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The effect of dental gel with IL-1β antagonist on indicators of nitrosative stress and antioxidant system in rats with experimental chronic generalized periodontitis

O. O. Dmytriieva[C,E,F], I. F. Bielenichev[A,E,D], I. B. Samura*[D,F], V. I. Salnykov[B], D. V. Robota[C]

Zaporizhzhia State Medical and Pharmaceutical University, Ukraine

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***E-mail:** Irinasamura77@gmail.com

The aim of the study was to evaluate the action of dental gel with IL-1β receptor antagonist on the indicators of nitrosative stress and antioxidant system in rats with experimental chronic generalized periodontitis.

Materials and methods. Experimental chronic generalized periodontitis (CGP) was modeled using a calcium-deficient peroxide diet with reduced masticatory function in Wistar rats weighing 190–220 g for 8 weeks. The studied pharmacological agents were administered within 30 days after the development of CGP: 1 % dental gel with IL-1β receptor antagonist (1 mg/kg, locally using a dispenser); and antioxidant Mexidol (250 mg/kg, intragastrically). To assess the condition of periodontal tissues, the levels of nitrotyrosine, iNOS, Cu/ZnSOD, and glutathione peroxidase-4 were determined by immunoenzymatic quantitative analysis; levels of stable metabolites of NO, reduced and oxidized glutathione were determined using biochemical methods.

Results. Course administration of dental gel with IL-1β antagonist in a therapeutic regimen to rats with CGP resulted in a decrease in the depth of periodontal pockets, almost complete elimination of bleeding and swelling of the gums, as well as to a decrease in iNOS expression by 37.8 % ($p < 0.05$), nitrotyrosine concentration – by 55.2 %, and NO metabolites – by 30 % ($p < 0.05$) in the blood of animals. Dental gel with IL-1β antagonist application resulted in an increase in concentration of reduced glutathione by 63 % ($p < 0.05$), glutathione peroxidase-4 expression – by 60.4 % ($p < 0.05$), and Cu/ZnSOD – by 31.2 % ($p < 0.05$) in the blood of animals with CGP. Mexidol, when administered to rats with CGP, affected only two studied indices – the level of nitrotyrosine and reduced glutathione ($p < 0.05$). However, it was inferior to the gel with the IL-1β receptor antagonist in terms of the degree of influence on these parameters ($p < 0.05$).

Conclusions. The obtained results provide experimental justification for further study of IL-1β antagonist as a promising agent for the treatment of CGP.

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Вплив стоматологічного гелю з антагоністом IL-1β на показники нітрозативного стресу й антиоксидантну систему в щурів з експериментальним хронічним генералізованим пародонтитом

О. О. Дмитрієва, І. Ф. Бєленічев, І. Б. Самура, В. І. Сальников, Д. В. Робота

Мета роботи – оцінити вплив гелю стоматологічного з рецепторним антагоністом IL-1β на показники нітрозативного стресу й антиоксидантну систему в щурів з експериментальним хронічним генералізованим пародонтитом.

Матеріали і методи. Експериментальний хронічний генералізований пародонтит (ХГП) відтворювали у щурів лінії Вістар масою 190–220 г за допомогою перекисної кальцій-дефіцитної дієти зі зниженою жувальною функцією протягом 8 тижнів. Фармакологічні агенти вводили протягом 30 діб після формування ХГП: 1 % гель стоматологічний із рецепторним антагоністом IL-1β (1 мг/кг) – місцево за допомогою дозатора; антиоксидант мексидол (250 мг/кг) – внутрішньогастрально. Для оцінювання стану тканин пародонта методом імуноферментного аналізу визначили рівні нітротирозину, iNOS, Cu/ZnSOD, глутатіонпероксидази-4 (GPx4); за допомогою біохімічних методів встановили рівні стабільних метаболітів NO, відновленого та окисненого глутатіону.

Результати. Курсове введення гелю стоматологічного з антагоністом IL-1β у лікувальному режимі щурам із ХГП призвело до зменшення глибини пародонтальних кишень, майже повного усунення кровоточивості та набряку ясен, а також до зниження експресії iNOS на 37,8 % ($p < 0,05$), концентрації нітротирозину – на 55,2 %, NOx – на 30,0 % ($p < 0,05$) у крові тварин. Стоматологічний гель з антагоністом IL-1β підвищив концентрацію відновленого глутатіону на 63,0 % ($p < 0,05$), експресію GPx4 – на 60,4 % на ($p < 0,05$), Cu/ZnSOD – на 31,2 % ($p < 0,05$) у крові тварин із ХГП. Мексидол при введенні щурам із ХГП впливав тільки на рівні нітротирозину та відновленого глутатіону ($p < 0,05$). Однак за ступенем впливу на ці показники він поступався гелю з рецепторним антагоністом IL-1β ($p < 0,05$).

Висновки. Результати дослідження є експериментальним обґрунтуванням для продовження вивчення антагоніста IL-1β як перспективного засобу для лікування хронічного генералізованого пародонтиту.

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It is known that periodontal tissue diseases are a prerequisite for tooth loss. Tooth loss due to periodontal disease is 5–10 times higher than the frequency of tooth extraction due to caries and its complications. Such early multiple loss of teeth causes significant suffering of patients, creates social and everyday inconvenience for them, and induces a number of functional disorders of the digestive tract and other body systems. According to WHO, about 80 % of people suffer from periodontal disease, while in adults the incidence of periodontitis and gingivitis ranges from 53.0 % to 97.5 %. However, the incidence of periodontal diseases is increasing, including among young people; already in childhood, the prevalence of both caries and gingivitis reaches 80–95 %. According to a study by Global Burden of Disease Study (2016), severe periodontal disease is the 11th most common disease worldwide [1,2,3]. The prevalence of periodontal disease ranges from 20 % to 50 % worldwide [1].

Considering the current knowledge of pathobiochemical and molecular mechanisms of the inflammatory process in periodontitis, it is promising to use medications that inhibit the main metabolic pathways of biosynthesis of pro-inflammatory molecules such as prostaglandins, leukotrienes, thromboxanes, cytokines, reactive oxygen and nitrogen species, lipid hydroperoxides [4,5,6].

Antioxidants such as thiotriazoline, alpha-tocopherol, selenium preparations, recombinant superoxide dismutase preparation, propolis preparations, mumiyo, and plant complexes with bioflavonoids are currently widely used in the treatment of periodontitis. These drugs are used in various dosage forms – parenteral solutions; oral capsules and tablets; gels, pastes, ointments; and films used locally for electrophoresis [7,8,9,10,11]. However, as practice shows, the impact on only one of the mechanisms of periodontitis formation – free radical – does not lead to a complete cure. There is a need for drugs that are able to influence several links, including the initial ones in the pathogenesis of inflammatory processes in the oral mucosa.

The impact of periodontal pathogens on the tissues of the periodontal complex triggers a number of immune mechanisms. It has also been established that the expression of pro-inflammatory cytokines, such as interleukin-1 beta (IL-1β), TNF-α, increased activity of inducible nitric oxide synthase (iNOS), and activation of nitrosative stress accompanied by an increase in cytotoxic forms of nitric oxide (NO) are important links in the pathogenesis of inflammatory processes in the oral mucosa [12]. These cytokines can activate osteoclastogenesis and bone resorption by osteoclasts. Increased migration of macrophages under the influence of cytokines and their constant presence in tissues aggravates destructive processes in the periodontium [13].

Cytotoxic forms of nitric oxide (NO), such as the peroxynitrite anion ($ONOO^-$) and the nitrosonium ion (NO^+) lead to chemical modification of macromolecules, inhibiting the reparative processes in tissues, suppressing immunity, and disrupting the molecular biochemical mechanisms of cellular signaling [14]. Nevertheless, only antibacterial medications, such as minocycline, doxycycline, roxithromycin, amoxicillin, and metronidazole with a very limited action on IL-1β are used in dentistry. Their effect disappears within 6 months [15].

IL-1β receptor antagonists / antibodies that interrupt IL-1β-dependent cascade mechanisms of ischemic neurodestruction and regulate glutathione-dependent mechanisms of HSP_{70} expression in brain mitochondria and cytosol during acute ischemia are of great interest to pharmacologists and clinicians [16,17].

Oromucosal gel with an IL-1β receptor antagonist (IL-1RA) was developed at the Department of Medicines Technology of Zaporizhzhia State Medical and Pharmaceutical University. Preclinical studies have shown its efficacy, safety, and harmlessness when administered to laboratory animals [18]. All of the above determines the relevance and prospects of the study.

Aim

To evaluate the effect of dental gel with IL-1β receptor antagonist on indicators of nitrosative stress and antioxidant system in rats with experimental chronic generalized periodontitis.

Materials and methods

The experiments were performed on 40 sexually mature white Wistar rats weighing 190–220 g, taken from the breeding center of the Institute of Pharmacology and Toxicology of the Academy of Medical Sciences of Ukraine. The duration of quarantine (acclimatization period) for all animals was 14 days. During the quarantine, each animal was examined daily (behavior and general condition). Twice a day the animals were observed in cages (morbidity and mortality). Before the start of the study, animals meeting the criteria for inclusion in the experiment were distributed into groups using a randomization method.

Experiments and all manipulations with animals were carried out in accordance with the regulations on the use of animals in biomedical experiments (Strasbourg, 1986, as amended in 1998) and the European Convention for the Protection of Vertebrate Animals Used for Experimental and Scientific Purposes. The experimental research protocols and their results were approved by the decision of the Zaporizhzhia State Medical and Pharmaceutical University Bioethics Commission.

Modeling of chronic generalized periodontitis (CGP) in laboratory animals was reproduced using a peroxide calcium-deficient diet with reduced masticatory function for 8 weeks. In the drinking bowls for drinking water, there was a 2 % solution of EDTA, and the pro-oxidant Delagil (chloroquine phosphate) was administered to the animals at a dose of 30 mg/kg in the form of a 0.59 % aqueous solution daily. During the experiment, the animals received soft food [19].

After the formation of CGP, the animals immediately received the studied agents. Forty rats were divided into four groups of ten animals each:

1. Intact group, that should receive isotonic saline solution (0.9 % NaCl) intragastrically for 30 days;

2. Control, animals with experimental CGP, which should receive isotonic saline solution (0.9 % NaCl) intragastrically for 30 days;

3. Animals with experimental CGP, which should be applied 1 % dental gel with IL-1β receptor antagonist at a dose of 1 mg/kg locally to the affected areas of the periodontium using a dispenser for 30 days [18];

4. Animals with experimental CGP, which should receive the reference drug Mexidol (PJSC "Technolog", Ukraine) at a dose of 250 mg/kg intragastrically daily for 30 days [17].

The study used 1 % oromucosal dental gel with IL-1β receptor antagonist, developed at the Department of Medicines Technology of Zaporizhzhia State Medical and Pharmaceutical University [18], and Mexidol (ethylmethylhydroxypyridine succinate, Mexicor, PrJSC "Tekhnolog", Ukraine). At the end of the study, rats from all experimental groups were taken out of the study under thiopental-sodium anesthesia (40 mg/kg). Following this, blood samples were obtained from the celiac artery for subsequent analysis.

Preparation of biological material. Blood was taken from the abdominal aorta by syringe, and serum was separated by centrifugation at +4 °C at 1500 rpm for 20 min [20] on an Ependorff 5804R centrifuge.

Enzyme-linked immunosorbent assay (ELISA). Nitrotyrosine in blood serum was determined by the solid-phase sandwich immunoassay method of ELISA. The ELISA Kit (Catalog No. HK 501-02 from Hycult Biotech, Uden, Netherlands) was used according to the instructions.

The activity of inducible nitric oxide synthase (iNOS) in the blood serum was assessed with enzyme immunoassay using the MyBioSource kit (SanDiego, CA, USA, #MBS023874), according to the instructions.

The activity of Cu-Zn-dependent superoxide dismutase in blood serum was evaluated with enzyme immunoassay using Rat SOD1 / Cu-Zn SOD (Sandwich ELISA). ELISA Kit – LS-F4234 from LSBio (USA) was used according to the instructions.

Glutathione peroxidase 4 activity in blood serum was assessed by enzyme immunoassay using GPX4 (Rat Glutathione Peroxidase 4, Phospholipid hydroperoxide glutathione) ELISA Kit from MyBioSource, Inc (USA). Catalog #MBS934198 was used according to the instructions. These analyses were conducted on a complete plate enzyme immunoassay analyzer (SIRIO-S, Seac, Italy).

Biochemical methods. The level of NO metabolites (NOx) in the blood was assessed using the Griess method. The supernatant obtained as above (1.0 mL) is deproteinized by adding 100 µL of 0.092 M zinc sulfate and 100 µL of 1 M NaOH, stirred, and left for 30–40 min. Then, it is centrifuged at 4000 g for 10 min (at 5 °C) using an Eppendorf™ 5430 G centrifuge (Hamburg, Germany). Subsequently, 100 µL of the resulting supernatant is transferred to a well in a microplate, and 0.5 mM of vanadium (III) chloride is added to each well to reduce nitrate to nitrite. Following this, 50 µm of sulfonamide and 0.2 µm of N-1-(naphthyl) ethylenediamine are added. The total volume of the incubation mixture is 300 µL. The next step is to incubate the samples for 30 min at 37 °C, and the optical density was measured at a wavelength of 540 nm. The concentration of NOx was determined using a linear standard curve within the range of 0–50 µµmol/L sodium nitrate. NOx level in the blood was expressed in µmol/L.

The level of reduced glutathione (GSH) and oxidized glutathione (GSSG) was determined fluorometrically. The principle of the method is based on the interaction of orthophthalic anhydride with reduced glutathione, resulting in the formation of a fluorescent complex which is registered fluorometrically at Ex/Em = 340/420 nm.

To a test tube containing 2.0 ml of 0.5 M phosphate buffer pH 8.0, 0.1 ml of blood serum and 0.5 ml of 1 % orthophthalic anhydride are added. The reaction mixture is stirred and incubated for 5 min, after which fluorometry was performed at Ex/Em = 340/420 nm.

To determine oxidized glutathione, 0.1 ml of blood serum is added to a test tube containing 2.0 ml of 0.5 M phosphate buffer pH 12.0. To mask the reduced glutathione, 0.04 ml of 0.5 mM 1-methyl-4-vinyl-pyridine is added. The reaction mixture is incubated for 60 min at room temperature.

To reduce oxidized glutathione, 0.1 ml of a reducing mixture (glutathione reductase 38 units, 7 mg NADPH dissolved in 20 ml of 0.5 m phosphate buffer pH 7.4) is added to the sample. Reduction is carried out for 2 min at a temperature of 37 °C. After that, 0.5 ml of orthophthalic anhydride is added to the sample, and fluorometry is performed at Ex/Em = 340/420 nm. Calculation of glutathione is carried out using a calibration curve.

Experimental data were statistically analyzed using Statistica for Windows 13 (StatSoft Inc., No. JPZ804I382130ARCN10-J), "SPSS16.0", and "Microsoft Office Excel 2010" software. Prior to statistical tests, we checked the results for normality (Shapiro–Wilk and Kolmogorov–Smirnov tests). In normal distribution, intergroup differences were considered statistically significant based on the parametric Student's t-test. If the distribution was non-normal, the comparative analysis was conducted using the non-parametric Mann–Whitney U-test. To compare independent variables in more than two selections, we applied ANOVA dispersion analysis for normal distribution, and the Kruskal–Wallis test for non-normal distribution. To analyze correlations between parameters, we used correlation analysis based on the Pearson or Spearman correlation coefficient. For all types of analysis, the differences were considered statistically significant at $p < 0.05$ (95 %).

Results

Our studies revealed that modeling of CGP resulted in typical manifestations of the disease [21]: gingival bleeding, hyperemia, swelling of the gums, and mobility of teeth. The depth of the periodontal pocket was 8 mm.

In rats with experimental CGP treated with a course of dental gel with IL-1β receptor antagonist in a daily dose of 1 mg/kg, a pronounced therapeutic effect was observed: a significant reduction in the size of the periodontal pocket to 2.5 mm, and an almost complete absence of bleeding and swelling. Rats with CGP receiving Mexidol 250 mg/kg intragastrically developed less pronounced therapeutic effect compared to the group receiving a gel with IL-1β receptor antagonist. In animals of this group,

Table 1. Molecular indicators of nitrosative stress and antioxidant system in the blood of animals with experimental chronic generalized periodontitis and after the treatment with pharmacological agents

Parameter, units of measurement	Intact group, n = 10	CGP (control), n = 10	CGP + gel with IL-1β receptor antagonist (1 mg/kg), n = 10	CGP + Mexidol (250 mg/kg), n = 10
Nitrotyrosine, ng/ml	50.50 ± 3.70	217.70 ± 15.20[1]	97.50 ± 5.77*[1#]	167.50 ± 9.70*[1]
iNOS, ng/ml	32.70 ± 2.55	76.40 ± 5.12[1]	47.50 ± 4.16*[1#]	72.30 ± 5.45[1]
GPX4, pg/ml	48.70 ± 2.33	17.70 ± 1.28[1]	28.40 ± 1.82*[1]	21.80 ± 2.02[1]
Cu/ZnSOD, pg/ml	88.50 ± 7.44	55.40 ± 3.40[1]	72.70 ± 6.44*[1]	62.80 ± 4.52[1]

*: compared to the control group (CGP) – p < 0.05; **1:** compared to the intact group – p < 0.05); **#:** compared to the Mexidol group – p < 0.05.

Table 2. Biochemical indicators of nitrosative stress and antioxidant system in the blood of animals with experimental chronic generalized periodontitis and after the treatment with pharmacological agents

Parameter, units of measurement	Intact group, n = 10	CGP (control), n = 10	CGP + gel with IL-1β receptor antagonist (1 mg/kg), n = 10	CGP + Mexidol (250 mg/kg), n = 10
NO metabolites (NOx), μmol/L	6.5 ± 0.47	11.2 ± 1.2[1]	7.8 ± 0.7*[1]	10.4 ± 1.7[1]
GSH, μmol/L	678.5 ± 45.0	321.8 ± 21.2[1]	524.5 ± 32.5*[1#]	419.4 ± 21.4*
GSSG, μmol/L	38.1 ± 2.8	88.5 ± 6.28[1]	63.5 ± 4.2*[1#]	77.1 ± 5.2[1]

*: compared to the control group (CGP) – p < 0.05; **1:** compared to the intact group – p < 0.05); **#:** compared to the Mexidol group – p < 0.05.

gingival swelling remained, but it was less compared to the control group; bleeding persisted when probing the periodontal pocket; the depth of the periodontal pocket was 6 mm, and tooth mobility persisted.

Molecular and biochemical studies of the peripheral blood of rats in the control group (CGP without treatment) revealed a significant decrease in the concentration of antioxidant enzymes, such as glutathione peroxidase-4 (GPX4) by 65.5 %, and Cu/Zn-dependent superoxide dismutase (Cu/ZnSOD) by 37.4 %, a decrease in the concentration of reduced glutathione by 52.5 %, and an increase in its oxidized form by 132 % (p < 0.05). Similar disturbances in the antioxidant system of the blood of animals with CGP occurred against the background of activation of nitrosative stress: the level of nitrotyrosine increased by 4.3 times, iNOS – by 2.3 times and NO metabolites – by 78.4 % (p < 0.05).

A course of treatment with dental gel containing IL-1β receptor antagonist in a dose of 1 mg/kg produced a significant effect on the molecular and biochemical parameters of antioxidant system and nitrosative stress *(Tables 1, 2)*. Thus, the concentration of nitrotyrosine was 55.2 % lower in the blood of animals of this group compared to the control group (p < 0.05). The concentration of iNOS and NOx decreased by 37.8 % and 30.5 %, respectively (p < 0.05).

Administration of dental gel with IL-1β receptor antagonist to rats with CGP produced a positive effect on the expression of antioxidant enzymes and the glutathione link of the thiol-disulfide system state in rats with CGP. Thus, the value of GPX4 and Cu/ZnSOD increased compared to the control by 60.4 % and 31.2 % (p < 0.05), respectively.

The more pronounced effect of dental gel on the expression of the GSH-dependent enzyme GPX4 corresponded to a 63 % increase in the level of reduced glutathione and a 28.2 % decrease in its oxidized form, respectively, in the blood of rats with CGP (p < 0.05). Administration of Mexidol to rats with CGP produced a significant effect only on the nitrotyrosine index, which is consistent with the results of previous studies [17].

The concentration of reduced glutathione in the blood of rats with CGP treated with Mexidol also increased (p < 0.05). However, in terms of the degree of influence on nitrosative stress and antioxidant system on these indicators, it was inferior to the gel with the IL-1β receptor antagonist (p < 0.05) *(Tables 1, 2)*.

Discussion

The experimental data obtained are consistent with the modern view of molecular-biochemical changes in the periodontium during inflammation. Thus, the interleukin (IL)-1 family plays a special role in the initiation and maintenance of periodontal inflammation. IL-1β at the site of inflammation is involved in enhancing local blood flow, leukocyte recruitment and neutrophil infiltration. IL-1β activates matrix metalloproteinase (MMP-9) expression in various cell types involved in periodontal inflammation, including osteoblasts, osteoclasts, neutrophils, and cementoblasts [22,23].

IL-1β stimulates the production of MMP-1 and/or MMP-3 in human periodontal ligament cells and gingival fibroblast cells, which promote extracellular matrix degradation and, in turn, lead to bone resorption and tissue destruction [24]. IL-1β also initiates the expression of iNOS mRNA, iNOS, and an increase in the production of reactive oxygen species (ROS), such as superoxide anion, hydroxyl anion, hypochloride anion, especially against the background of L-arginine deficiency and Cu/ZnSOD deprivation [25,26]. Many authors consider Cu/ZnSOD deficiency as the main reason for the increased production of superoxide anion in the periodontium during inflammation, which is involved in alveolar

bone resorption [27,28]. The increased expression of iNOS that we detected against the background of increased concentrations of NOx and nitrotyrosine demonstrates the enhanced production of cytotoxic forms of NO, which are active participants in nitrosative stress, initiate apoptosis of periodontal ligament cells, the formation of localized microvasculopathy, periodontal ischemia, and endothelial dysfunction of periodontal microvessels [29].

An excess of cytotoxic forms of NO with insufficient activity of antioxidant system, especially thiol-disulfide system, plays a role in the occurrence and development of periodontal diseases due to the re-increased activity of pro-inflammatory cytokines [30].

The revealed decrease in GSH and GPX4 expression, and an increase in GSSG level in the blood of rats with CGP may also be associated with nitrosative stress leading to S-nitrosation of the γ-glutamylcysteine synthetase (GCS) protein, and a decrease in GSH synthesis. GSH is quickly consumed in reactions with ROS [31]. A decrease in the specific isoform of glutathione peroxidase-4 (GPX4), which is of great importance in the metabolism of lipid peroxides in the blood of rats with CGP, indicates activation of lipid peroxidation reactions, uncontrolled formation of pro-inflammatory prostaglandins, 4-hydroxy-2-trans-nonenal, aldehydes, and ketones resulting to increased local ischemia, apoptosis and tissue destruction [32].

It is known that increased production of TNF-α, IL-1β, IL-6 and IL-8 occurs against the background of GSH deficiency. Currently, members of the IL-1 family are considered as new promising therapeutic medications in the treatment of inflammatory diseases of the oral cavity. The observed effect of dental gel with an IL-1β receptor antagonist on nitrosative stress parameters in modeling CGP can be explained by the interruption of IL-1β-dependent mechanisms of iNOS expression, which produces a high amount of ROS and NO in periodontal diseases. Perhaps the IL-1β receptor antagonist prevents the interaction of IL-1β with its receptors that triggers the expression of the nuclear transcription factors AP-1 and NF-κB. These factors change the function of target cells and result in the development of an acute-phase cellular response, the expression of other pro-inflammatory factors, stimulation of the expression of iNOS, NO cytotoxic metabolites, as well as an increase in the permeability of the mitochondrial pore and initiation of apoptosis [33].

The IL-1β receptor antagonist can prevent the nitrosylation of enzymes that ensure the stability of the intracellular concentration of GSH (γ-glutamyltransferase, γ-glutamylcysteine synthetase) by reducing the expression of iNOS. In addition, based on the studies of other authors and our own, we can assume that dental gel with IL-1β receptor antagonist can influence GSH transport through modulation of IL-1β level. We also assume that the IL-1β antagonist may exert antioxidant effect by stimulating the expression of 70 kDa heat shock proteins and affecting the structural integrity of antioxidant enzymes [34,35].

Mexidol is able to reduce the biosynthesis of pro-inflammatory mediators, such as metabolites of arachidonic acid, aldehydes, 4-hydroxy-2-trans-nonenal, and lipid peroxides due to its properties of direct antioxidant and inhibitor of lipid peroxidation processes. These properties may provide it with certain therapeutic effects in CGP. It is also possible that Mexidol can interrupt ROS-dependent mechanisms of IL-1β expression [9,17].

Conclusions

1. Experimental modelling of CGP using a calcium-deficient prooxidant model of CGP resulted in a typical clinical picture of periodontitis with symptoms of severe gingival inflammation, including hyperemia, bleeding and swelling of the gums, formation of periodontal pockets, tooth mobility, as well as an increase in molecular biochemical markers of nitrosative stress, such as iNOS, NOx and nitrotyrosine, which developed against the background of reduced glutathione deficiency, decrease of glutathione peroxidase-4 and Zn-Cu-dependent superoxide dismutase expression in the blood of animals.

2. The course of animals with CGP treatment with 1 % dental gel containing interleukin-1β receptor antagonist (1 mg/kg) in the therapeutic regimen resulted in reduction of gingival pockets to 2.5 mm, almost complete cessation of bleeding, and disappearance of swelling.

3. The course of animals with CGP treatment with 1 % dental gel containing interleukin-1β receptor antagonist (1 mg/kg) resulted in decrease in iNOS expression by 37.8 % ($p < 0.05$), nitrotyrosine concentration – by 55.2 %, and NOx – by 30.0 % ($p < 0.05$) in the blood of animals.

4. The course of animals with CGP treatment with 1 % dental gel containing interleukin-1β receptor antagonist (1 mg/kg) resulted in increase in the concentration of reduced glutathione by 63.0 % ($p < 0.05$), expression of glutathione peroxidase-4 – by 60.4 % ($p < 0.05$), and Cu/ZnSOD level – by 31.2 % ($p < 0.05$) in the blood of animals.

5. Treatment with Mexidol (250 mg/kg, intragastrically) produced an effect on only two studied parameters: decrease in nitrotyrosine level by 23 %, and an increase in the concentration of reduced glutathione by 30 % in the blood of rats with CGP ($p < 0.05$). However, it was inferior to 1 % dental gel with IL-1β receptor antagonist in terms of its effect on these indices ($p < 0.05$).

Prospects for further research. The results obtained provide an experimental basis for further research of the dental gel with IL-1 β receptor antagonist.

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Information about the authors:
Dmytriieva O. O., Postgraduate student of the Department of Propaedeutic and Surgical Dentistry, Zaporizhzhia State Medical and Pharmaceutical University, Ukraine.
ORCID ID: 0009-0003-5259-2212
Bielenichev I. F., PhD, DSc, Professor, Head of the Department of Pharmacology and Medical Formulation with Course of Normal Physiology, Zaporizhzhia State Medical and Pharmaceutical University, Ukraine.
ORCID ID: 0000-0003-1273-5314
Samura I. B., MD, PhD, Associate Professor of the Department of Pharmacology and Medical Formulation with Course of Normal Physiology, Zaporizhzhia State Medical and Pharmaceutical University, Ukraine.
ORCID ID: 0000-0001-5352-3209
Salnykov V. I., MD, Assistant of the Department of Propaedeutic and Surgical Dentistry, Zaporizhzhia State Medical and Pharmaceutical University, Ukraine.
ORCID ID: 0009-0002-9199-2174
Robota D. V., MD, Assistant of the Clinical Laboratory Diagnostics, Zaporizhzhia State Medical and Pharmaceutical University, Ukraine.
ORCID ID: 0000-0003-2205-3579

Відомості про авторів:
Дмитрієва О. О., аспірант каф. пропедевтичної та хірургічної стоматології, Запорізький державний медико-фармацевтичний університет, Україна.
Бєленічев І. Ф., д-р біол. наук, професор, зав. каф. фармакології та медичної рецептури з курсом нормальної фізіології, Запорізький державний медико-фармацевтичний університет, Україна.
Самура І. Б., канд. мед. наук, доцент каф. фармакології та медичної рецептури з курсом нормальної фізіології, Запорізький державний медико-фармацевтичний університет, Україна.
Сальников В. І., асистент каф. пропедевтичної та хірургічної стоматології, Запорізький державний медико-фармацевтичний університет, Україна.
Робота Д. В., асистент каф. клінічної лабораторної діагностики, Запорізький державний медико-фармацевтичний університет, Україна.

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