02^{xxx}	1.37±0.12^{xxx}
	14	0.17±0.01^{xxx}	0.15±0.01^{xxx}	0.66±0.05^{xxx}
	21	0.06±0.01	0.06±0.003	0.33±0.01
Injection of indolicidin No.7	7	0.37±0.03**	0.34±0.02**	2.05±0.06**
	14	0.10±0.01**	0.09±0.01**	0.52±0.05
	21	0.05±0.01	0.06±0.003	0.35±0.02
Injection of indolicidin No.8	7	0.39±0.02**	0.37±0.02**	1.84±0.06**
	14	0.11±0.01**	0.10±0.01**	0.53±0.04
	21	0.04±0.003	0.05±0.003	0.31±0.03
Injection of lincomycin	7	0.21 ± 0.02	0.19 ± 0.02	1,14 ± 0,07
	14	0.06±0.01***	0.07±0.004***	0.37±0.04**
	21	0.05±0.003	0.05±0.003	0.30±0.01

Note: ^{x} – p<0.05, ^{xx} – p<0.01, ^{xxx} – p<0.001 compared to intact animals; * – p<0.05, ** – p<0.01, *** – p<0.001 compared to control animals; ^{1} – p<0.05, ^{11} – p<0.01, ^{111} – p<0.001 compared to animals treated with a solution of lincomycin.

tration was 33.0% lower (p<0.001), and AHP – by 17.0% (p<0.05), and on the 14th day: MDA content – by 16.9% (p<0.01), and AHP content – by 16.7% (p<0.05) lower than in the animals of the control group. On the 21st day, the content of AHP in blood plasma was by 22.7% (p<0.05) lower than that in the control group. The concentration of MDA did not differ significantly from the corresponding parameters in the control rats. The administration of lincomycin did not significantly affect the activity of antioxidant enzymes in any period of observation.

A significant increase in acute phase proteins (APP) was revealed on 7th day after the removal of the suture thread compared to the intact animals. Thus, the content of CRP increased 5 times, of ceruloplasmin – 4 times, of alpha$_1$-antitrypsin – 4.03 times (p<0.001) (Table 7). On the 14th day, the concentration of these proteins reduced, but remained still substantially higher than those in intact rats: of CRP – 3.4 times, of ceruloplasmin – 2.5 times, of alpha$_1$-antitrypsin – 1.94 times (p<0.001). Twenty-one days after removal of the suture thread, there were no differences between the rats of the intact and control groups (p>0.05).

The injection of indolicidins No.7 and No.8 had a stimulating effect on the content of APP 7 days after the end of the periodontitis simulation (Table 7). Thus, the CRP content increased by 48.0% (p<0.01), of ceruloplasmin – by 41.7% (p<0.01), of alpha$_1$-antitrypsin – by 49.6% (p<0.01) in the group treated by indolicidin No.7. Indolicidin No.8 had a similar effect. An increase in CRP

concentration was 56.0% ($p<0.01$), in ceruloplasmin concentration – by 54.2% ($p<0.01$), and in alpha$_1$-antitrypsin concentration – by 34.3% ($p<0.01$).

On the 14th day, the APP content in blood plasma of rats with periodontitis, which had received analogues of indolicidins, significantly reduced compared to that in the control group. The administration of indolicidin No.7 led to a decrease in the concentration of CRP by 41.2% ($p<0.01$), of ceruloplasmin – by 40.0% ($p<0.01$), whereas administration of indolicidin No.8 caused a decrease in the content of CRP by 35.3 % ($p<0.01$) and of ceruloplasmin – by 33.3% ($p<0.01$). There were no statistically significant differences in alpha$_1$-antitrypsin concentrations between the rats of the control group and the rats treated with indolicidin. On the 21st day, the APP content in the experimental groups did not differ significantly from the corresponding indices in the control group.

Simulation of periodontitis was accompanied with an increase in the concentration of endothelin-1 in gingival tissue by 59.0% ($p<0.001$) and of stable nitric oxide metabolites – by 39.7% ($p<0.001$) compared to the intact group on the 7th day of the experiment (Table 8).

A statistically significant increase in the investigated indices was observed on both the 14th and 21st days: on the 14th day, the content of endothelin-1 increased by 24.8% ($p<0.01$), and stable nitric oxide metabolites – by 91.0% ($p<0.001$) compared to the intactgroup; on the 21st day: endothelin-1 concentration increased by 15.6% ($p<0.01$), and stable nitric oxide metabolites – by 31.3% ($p<0.001$).

The administration of synthetic analogues of indolicidins did not significantly affect the content of endothelin-1 and stable nitric oxide metabolites on the 7th day of the experiment. But as early as on the 14th day, there was a decrease in the concentrations of endothelin-1 and stable nitric oxide metabolites in the groups treated with peptides No.7 and No.8 compared to the control group: the content of stable nitric oxide metabolites decreased by 12.5% when administering No.7 ($p<0.05$) and endothelin-1 – by 25.8% ($p<0.001$), and when injecting indolicidin No.8 – by 11.4% ($p<0.05$) and 25.0% ($p<0.001$), respectively. On the 21st days, this tendency remained: the concentrations of endothelin-1 and of stable metabolites were statistically significantly lower than those in rats of the control group ($p<0.05$–0.01).

The administration of lincomycin did not significantly affect the contents of stable nitric oxide metabolites and endothelin-1 on the 7th days: there were no statistically significant differences in the concentrations of the investigated substances between the control group and the rats treated with lincomycin. Only on the 14th day the administration of lincomycin decreased endothelin-1 content by 8.6% ($p<0.01$).

Thus, the change in the content of vasoactive substances endothelin-1 and stable nitric oxide metabolites in rats with periodontitis was established. An increase in the concentration of endothelin-1 in periodontal tissue indicates an increase in the production of this substance by endothelium cells, and an increase in the content of stable nitric oxide metabolites should be regarded as an acceleration of the destruction of nitrogen oxide during periodontitis.

Indolicidin analogues have an effect on the content of vasoactive substances in the periodontal tissue and plasma in rats with periodontitis, starting from 14 days after completing the periodontal simulation. The effect of peptides No.7 and No.8 on the production and disintegration of vasoactive substances seems to influence indirectly the change in the activity of macrophages, as shown in the experiments described in this paper.

The investigation established that the development of acute periodontitis is accompanied with macroscopic changes: hyperemia, erosions and swollen mucosa of gums, dental plaque, an increase in the size of the interdental papillae, spindle-shaped form of gum, the distorted dentogingival junction, the formation of periodontal pockets with purulent content. SBI increased 6.5–8.0 times, a Schiller-Pisarev test was positive in all the animals, and its index increased 10–12 times. AGF increased 3.64–4.29 times throughout the experiment.

The development of periodontitis was manifested by the following inflammatory morphological changes: swollen epithelium and lamina propria, the formation of inflammatory infiltrates, the hydropic cell degeneration, and the reduced vascular network. At the same time, loose connective tissue was formed, which as early as on the 14th day filled about half of the section. On the 21st day, the normal connective tissue was observed.

The investigation established the presence of the corrective influence of indolicidin analogues No.7 and No.8 on periodontitis, which was manifested by a decreased swelling, a decrease in the relative area of cellular infiltrates and a significant increase in the relative area of normal tissue. These changes were recorded as early as on the 14th day after removal of the suture thread. On the 21st day of the experiment, the administration of indolicidin analogues No.7 and No.8 eliminated epithelial edema: its thickness did not differ significantly from that in intact rats. The relative area of cell infiltrates in these groups was significantly less. More than one-third of the section represented normal tissue in the groups treated with indolicidin analogs No.7 and 8. At the same time, the relative area of loose fibrous connective tissue was lower than that in the rats of the control group.

Although no influence of indolicidin analogues No.7 and No.8 on SBI was established, the Schiller-Pisarev test and AGF indices were lower on the 7th day after removing the suture thread; on the 14th day there was a decrease in AGF; on the 21st day, all of these indices decreased statistically significantly in the animals treated with indolicidin No.7 or No.8.

It is shown that an increase in the overall positive charge of the molecule contributes to the enhancement of antimicrobial properties (Smirnova et al. 2004). Indolicidin No.8 has a higher positive charge (+7) than indolicidin No.7 (+6), and its corrective effect on the development of regenerative processes in acute periodontitis are more pronounced.

Table 8. The Effect of Indolicidin Analogues on the Content of Endothelin-1 in Gingival Tissue and Nitrogen Oxide Metabolites in Plasma in Rats with Periodontitis (M±m, n=8).

Group	Indices		
	Period of experiment, day	Content of stable nitric oxide metabolites in plasma, mcmol/l	Content of endothelin-1 in tissue of gums, fg/mg of protein
Intact		14.1±0.4	1.34±0.06
Control	7	19.7±0.7^{xxx}	2.13±0.05^{xxx}
	14	17.6±0.6^{xx}	2.56±0.04^{xxx}
	21	16.3±0.4^{xx}	1.76±0.04^{xxx}
Injection of indolicidin No.7	7	18.9±0.6	2.07±0.06
	14	15.4±0.7*	1.90±0.05***
	21	14.0±0.4**	1.39±0.03***
Injection of indolicidin No.8	7	20.1±0.6	2.12±0.06
	14	15.6±0.6*	1.92±0.05***
	21	14.7±0.5*	1.41±0.05***
Injection of lincomycin	7	20.3±0.6	2.18±0.05
	14	16.4±0.7	2.34±0.03**
	21	13.8±0.4	1.75±0.05

Note: ^{x} – p<0.05, ^{xx} – p<0.01, ^{xxx} – p<0.001 compared to intact animals; * – p<0.05, ** – p<0.01, *** – p<0.001 compared to control animals; ^{1} – p<0.05, ^{11} – p<0.01, ^{111} – p<0.001 compared to animals treated with a solution of lincomycin.

The obtained results confirm the literature data on the reduction of neutrophil phagocytic activity in the early period of periodontitis (Vokhmintseva et al. 2009). The investigation established that the phagocytic activity of neutrophils reduced on the 7th and 14th days of the experiment, which was manifested by a decrease in PI and OPI. Only on the 21st day, the number of phagocytic neutrophils did not differ significantly from the corresponding index in intact rats. At the same time, no oppression of the absorptive capacity of phagocytes in rats with periodontitis was established.

Simulation of periodontitis led to a decrease in the relative number of macrophages in the peritoneal lavage, while a decrease in their adhesive ability was found only on the 14th day of the experiment, and there was even an increase in the number of adherent cells on the 7th day. Previously, it was shown that the change in the adhesive properties of macrophages in various pathological processes has a phase character (Lazarenko et al. 2017). Taking into account the fact that macrophages are the central link of intercellular cooperation in inflammatory diseases; it can be argued that such a change in their functional activity is the result of a wide range of active factors.

The administration of indolicidin analogues led to a pronounced increase in the number of phagocytic neutrophils and their absorptive capacity as early as on the 7th days after the removal of the suture thread. The stimulating effect of the investigated indolicidins on the phagocytic activity of neutrophils established in the investigation is apparently due to the peculiarities of the bactericidal action of antimicrobial peptides, namely, rapid damage of membranes of microorganisms with a subsequent increase in their permeability (Artamonov et al. 2014). Such cells are actively absorbed by phagocytes. In addition to the rapid and pronounced bactericidal effect, natural indolicidin and its analogs have an immunomodulatory effect (Artamonov et al. 2009), which,considering close cooperation of immunocompetent cells, macrophages and neutrophils in the development of defense reactions, also contributes to an increase in the functional activity of neutrophils and macrophages.

Natural indolicidin is known to be poorly resistant to peptidases (Guani-Guerra et al. 2010). Synthetic analogues used in the investigation have a longer half-life period compared to the natural peptide, which leads to an increase in the time of action of the drugs, which probably causes their pronounced effect.

The results obtained in the investigation confirm the literature data on the activation of LPO in the gum tissues during acute periodontitis (Leontyev and Galenko-Yaroshevsky 2013). The development of the inflammatory process is accompanied by disorders that lead to the formation of an excessive amount of free radicals: microcirculation disorders, hypoxia, mitochondrial damage, and phagocyte activation (Pinegin and Mayansky 2007). A number of researchers point to a close relationship between the reduction of proteins in the composition of CTM and the activation of LPO (Omarov et al. 2011).

Previously, it was shown that the use of indolicidins leads to increased LPO in inflammatory diseases (Lazarenko et al. 2017), which is apparently connected with an increase in the functional activity of phagocytes (Lazarenko et al. 2017). In the current investigation, the activation of LPO in the periodontal tissue was established on the 7th day after the periodontitis simulation. This is apparently due to the stimulating effect of indolicidins on the functional activity of phagocytes, which is accompanied by an increase in the production of free radicals by cells. On the 14th day, the content of LPO products did not differ significantly from similar indices in the animals of the control group; and then on the 21st day, the concentrations of MDA and AHP were lower. This seems to be due to the stimulating effect of the investigated indolicidins on the production of catalase by leukocytes.

On the 21st day, there was an increase in the content of CTM components in the groups of animals treated with indolicidin analogues. It is known that macrophages play a key role in the development of inflammation, which not only realize the phagocytic function, but also secrete cytokines that stimulate fibroblasts. Thus, it can be assumed that the injection of the investigated indolicidins has a stimulating effect on macrophages that secrete cytokines that activate fibroblasts.

In the current study, the stimulating effect of indolicidin on the restoration of CTM in rats with periodontitis was established. At the same time, a comparative analysis of the action of analogues No.7 and No.8 revealed the following features of the realization of this effect – the effect of peptide No.8 is higher than that of peptide No.7, and indolicidin No.8 has a higher positive charge (+7) than indolicidin No.7 (+6).

The results obtained in the investigation also confirm the literature data on increasing the concentration of LPO products in plasma in periodontitis (Leontiev et al. 2013). In the current investigation it was established that the concentrations of the final and intermediate LPO products: MDA and AHP – remained higher than those of intact rats throughout the experiment (21 days). Activation of LPO during inflammation is associated with impaired microcirculation and the development of hypoxia, as well as an increased production of free radicals by activated leukocytes (Pinegin and Mayansky 2007).

The control group showed a decrease in the activity of antioxidant enzymes: SOD and catalase. Suppression of SOD activity apparently develops due to the toxic effects of excess MDA and AHP in acute periodontitis, as well as due to possible structural changes in the enzyme molecule, in particular, its glycation (Zorkina et al. 1997). The decrease in catalase activity can be explained by the depletion of the enzyme when exposed to an excess of free radicals.

The investigation found that the administration of indolicidin analogues No.7 and No.8 causes an increase in the content of MDA and AHP in the plasma 7 days after the removal of the suture thread. The activation of LPO at this period seems to be associated with the excessive production of free radicals by activated leukocytes (Pinegin and Mayansky 2007), the number of which significantly increases in the rats, which had been treated with indolicidin analogues. An increased activity of SOD and catalase when administering the investigated indolicidins can be explained by the stimulating effect of peptides on macrophages and neutrophils, which are known to produce these enzymes.

On days 14 and 21, the concentrations of both MDA and AHP in plasma in the groups of animals treated with indolicidin analogues did not significantly differ from the corresponding values in the control rats and were statistically significantly lower than on the 7th day of observation. The fall in the content of LPO products during this period can be explained, by the reduction of the pro-oxidant-antioxidant balance, which is confirmed by an increase in both SOD and catalase activity in plasma.

The fact that synthetic analogues of indolicidin No.7 and No.8 have a correcting action in acute periodontitis is confirmed by investigations of the APP content in the blood. A statistically significant increase in the concentration of CRP, alpha$_1$-antitrypsin and ceruloplasmin was observed as early as on the 7th day after removing the suture thread. Only on the 21st day, the APP content in plasma significantly reduced and did not differ significantly from the corresponding indices in intact rats.

The investigated indolicidin analogues had a two-phase effect on the APP concentration in plasma in rats with periodontitis. On the 7th day, the contents of CRP, alpha -antitrypsin and ceruloplasmin in plasma of rats with periodontitis treated with peptides were significantly higher than that of the control animals which had received normal saline. On the 14th day, the concentrations of CRP and ceruloplasmin in the rats with periodontitis treated with indolicidins No.7 or No.8, were significantly reduced and was significantly lower than in the control group. The stimulating effect of indolicidin analogues on the content of APP can be explained by the increased production of interleukins by macrophages that activate the synthesis of APP. The decrease in the concentration of APP on the 14th day of the experiment developed due to the fact that macrophages began secretion of biologically active substances stimulating proliferation, which is confirmed by the stimulating effect of synthetic analogues of indolicidin on the functional activity of macrophages.

The corrective effect of the investigated indolicidin analogues on the content of vasoactive substances in the blood plasma: endothelin-1 and stable nitric oxide metabolites – was established in the paper. While the ecperiments showed an increase in the concentration of endothelin-1 and a decrease in the content of stable nitric oxide metabolites up to and including the 14th day of the experiment, in rats with periodontitis treated with the investigated peptides, there was an increase in the production of stable nitric oxide metabolites and a decrease in endothelin-1 on the 14th day. The data presented indicate that indolicidin analogues can have the corrective influence on the microcirculation condition in damaged periodontium. The mechanism of this effect of the investigated peptides can be mediated and is associated with the influence on the production of vasoactive substances: arachidonic acid cascade products, growth factors, etc. – by macrophages and platelets.

In the current investigation, the correcting effect of the synthetic analogues of indolicidin No.7 and No.8 was compared with the action of antibiotic lincomycin, which is widely used in the treatment of periodontitis. Lincomycin was used at a dose of 0.5 g/kg, as recommended in previous studies (Leontyev and Galenko-Yaroshevsky 2013). It was found that the correcting effect of indolicidin analogues on the development of periodontitis is more pronounced than that of lincomycin, which is confirmed by an increase in the relative area of normal tissue in the periodontium, a decrease in epithelial edema, an increase in OP and GAG in periodontal tissue on the 21st day of

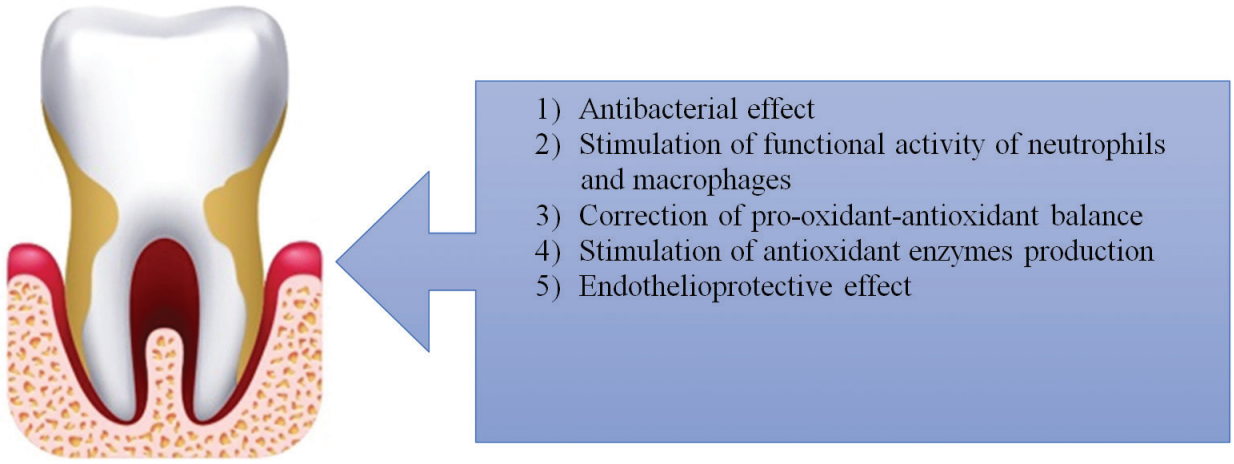

Figure 1. Mechanism of correcting effect of indolicidin analogues in periodontitis.

the experiment in rats treated with the investigated peptides. Unlike lincomycin, synthetic analogues of indolicidin No.7 and No.8 also increase the functional activity of neutrophils and macrophages and correct the vasoactive BAS content in plasma in rats with periodontitis.

The results obtained in this investigation confirm the effecacy of the administration of synthetic analogues of indolicidin No.7 and No.8 in rats with periodontitis. Parenteral administration of peptides at a dose of 500 mcg/kg of body weight for 7 days after simulating periodontitis reduces the appearance of inflammation and activate reparative processes in the rats with periodontitis. The mechanism of the correcting action of indolicidin analogues in addition to their pronounced antimicrobial action, as shown previously by numerous investigations, includes the stimulation of the functional activity of macrophages and peripheral blood neutrophils, normalization of pro-oxidant-antioxidant balance, activation of antioxidant enzymes both in plasma and in the periodontium, and the correction of the production of vasoactive substances and microcirculation.

Conclusion

The investigation revealed that the administration of synthetic analogues of indolicidin No.7 and No.8 is an effective method of pharmacological correction of periodontitis (Fig. 1). When administering the investigated peptides, there was a decrease in inflammatory manifestations and the stimulation of regenerative processes in periodontium in rats: a decrease in the swelling of the gingival mucosa, a decrease in the relative area of cellular infiltrates in the gingival tissue, and an increase in the relative area of normal tissue. There was a decrease in SBI, a decrease in the Schiller-Pisarev test values, AGF under the influence of indolicidin analogues No.7 and No.8. The investigated peptides had a correcting effect on the composition of CTM: an increase in the content of free and bound OP and GAG in rats with periodontitis was established.

The administration of the investigated peptides causes an increase in the functional activity of neutrophils and macrophages. The pharmacological effects of synthetic analogues of indolicidin No.7 and No.8 on the development of a systemic inflammatory reaction, pro-oxidant-antioxidant balance and the activity of antioxidant enzymes in periodontium and plasma were proven. The correcting effect of the investigated indolicidin analogues on the synthesis of vasoactive substances (endothelin-1, nitric oxide) and microcirculation in periodontium was revealed.

The results obtained allow to recommend further preclinical investigation of the correcting effect of synthetic analogues of indolicidin and the creation of drugs based on them in order to include such drugs in the periodontitis treatment, to reduce the severity of local and general inflammatory reactions in periodontitis, and to activate the formation of normal connective and epithelial tissue in the most effective dose of 500 mcg/kg.

The administration of indolicidin analogues No.7 and No.8 has a stimulating effect on the functional activity of neutrophils and macrophages, which allows to recommend the administration of the peptide for the prevention of purulent complications in various forms of pathology, in pathological processes accompanied with the inhibited phagocytic immunity.

The results obtained open up prospects for preclinical and clinical trials of synthetic analogues of indolicidin No.7 and No.8 as drugs that have an antimicrobial, anti-inflammatory effects, stimulating influence on reparative processes in periodontitis. The data obtained in the investigation can be used to create new drugs, both for local and systemic administration, which can be used for the treatment and prevention of periodontal lesions and the stimulation of reparative processes.

Conflict of interest

Authors declare neither evident nor potential conflicts of interest which can be caused by publishing this article.

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