DOI: 10.14739/mmt.2025.2.322333 Оригінальні дослідження / Original research
UDC 615.454.1:615.242].015:616.314.17-008.1-085-092.9

Some aspects of the therapeutic effect of dental gel with IL-1β antagonist in experimental chronic generalized periodontitis

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

Zaporizhzhia State Medical and Pharmaceutical University, Ukraine

A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation; D – writing the article; E – critical revision of the article; F – final approval of the article

Keywords: chronic generalized periodontitis, oromucosal gel, heat shock proteins, IL-1β receptor antagonist.

Ключові слова: хронічний генералізований пародонтит, гель оромукозний, білки теплового шоку, антагоніст рецепторів IL-1β.

Надійшла до редакції / Received: 05.02.2025

Після доопрацювання / Revised: 03.03.2025

Схвалено до друку / Accepted: 20.03.2025

Конфлікт інтересів: відсутній.

Conflicts of interest: authors have no conflict of interest to declare.

The aim of the current study was to evaluate the therapeutic efficacy of 1 % IL-1β antagonist dental gel by its effect on biomarkers of inflammation and cytoprotection under conditions of modelling chronic generalized periodontitis in rats.

Materials and methods. Chronic generalized periodontitis (CGP) was reproduced in Wistar rats weighing 180–210 g by 8-week administration of the prooxidant Delagil (chloroquine phosphate, 30 mg/kg) and addition of EDTA (2 %) to drinking water using a calcium-deficient peroxide diet with reduced chewing function. The studied pharmacological medications were administered after the development of CGP in rats for 30 days: 1 % oromucosal gel with IL-1β receptor antagonist (RAIL-1β, 1 mg/kg) locally using a dispenser; and the antioxidant Mexidol (250 mg/kg) intragastrically for 30 days. The condition of periodontal tissues and the effect of the studied medications on the levels of inflammatory markers IL-1β, tumour necrosis factor alpha (TNF-α), matrix metalloproteinase-2 (MMP-2) and markers of endogenous neuroprotection hypoxia-induced factor 1-alpha (HIF-1α) and heat shock proteins (HSP_{70}) were evaluated using enzyme-linked immunosorbent assay (ELISA).

Results. Modelling of CGP in rats by 8-week administration of the prooxidant Delagil and addition of EDTA to drinking water resulted in typical manifestations of the disease: bleeding, hyperaemia, and swelling of the gums; tooth mobility; formation of gingival pockets up to 8 mm against the background of increased levels of inflammation markers (TNF-α, IL-1β), and molecular markers (HIF-1α and HSP_{70}) in the blood indicated a homeostatic response of the periodontium to inflammation and subsequent hypoxia by an increase in the synthesis of HIF-1α and HSP_{70}. Course application of 1 % oromucosal gel with IL-1β receptor antagonist (1 μg/kg) to rats with CGP in a therapeutic regimen led to an improvement in the clinical picture of the disease: significant reduction in the size of the gingival pocket to 2.2 mm, and a significant reduction of bleeding and swelling against the background of lowering the levels of inflammatory markers in the blood: TNF-α – by 82 % ($p < 0.05$), metalloproteinase-2 – by 65 % ($p < 0.05$), and IL-1β – by 71.4 % ($p < 0.05$) compared to the group of untreated animals. Application of 1 % oromucosal gel with IL-1β receptor antagonist resulted in an increase in HIF-1α levels by 42 % ($p < 0.05$) in comparison to control indicators, and an increase in HSP_{70} levels by 62.8 % compared to the control group, and in 2.4 times ($p < 0.05$) compared to the intact group that indicated a significant impact of IL-1β receptor antagonist on the HSP_{70}-dependent mechanisms of endogenous cytoprotection. Oromucosal gel with 1 % IL-1β receptor antagonist (1 μg/kg) was significantly superior to the reference drug Mexidol (250 mg/kg) in terms of its action on the studied parameters under conditions of CGP.

Conclusions. The obtained results substantiate the further in-depth pharmacological study of the new oromucosal gel with IL-1β receptor antagonist (1 μg/kg) for the purpose of clinical use in the treatment of generalized periodontitis. We have found that the use of IL-1β receptor antagonist in experimental CGP is more effective than Mexidol.

Modern medical technology. 2025;17(2):124-131

Деякі аспекти терапевтичної дії стоматологічного гелю з антагоністом IL-1β при експериментальному хронічному генералізованому пародонтиті

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

Мета роботи – оцінити терапевтичну ефективність 1 % оромукозного гелю з антагоністом інтерлейкіну 1β (IL-1β) за його впливом на маркери запалення та цитопротекції в умовах моделювання хронічного генералізованого пародонтиту у щурів.

Матеріали і методи. Хронічний генералізований пародонтит (ХГП) моделювали у щурів лінії Вістар масою 180–210 г за допомогою перекисної кальцій-дефіцитної дієти зі зниженою жувальною функцією протягом 8 тижнів. У питну воду додавали 2 % розчин ЕДТА та внутрішньошлунково вводили прооксидант «Делагіл» (хлорохіну фосфат, 30 мг/кг) щодня. Препарати, що вивчали, вводили після розвитку ХГП протягом 30 діб: оромукозний гель 1 % із рецепторним антагоністом IL-1β (1 мкг/кг) наносили місцево за допомогою доза-

тора; «Мексидол» (250 мг/кг) вводили внутрішньошлунково. Методами твердофазного імуноферментного аналізу оцінювали стан тканин пародонта та вплив досліджених сполук на рівні маркерів запалення IL-1β, фактора некрозу пухлини альфа (TNF-α), матриксної металопротеїнази-2 (MMP-2) та маркерів ендогенної нейропротекції – індукованого гіпоксією фактора 1-альфа (HIF-1α) та білків теплового шоку (HSP_{70}).

Результати. Моделювання ХГП у щурів шляхом 8-тижневого введення прооксиданта «Делагілу» та додавання ЕДТА до питної води спричиняло типові прояви хвороби: кровоточивість, гіперемію та набряк ясен; рухливість зубів; утворення ясенних кишень до 8 мм на тлі підвищення рівня маркерів запалення (TNF-α, IL-1β), а також молекулярних маркерів (HIF-1α та HSP_{70}) у крові. Це свідчить про гомеостатичну відповідь пародонта на запалення та наступну гіпоксію шляхом збільшення синтезу HIF-1α і білка HSP_{70}. Курсове введення 1 % оромукозного гелю з антагоністом рецепторів IL-1β (1 мкг/кг) щурам із ХГП сприяло покращенню клінічної картини захворювання: значному зменшенню глибини пародонтальних кишень до 2,2 мм, зменшенню кровоточивості та набряку ясен на тлі зниження рівнів маркерів запалення в крові (TNF-α – на 82 %, $p < 0,05$, MMP-2 – на 65 %, $p < 0,05$, IL-1β – на 71,4 %, $p < 0,05$) порівняно з групою нелікованих тварин. Введення 1 % оромукозного гелю з антагоністом рецепторів IL-1β призводило до підвищення рівнів HIF-1α на 42 % ($p < 0,05$) порівняно з контрольними показниками і збільшення рівнів HSP_{70} на 62,8 % порівняно з контролем та у 2,4 раза ($p < 0,05$) порівняно з інтактною групою. На підставі цих даних зробили висновок про значний вплив антагоніста рецепторів IL-1β на HSP_{70}-залежні механізми ендогенної цитопротекції. За дією на досліджені показники в умовах ХГП 1 % оромукозний гель з антагоністом рецепторів IL-1β (1 мкг/кг) вірогідно переважав препарат порівняння «Мексидол» (250 мг/кг).

Висновки. Результати дослідження обґрунтовують доцільність продовження фармакологічного вивчення нового оромукозного гелю з антагоністом рецепторів IL-1β (1 мкг/кг) для клінічного використання під час лікування генералізованого пародонтиту.

Сучасні медичні технології. 2025. Т. 17, № 2(65). С. 124-131

Inflammatory periodontal diseases are the most complex problem from the point of view of therapeutic dentistry as they can be complicated by the development of unresolved hyperinflammation; dysbiosis of the oral, intestinal and other location's microbiota; disruption of innate and adaptive immunity, and other systemic alterations that may cause or exacerbate other health problems associated with increased morbi-mortality [1]. According to international statistics, periodontitis ranks second after dental caries. Thus, according to the scientific and analytical group of WHO, inflammatory periodontal diseases are significantly widespread among the world population and their significant growth continues [2,3].

Despite the promotion of a healthy lifestyle, hygiene and preventive measures, incidence and prevalence of periodontitis has constantly increased (55–80 %) in young people [4]. Periodontitis has a multifactorial etiology, in which the presence of aggressive gingival microbiota, weakened immune and antioxidant systems against the background of a significant surge in free-radical and inflammatory reactions leads to the disease and its progression [5]. All this knowledge about the pathogenesis of periodontitis justified the widespread use of antiseptics, antibiotics, anti-inflammatory agents, and antioxidants for the treatment of the disease [6].

The therapeutic effectiveness of anti-inflammatory and antioxidant medications from medicinal plant materials (chamomile, turmeric, calendula, and ginger), and synthetic medications (COX inhibitors, leukotriene modulators, lipid peroxidation inhibitors, and membrane stabilizers) has been studied in detail, and these are among the most widely used classes of medications for the management of chronic generalized periodontal disease [7,8,9]. Dosage forms containing essential oils of medicinal plants, which exhibit anti-inflammatory, wound-healing, and antibacterial properties, have found application in the treatment of periodontitis [10,11]. In our country and abroad, preclinical justification has been obtained for the use of noble metals and noble metal-based nanoparticles in the management of periodontal disease [12,13,14].

It has been established recently that the pro-inflammatory mediators IL-1β and TNF-α; increased enzymatic and non-enzymatic production of reactive oxygen species (ROS) are of great importance in the formation and progression of inflammatory periodontal diseases, among which the leading place is occupied by generalized periodontitis.

ROS and cytotoxic forms of NO cause an active burst of free-radical reactions that lead to oxidative modification of protein molecules, complex lipids, and nucleic acids. The interleukin 1 family includes key signalling molecules that trigger and maintain periodontal inflammation, initiate some mechanisms of apoptosis, inhibit protein synthesis and reparative regeneration [15,16,17]. IL-1β is a master regulator of inflammation and plays essential roles in enhancing local blood flow, and neutrophil infiltration at the site of inflammation. Further studies demonstrated that the IL-1 family is a diverse group of mediators that includes pro-inflammatory and anti-inflammatory cytokines. IL-1β enhances the secretion and activity of matrix metalloproteinases (MMP), particularly MMP-9, in different types of cells involved in periodontal inflammation, including neutrophils, osteoclasts, and osteoblasts; this represents an important amplification loop of the inflammatory response [18,19]. In addition, The IL-1β molecules enhance the production of other MMP, such as MMP-1 and MMP-2, in gingival fibroblast cells and periodontal ligament cells promoting extracellular matrix degradation and leading to tissue destruction and bone resorption [20,21]. Novel IL-1 modulators have shown significant promise as adjuncts to traditional local therapy in the clinical management of periodontal disease.

Currently, FDA approved recombinant IL-1β antagonists Rilonacept, Canakinumab, Infliximab, Bortezomib and Anakin-

ra. IL-1β inhibiting properties have been identified in curcumin, metformin, and metronidazole [22]. The most encouraging results have been found with clinical use of Anakinra in various pathological conditions. Anakinra and its generics interrupt IL-1β-dependent pathways of neurodestruction, modulate glutathione (GSH)-dependent mechanisms of mitochondrial and cytosolic heat shock protein 70 (HSP_{70}) expression in the brain during acute ischemia. Oromucosal gel with an IL-1β inhibitor was developed at the Department of Medicines Technology of Zaporizhzhia State Medical and Pharmaceutical University. Numerous preclinical studies demonstrated its effectiveness, safety and harmlessness in experimental periodontitis [23,24]. The above predetermined the prospects for further studies of oromucosal gel with IL-1β antagonist.

Aim

To evaluate the therapeutic efficacy of 1 % IL-1β antagonist dental gel by its effect on biomarkers of inflammation and cytoprotection under conditions of modeling chronic generalized periodontitis in rats.

Materials and methods

Laboratory animals. The studies were conducted on forty 3-month-old white female Wistar rats weighing 180–210 g, obtained from the nursery of the Institute of Pharmacology and Toxicology of the Academy of Medical Sciences of Ukraine. Experiments and all manipulations with animals were conducted 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 [25,26]. The experimental research protocols and their results were approved by the decision of the Zaporizhzhia State Medical and Pharmaceutical University Bioethics Commission. The Zaporizhzhia State Medical and Pharmaceutical University Commission on Bioethics decided to adopt the experimental study protocols and outcomes (Protocol No. 3, dated March 22, 2021).

Before the start of the experiment, animals that met the criteria for inclusion in the experiment were randomly assigned to the groups. Animals that did not meet the criteria were excluded from the experiment during quarantine. The animals were placed in polycarbonate cages measuring 550 × 320 × 180 mm with galvanized steel covers measuring 660 × 370 × 140 mm and glass drinkers. Five rats were kept in each cage. Each cage was labelled with study number, species, sex, animal numbers, and dose. Cages were placed on racks according to the dose levels and cage numbers indicated on the labels. All rats were fed *ad libitum* standard ration for laboratory animals, supplied by the company "Phoenix", Ukraine. Water from the municipal water supply network (after reverse osmosis and sterilization by UV radiation) was given without restrictions. Alder (*Alnus glutinosa*) sawdust, previously treated by autoclaving, was used as litter.

Experimental model. Experimental modelling of chronic generalized periodontitis (CGP) was reproduced for 8 weeks using a calcium-deficient peroxide diet with reduced masticatory function. The drinking water bottles contained a 2 % EDTA solution, and the prooxidant Delagil (chloroquine phosphate) at a dose of 30 mg/kg in the form of a 0.59 % aqueous solution was administered daily to the animals. The animals were given soft food throughout the experiment [27]. After the development of CGP simulation, the animals received the studied medications intragastrically using a metal probe. All animals were divided into 4 groups (10 animals each):

1. Intact group – received isotonic saline solution (0.9 % NaCl) intragastrically for 30 days;
2. Control group – animals with experimental CGP received isotonic saline solution (0.9 % NaCl) intragastrically for 30 days;
3. Animals with experimental CGP, which were applied 1 % oromucosal 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 [23];
4. Animals with experimental CGP, which received the reference drug Mexidol (PJSC "Technolog", Ukraine) at a dose of 250 mg/kg intragastrically daily for 30 days [27].

In the work we used 1 % oromucosal dental gel with IL-1β receptor antagonist, developed at the Department of Medicines Technology of Zaporizhzhia State Medical and Pharmaceutical University. Antagonist of interleukin-1β was used as an active pharmaceutical ingredient in the recipe of oromucosal gel, Excipients were D-panthenol (a plasticizer), carboxymethyl cellulose sodium salt (a viscosity modifier and mucoadhesive component), Tween-80 (an absorption enhancer), benzalkonium chloride (a preservative), sodium hydrophosphate + citric acid (a phosphate buffer solution), and purified water.

For the experiments, only pharmaceutical-grade active and auxiliary ingredients were utilized, which were obtained from Ukraine suppliers such as SINBIAS LLC, Istok-Plus LLC, and MOBIL MEDICAL LLC.

Under thiopental anaesthesia (40 mg/kg), rats of all experimental groups were withdrawn from the study. Following this, blood samples were obtained from the celiac artery for subsequent analysis.

Blood was taken from the abdominal aorta by syringe, and serum was separated by centrifugation at +4 C° at 1500 rpm for 20 min [28].

Enzyme-Linked Immunosorbent Assay (ELISA). The concentration of hypoxia-inducible factor 1-alpha (HIF-1α) in the blood serum was assessed using the solid phase immunoassay sandwich method of ELISA. The ELISA Kit HIF-1 alpha ELISA kit ab275103 (Abcam Limited, UK) was used according to the instructions.

The concentration of heat shock protein HSP70 was determined by enzyme immunoassay using the HSP_{70} High-Sensitivity StressXpress ELISA Kit #MBS806878 (MyBioSource, Canada) according to the instructions included with the kits.

The content of IL-1β was determined using solid phase enzyme-linked immunosorbent assay (ELISA) using the Rat Interleukin 1β, IL-1β ELISA Kit # CSB-E08055r (CUSABIO TECHNOLOGY, USA) test kit in accordance with the instructions included with the kits.

The content of TNF-α was determined by solid-phase enzyme-linked immunosorbent assay (ELISA) using Rat TNF alpha ELISA Kit #ab 108913 (Abcam, USA) in accordance with the instructions supplied with the kits.

Table 1. Effect of 1 % oromucosal gel with IL-1β receptor antagonist and reference drug Mexidol on the concentration of inflammatory markers in the blood of rats with CGP

Parameter, unit of measurement	Intact group, n = 10	CGP (control), n = 10	CGP + Mexidol (250 mg/kg), n = 10	CGP + gel with IL-1β receptor antagonist (1 mg/kg), n = 10
Gingival pocket depth, mm	–	8.00 ± 0.43[1]	6.00 ± 0.93*[1]	2.20 ± 0.42*[1]
TNF-α, ng/ml	0.112 ± 0.053	0.907 ± 0.107[1]	0.577 ± 0.030*[1]	0.162 ± 0,012*[1#]
IL-1β, ng/ml	0.130 ± 0.014	0.560 ± 0.109[1]	0.397 ± 0.06[1]	0.160 ± 0.017*[1#]
MMP-2, ng/ml	0.800 ± 0.078	16.300 ± 1.120[1]	14.000 ± 1.520[1]	5.700 ± 0.520*[1#]

*: the difference is significant compared to the control group (CGP) – $p < 0.05$); [1]: the difference is significant compared to the intact group – $p < 0.05$; [#]: the difference is significant compared to the Mexidol group – $p < 0.05$.

Table 2. Effect of 1 % oromucosal gel with IL-1β receptor antagonist and reference drug Mexidol on the concentration of cytoprotection markers in the blood of rats with CGP

Parameter, unit of measurement	Intact group, n = 10	CGP (control), n = 10	CGP + Mexidol (250 mg/kg), n = 10	CGP + gel with IL-1β receptor antagonist (1 mg/kg), n = 10
HSP_{70}, ng/ml	17.40 ± 0.82	24.20 ± 1.32[1]	27.00 ± 4.1[1]	39.40 ± 3.4*[1#]
HIF-1α, pg/ml	1874.1 ± 121.1	2761.2 ± 117.1[1]	3117.5 ± 112.3*[1]	3922.3 ± 115.2*[1#]

*: the difference is significant compared to the control group (CGP) – $p < 0.05$); [1]: the difference is significant compared to the intact group – $p < 0.05$; [#]: the difference is significant compared to the Mexidol group – $p < 0.05$.

The content of matrix metalloproteinase-2 (MMP-2) was determined by solid phase enzyme-linked immunosorbent assay (ELISA) using the Rat MMP-2(Matrix Metalloproteinase 2) ELISA Kit (E-EL-R0618) (Elabscience, USA) according to the instructions supplied with the kits. These analyses were conducted on a complete plate enzyme immunoassay analyser (SIRIO-S, Seac, Italy).

Experimental data were statistically analysed 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 not 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 analyse 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 demonstrated that modelling of CGP resulted in typical manifestations of the disease: bleeding, hyperaemia, gingival swelling, and tooth mobility. The depth of the gingival pocket was 8 mm. A significant therapeutic effect was developed with a decrease in the size of the gingival pocket to 2.5 mm, and an almost complete absence of bleeding and swelling in rats with CGP, receiving a oromucosal gel with IL-1β inhibitor in a dose of 1 mg/kg in a therapeutic regimen.

Mexidol in a dose of 250 mg/kg produced a less pronounced therapeutic effect in comparison to the group receiving gel with IL-1β antagonist. The animals still had gum swelling, although it was smaller than in the control group, and bleeding persisted during probing of the periodontal pocket with a button probe. The depth of the gingival pocket was approximately 6 mm, and tooth mobility persisted.

The molecular studies of control group (CGD without treatment) revealed a significant increase (several times) in the levels of pro-inflammatory mediators TNF-α ($p < 0.05$) and IL-1β ($p < 0.05$), as well as MMP-2 compared to the intact group. The results obtained indicate a pronounced inflammatory process in the periodontium under the conditions of reproducing this model of CGD that was also confirmed by our earlier works [27,29].

Administration of 1 % oromucosal gel with IL-1β receptor antagonist to rats with CGP led to a decrease in TNF-α by 82.1 % ($p < 0.05$), IL-1β –by 71.4 % ($p < 0.05$), and MMP-2 – by 65 % ($p < 0.05$) compared to the group of untreated animals. Administration of Mexidol to rats with CGP led to a decrease in the concentration of TNF-α by 36.3 % ($p < 0.05$) compared to the control group, without affecting the concentration of IL-1β and MMP-2.

As can be seen from *Table 1*, in terms of the degree of reduction of inflammation markers (MMP-2, IL-1β, TNF-α) in the blood of rats with CGP, the oromucosal gel with IL-1β receptor antagonist was significantly superior to Mexidol ($p < 0.05$).

The data presented in *Table 2* demonstrate changes in the molecular markers of endogenous cytoprotection HIF-1α and HSP70. The results obtained in this study are completely

consistent with our previous studies, which showed that chronic periodontal inflammation in rats led to an increase in the levels of HIF-1α and HSP70 [27,29]. Thus, the level of HSP_{70} in the blood serum of rats with CGP was significantly higher than the similar value of intact animals by 140 %, while the level of HIF-1α was significantly higher by 47.3 % than the similar values of the intact group. These data demonstrate that chronic inflammation can lead at a certain stage to the activation of HSP_{70} mechanisms of endogenous cytoprotection. This is also evidenced by our earlier publications [30].

Course application of oromucosal gel with IL-1β receptor antagonist to rats with CGP led to an increase in endogenous cytoprotection mechanisms, as evidenced by an increase in HIF-1α by 42 % ($p < 0.05$) in the blood of rats compared to the control values, and by 109 % ($p < 0.05$) compared to similar values in animals of the intact group. In the blood of animals with CGP that received a gel with an IL-1β receptor antagonist, the HSP70 values were 62.8 % ($p < 0.05$) higher compared to the values of animals with CGP without treatment and 126.4 % ($p < 0.05$) higher compared to the same value of animals in the intact group. This fact confirms the possibility of pharmacological modulation of endogenous cytoprotection by influencing IL-1β, which we wrote about in our other studies [31].

The introduction of Mexidol resulted in an increase in HIF-1α levels by 12.9 % ($p < 0.05$) compared to the control, without affecting the concentration of HSP_{70} in the blood of rats with CGP.

Discussion

This model of chronic periodontal disease leads to persistent inflammatory processes in the periodontium against the background of activation of oxidative stress, disruption of the NO system and, ultimately, to structural changes in periodontal tissues and tooth loss, as shown by numerous studies and our research [27,29,32,33]. It is known that in periodontitis, inflammatory changes develop in the tissues that support the teeth, and bacteria, fungi and viruses are responsible for these processes. Microorganisms involved in periodontitis increase the expression of genes involved in immunological and inflammatory reactions, the cell cycle and apoptosis. The greatest role in the activation of inflammatory reactions belongs to periopathogens, represented by such species as *Porphyromonas gingivalis, Treponema denticola, Tanerella forsythia* and *Aggregatibacter actinomycetemcomitans*. Genetic loci may be associated with bacterial colonization occurring in periodontitis. Genetic markers reveal host genetic variants associated with periodontitis. Lipopolysaccharides of gram-negative bacteria are an important agent inducing inflammatory changes and bone resorption in periodontitis [5].

Dysregulation between proinflammatory and anti-inflammatory cytokines is another molecular mechanism that contributes to periodontal tissue damage. Cytokines are known to play an important role in maintaining tissue homeostasis, immunity, and cellular signalling. In periodontitis, lipopolysaccharides induce the production of proinflammatory cytokines [21].

Many proinflammatory cytokines, such as interleukin IL-1β, TNF-α, play an important role in the pathogenesis of periodontitis. IL-1β promotes the activation of both Th1 and Th2 cells, which are involved in the host immune response associated with the pathogenesis of periodontitis [34].

IL-1β is one of the most important molecules associated with the development and progression of periodontal inflammation. IL-1β is involved in a number of processes necessary for the initiation and maintenance of the inflammatory response. IL-1β enhances the production of adhesion molecules, facilitates the migration of leukocytes, stimulates the production of other inflammatory mediators and metalloproteinases, activates T- and B-lymphocytes, stimulates osteoblasts, leading to bone resorption, and enhances programmed death of cells producing extracellular matrix, thereby limiting the regenerative capacity of tissues. The concentration of IL-1β in gingival fluid and saliva directly correlates with the severity of periodontitis, and a decrease in IL-1β levels may indicate the effectiveness of treatment [35].

IL-1β and TNF-α are two of the most important and earliest cytokines activated during periodontal infection. Systemic activation of these two cytokines contributes to the creation of a proinflammatory environment of nitrosative and oxidative stress, activation of matrix metalloproteinases. Systemic elevation of TNF-α levels, in addition to its important role in periodontal destruction, is extremely toxic to the cell and has therefore been called the “suicide hormone” [36].

Metalloproteinases (MMPs) are proteolytic enzymes that play a central role in normal tissue turnover and degradation of tissues surrounding teeth and implants. Both collagenases (MMP-8 and MMP-13) and gelatinases (MMP-2 and MMP-9) have been isolated in the pathological processes of periodontitis and peri-implantitis. MMP-2 is secreted into the extracellular matrix as inactive proenzymes and is activated by IL-1β, TNF-α, and iNOS [37,38]. Proinflammatory cytokines (such as TNF-α and IL-1β) activate MMP-2 genes through binding of transcription factors (such as activator protein-1 and mitogen-activated protein kinase.

Evaluation of IL-1β, TNF-α and MMP-2 levels may be important for the diagnosis of periodontitis. MMP-2 levels are elevated in sites with peri-implantitis compared to healthy implants, suggesting that this biomarker may be a potential candidate for early disease prediction [39,40]. Therefore, a proper understanding of the role of IL-1β in the pathogenesis of inflammatory periodontal diseases is necessary for the development of future drug targets.

In this and previous studies, we found that modelling CGP in rats resulted in changes in the expression of proteins that play a general protective role [27,29]. We are talking, first of all, about heat shock proteins. Heat shock proteins protect cells from damaging factors, including inflammatory and infectious diseases. 70-kDa HSPs (HSP_{70}) are the main HSP expressed in inflamed tissues. HSP_{70} can be considered as a potential marker of the severity of periodontal disease [41].

IL-1β, TNF-α, and INF-γ are produced in inflamed periodontal tissues, and their elevated concentrations can induce HSP_{70} expression. However, ultra-high concentrations of IL-1β can suppress HSP_{70} expression and reduce endogenous cytoprotection [42,43]. HSP_{70} can provide protection of protein molecules from oxidative modification, regulate calcium transport, reduce energy deficiency in generalized ischemia due to inflammation. HSP_{70} also prolongs the life of the factor induced by hypoxia and provides its higher bioavailability [30].

There are works on changes in the expression of HIF-1α in periodontal tissues during inflammation. There is evidence for an important protective role of HIF-1α in infection and immunity. In inflammatory periodontal diseases, the expression of HIF-1α is launched by TNF-α. Subsequently, HIF-1α in the inflammatory periodontium plays a protective role, regulates the expression of vascular endothelial growth factor, erythropoiesis, and increases the effectiveness of compensatory energy shunts [44,45,46]. We have already written that this model of CGP led to a decrease in the expression of HIF-1α mRNA and an increase in HIF-1α protein [27].

Our results are consistent with other studies that have shown a marked increase in the proportion of fibroblast-like cells and leukocyte-like cells expressing HIF-1α. HIF-1α, vascular endothelial growth factor, and TNF-α protein levels were significantly higher in periodontal pockets in advanced periodontitis [47,48].

Expression of HIF-1α in periodontitis promotes reorganization of periodontal tissue energy metabolism to reduce oxygen consumption by switching energy metabolism from aerobic respiration to glycolysis. Expression of HIF-1α also increases expression of pyruvate dehydrogenase kinase that reduces the incorporation of pyruvate into the citric acid cycle. This metabolic switch is essential for periodontal protection because such HIF-1α-regulated glycolytic metabolism is required for B cell development and T cell metabolism [49]. HIF-1α is a critical mediator of neoangiogenesis, which is essential for bone regeneration. HIF-1α treatment of periodontal ligament stem cells improved their osteogenic potential and mineralization [50].

We have demonstrated new interesting mechanisms of the protective effect of gel with IL-1β receptor antagonist, contributing to both a reduction in periodontal inflammation and an enhancement of endogenous cytoprotection. The review [30] shows that selective blockade of the IL-1β receptor leads to a reduction of hyperactivity of nuclear transcription factors AP-1 and NF-κB that changes the behavior of target cells and leads to suppression of the acute inflammatory response, expression of other proinflammatory factors, a decrease in the hyperexpression of iNOS and cytotoxic NO derivatives, a decrease in the permeability of mitochondrial pores and inhibition of apoptosis.

Interruption of the IL-1β signalling pathway, which is involved in the formation of generalized tissue ischemia, can occur against the background of an increase in HSP70 levels. HSP70 weakens the expression of IL-1β by inhibiting the transcription factors C/EBPβ and C/EBPδ. A decrease in IL-1β protein expression leads to an enhancement of the mechanisms that initiate HSP_{70} expression; and an increase in HSP_{70} concentration, and overexpression of IL-1β occur against the background of HSP_{70} deficiency. Currently, there is convincing evidence of the neuro- and cytoprotective action of the IL-1β antagonist. Parenteral introduction of IL-1β to animals with stroke was shown to reduce mortality, neurological deficits, and the area of cerebral infarction [51]. We also found that the IL-1β receptor antagonist is able to regulate the expression of HSP_{70} and GSH by blocking the action of IL-1β. This is a new and promising mechanism of action. HSP_{70} blocks the Fas/Apo-1 receptor launching apoptosis and inhibits apoptosis in mitochondria at the stage between the release of cytochrome C and the cleavage of procaspase-9, and prevents the binding of cytochrome C to the proapoptotic protein Apaf-1 in mitochondria [30].

It is known that IL-1β receptor antagonist can regulate IL-1β-dependent GSH transport. It is possible that the IL-1β receptor antagonist, by increasing the concentration of HSP_{70}, affects the activation of redox-sensitive transcription factors AP-1, NF-κB and NF-1, which increase the expression of GPX, as evidenced by our previous work [24].

We established in this and previous work [20] that the administration of Mexidol to animals with CGP did not affect the levels of IL-1β but significantly reduced the expression of TNF-α that could be due to suppression of succinate receptor expression SUCNR1/GPR91 [52]. Mexidol did not affect the indicators of endogenous cytoprotection, without affecting the concentration of HSP_{70} in the blood of rats with CGP. We have identified a certain effect of Mexidol on the expression of HIF-1α in the blood of rats with CGP that was associated with the action of its structural fragment – succinate [53]. Mexidol inhibits the oxidative modification of macromolecules and produces membrane-protective, antioxidant, and anti-inflammatory action. Mexidol reduced the levels of pro-inflammatory cytokines (TNF-α) by suppressing the expression of the succinate receptor SUCNR1/GPR91 [52].

Conclusions

1. Modelling of CGP in rats by 8-week prooxidant Delagil administration and addition of EDTA to drinking water resulted in development typical signs of the disease such as bleeding, swelling and hyperaemia of the gums; formation of gingival pockets up to 8 mm, tooth mobility against the background of increased levels of inflammatory markers (TNF-α, IL-1β), and molecular markers (HIF-1α and HSP_{70}) in the blood indicate a homeostatic response of the periodontium to inflammation and subsequent hypoxia by an increase in the synthesis of the hypoxia-induced factor HIF-1α and heat shock protein HSP_{70}.

2. Course application of 1 % oromucosal gel with IL-1β receptor antagonist (1 μg/kg) to rats with CGP led to an improvement in the clinical picture of the disease: a significant reduction in the size of the gingival pocket to 2.2 mm, and a significant reduction of bleeding and swelling against the background of lowering the levels of inflammatory markers in the blood: TNF-α – by 82 % ($p < 0.05$), metalloproteinase-2 – by 65 % ($p < 0.05$) and IL-1β – by 71.4 % ($p < 0.05$) compared to the group of untreated animals.

3. The application of 1 % oromucosal gel with IL-1β receptor antagonist contributed to an increase in HIF-1α levels by 42 % ($p < 0.05$) in comparison with to control group, and an increase in HSP_{70} levels by 62.8 % in comparison with the control, and by 2.4 times ($p < 0.05$) in comparison with the intact group that indicates a significant effect of IL-1β receptor inhibitor on the HSP_{70}-dependent mechanisms of endogenous cytoprotection.

4. In terms of its action on the studied parameters in conditions of CGP, 1 % oromucosal gel with IL-1β receptor antagonist (1 μg/kg) was significantly superior to the reference drug Mexidol (250 mg/kg).

5. We have found that the use of IL-1β receptor antagonist in experimental CGP is more effective than Mexidol.

Prospects for further research. The obtained results substantiate the further in-depth pharmacological study of the new oromucosal gel with IL-1β receptor antagonist (1 μg/kg) for the purpose of clinical use in the treatment of generalized periodontitis.

Funding
This study was funded by the Ministry of Health of Ukraine from the state budget.

Information about the authors:
Dmytriieva O. O., MD, Postgraduate Student of the Department of Surgical and Propaedeutic 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 Surgical and Propaedeutic Dentistry, Zaporizhzhia State Medical and Pharmaceutical University, Ukraine.
ORCID ID: 0009-0002-9199-2174

Відомості про авторів:
Дмитрієва О. О., аспірант каф. хірургічної та пропедевтичної 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. хірургічної та пропедевтичної стоматології, Запорізький державний медико-фармацевтичний університет, Україна.


Iryna Samura (Ірина Самура)
irinasamura77@gmail.com

References

1. Martínez-García M, Hernández-Lemus E. Periodontal inflammation and systemic diseases: an overview. Front Physiol. 2021;12:709438. doi: 10.3389/fphys.2021.709438
2. Li Y, Xiang Y, Ren H, Zhang C, Hu Z, Leng W, et al. Association between periodontitis and dental caries: a systematic review and meta-analysis. Clin Oral Investig. 2024;28(6):306. doi: 10.1007/s00784-024-05687-2
3. Huang D, Wang YY, Li BH, Wu L, Xie WZ, Zhou X, et al. Association between periodontal disease and systemic diseases: a cross-sectional analysis of current evidence. Military Med Res. 2024;11(1):74. doi: 10.1186/s40779-024-00583-y
4. Janakiram C, Dye BA. A public health approach for prevention of periodontal disease. Periodontol 2000. 2020;84(1):202-14. doi: 10.1111/prd.12337
5. Bhuyan R, Bhuyan SK, Mohanty JN, Das S, Juliana N, Juliana IF. Periodontitis and its inflammatory changes linked to various systemic diseases: a review of its underlying mechanisms. Biomedicines. 2022;10(10):2659. doi: 10.3390/biomedicines10102659
6. López-Valverde N, López-Valverde A, Montero J, Rodríguez C, Macedo de Sousa B, Aragoneses JM. Antioxidant, anti-inflammatory and antimicrobial activity of natural products in periodontal disease: a comprehensive review. Front Bioeng Biotechnol. 2023;11:1226907. doi: 10.3389/fbioe.2023.1226907
7. Gonçalves S, Fernandes L, Caramelo A, Martins M, Rodrigues T., Matos RS. Soothing the itch: the role of medicinal plants in alleviating pruritus in palliative care. Plants. 2024;13(24):3515. doi: 10.3390/plants13243515
8. Emerald M. Medicinal plants: Therapeutic potential, safety, and toxicity. In: Drug Discovery and Evaluation: Safety and Pharmacokinetic Assays. Cham: Springer International Publishing; 2024. p. 1327-97. doi: 10.1007/978-3-031-35529-5_90
9. Pázmándi K, Szöllősi AG, Fekete T. The «root» causes behind the anti-inflammatory actions of ginger compounds in immune cells. Front Immunol. 2024;15:1400956. doi: 10.3389/fimmu.2024.1400956
10. Siddiqui R, Badran Z, Boghossian A, Alharbi AM, Alfahemi H, Khan NA. The increasing importance of the oral microbiome in periodontal health and disease. Future Sci OA. 2023;9(8):FSO856. doi: 10.2144/fsoa-2023-0062
11. Sabaoui Z, Lakhdar L. Essential Oils in Periodontics. What is the Interest? Integr J Med Sci. 2021;8:1-3. doi: 10.15342/ijms.2021.499
12. Menéndez SG, Manucha W. Nanopharmacology as a new approach to treat neuroinflammatory disorders. Transl Neurosci. 2023;14(1):20220328. doi: 10.1515/tnsci-2022-0328
13. Nasiri K, Masoumi SM, Amini S, Goudarzi M, Tafreshi SM, Bagheri A, et al. Recent advances in metal nanoparticles to treat periodontitis. J Nanobiotechnology. 2023;21(1):283. doi: 10.1186/s12951-023-02042-7
14. Bapat R, Chaubal TV, Dharmadhikari S, Abdulla AM, Bapat P, Alexander A, et al. Recent advances of gold nanoparticles as biomaterial in dentistry. Int J Pharm. 2020;586:119596. doi: 10.1016/j.ijpharm.2020.119596
15. Brierly G, Celentano A, Breik O, Moslemivayeghan E, Patini R, McCullough M, et al. Tumour necrosis factor alpha (TNF-α) and oral squamous cell carcinoma. Cancers (Basel). 2023;15(6):1841. doi: 10.3390/cancers15061841
16. Afzal S, Abdul Manap AS, Attiq A, Albokhadaim I, Kandeel M, Alhojaily SM. From imbalance to impairment: the central role of reactive oxygen species in oxidative stress-induced disorders and therapeutic exploration. Front Pharmacol. 2023;14:1269581. doi: 10.3389/fphar.2023.1269581
17. Papathanasiou E, Conti P, Carinci F, Lauritano D, Theoharides TC. IL-1 superfamily members and periodontal diseases. J Dent Res. 2020;99(13):1425-34. doi: 10.1177/0022034520945209
18. Pyrillou K, Burzynski LC, Clarke MC. Alternative pathways of IL-1 activation, and its role in health and disease. Front. Immunol. 2020;11:613170. doi: 10.3389/fimmu.2020.613170
19. Herrero-Cervera A., Soehnlein O, Kenne E. Neutrophils in chronic inflammatory diseases. Cell Mol Immunol. 2022;19(2):177-91. doi: 10.1038/s41423-021-00832-3
20. Behm C, Nemec M, Blufstein A, Schubert M, Rausch-Fan X, Andrukhov O, et al. Interleukin-1β induced matrix metalloproteinase expression in human periodontal ligament-derived mesenchymal stromal cells under *in vitro* simulated static orthodontic forces. Int J Mol Sci. 2021;22(3):1027. doi: 10.3390/ijms22031027
21. Cheng R, Wu Z, Li M, Shao M, Hu T. Interleukin-1β is a potential therapeutic target for periodontitis: a narrative review. Int J Oral Sci. 2020;12(1):2. doi: 10.1038/s41368-019-0068-8
22. Geng J, Wang F, Huang Z, Chen X, Wang Y. Perspectives on anti-IL-1 inhibitors as potential therapeutic interventions for severe COVID-19. Cytokine. 2021;143:155544. doi: 10.1016/j.cyto.2021.155544
23. Dmytriieva OO, Belenichev IF, Burlaka BS. Optimisation of the composition of safe dental gel with IL-1β antagonist for the treatment of inflammatory periodontal diseases Zaporozhye medical journal. 2024;26(2):134-43. doi: 10.14739/2310-1210.2024.2.292521
24. Dmytriieva OO, Bielenichev I.F, Samura IB, Salnykov VI, Robota DV. The effect of dental gel with IL-1β antagonist on indicators of nitrosative stress and antioxidant system in rats with experimental chronic generalized periodontitis. Modern medical technology. 2024;16(3):214-9. doi: 10.14739/mmt.2024.3.303351
25. Kiani AK, Pheby D, Henehan G, Brown R, Sieving P, Sykora P, et al. Ethical considerations regarding animal experimentation. J Prev Med Hyg. 2022 Oct 17;63(2 Suppl 3):E255-66. doi: 10.15167/2421-4248/jpmh2022.63.2S3.2768
26. American Veterinary Medical Association. AVMA guidelines for the euthanasia of animals: 2020 edition. Version 2020.0.1. Available from: https://www.avma.org/sites/default/files/2020-02/Guidelines-on-Euthanasia-2020.pdf
27. Parkhomenko D, Belenichev IF, Kuchkovskyi OM, Ryzhenko V. Characteristics of HIF-1A and HSP70 MRNA expression, level, and interleukins in experimental chronic generalized periodontitis. MicroRNA. 2024;13(2):132-9. doi: 10.2174/0122115366264794240327073739
28. McPherson RA, Pincus MR. Henry's clinical diagnosis and management by laboratory methods. 24th ed. Philadelphia, PA: Elsevier – Health Sciences Division; 2021.

29. Belenichev I, Salnykov V, Samura I. The effect of selenase on inflammatory and cytoprotective markers in experimental chronic generalized periodontitis. Biomed Pharmacol J. 2024;17(3):1911-9. doi: 10.13005/bpj/2993
30. Belenichev IF, Aliyeva OG, Popazova OO, Bukhtiyarova NV. Involvement of heat shock proteins HSP_{70} in the mechanisms of endogenous neuroprotection: the prospect of using HSP_{70} modulators. Front Cell Neurosci. 2023;17:1131683. doi: 10.3389/fncel.2023.1131683
31. Belenichev I, Popazova O, Bukhtiyarova N, Savchenko D, Oksenych V, Kamyshnyi O. Modulating nitric oxide: implications for cytotoxicity and cytoprotection. Antioxidants (Basel). 2024;13(5):504. doi: 10.3390/antiox13050504
32. Leira Y, Iglesias-Rey R, Gómez-Lado N, Aguiar P, Sobrino T, D'Aiuto F, et al. Periodontitis and vascular inflammatory biomarkers: an experimental in vivo study in rats. Odontology. 2020;108(2):202-12. doi: 10.1007/s10266-019-00461-3
33. Lee J, Lee JB, Song HY, Son MJ, Li L, Rhyu IC, et al. Diagnostic Models for Screening of periodontitis with inflammatory mediators and microbial profiles in saliva. Diagnostics (Basel). 2020;10(10):820. doi: 10.3390/diagnostics10100820
34. Alayash Z, Baumeister S-E, Holtfreter B, Kocher T, Baurecht H, Ehmke B, et al. Inhibition of tumor necrosis factor receptor 1 and the risk of periodontitis. Front. Immunol. 2023;14:1094175. doi: 10.3389/fimmu.2023.1094175
35. Brodzikowska A, Górski B, Bogusławska-Kapała A. Association between IL-1 gene polymorphisms and stage III grade B periodontitis in Polish population. Int J Environ Res Public Health. 2022;19(22):14687. doi: 10.3390/ijerph192214687
36. Wang RP, Huang J, Chan KW, Leung WK, Goto T, Ho YS, et al. IL-1β and TNF-α play an important role in modulating the risk of periodontitis and Alzheimer's disease. J Neuroinflammation. 2023;20(1):71. doi: 10.1186/s12974-023-02747-4
37. Radzki D, Negri A, Kusiak A, Obuchowski M. Matrix metalloproteinases in the periodontium – vital in tissue turnover and unfortunate in periodontitis. Int J Mol Sci. 2024;25(5):2763. doi: 10.3390/ijms25052763
38. Jansson L, Lundmark A, Modin C, Abadji D, Yucel-Lindberg T. Intra-individual cytokine profile in peri-implantitis and periodontitis: A cross-sectional study. Clin Oral Implants Res. 2021;32(5):559-68. doi: 10.1111/clr.13725
39. Jansson L, Lundmark A, Modin C, Gustafsson A, Yucel-Lindberg T. Levels of matrix metalloproteinase-1 (MMP-1), MMP-2, MMP-3, osteopontin, pentraxin-3, and thymic stromal lymphopoietin in crevicular fluid samples from peri-implantitis, periodontitis, and healthy sites. J Periodontal Res. 2024;00:1-11. doi: 10.1111/jre.13338
40. Bernal-Sánchez KK, Lara-Carrillo E, Velázquez-Enriquez U, Casanova-Rosado JF, Casanova-Rosado AJ, Morales-Valenzuela AA, et al. Clinical and socio-demographic factors associated with dental extractions in a clinical sample. Braz Dent J. 2023;34(6):121-9. doi: 10.1590/0103-6440202305355
41. Furuse N, Takai H, Ogata Y. Effects of initial periodontal therapy on heat shock protein 70 levels in gingival crevicular fluid from periodontitis patients. J Clin Med. 2020;9(10):3072. doi: 10.3390/jcm9103072
42. Li M, Huang S, Zhang Y, Song Z, Fu H, Lin Z, et al. Regulation of the unfolded protein response transducer IRE1α by SERPINH1 aggravates periodontitis with diabetes mellitus via prolonged ER stress. Cell Signal. 2022;91:110241. doi: 10.1016/j.cellsig.2022.110241
43. Takai H, Furuse N, Ogata Y. Anti-heat shock protein 70 levels in gingival crevicular fluid of Japanese patients with chronic periodontitis. J Oral Sci. 2020;62(3):281-4. doi: 10.2334/josnusd.19-0159
44. Kzar WA, Abbas RF. Association of Polymorphism with Periodontitis and Salivary Levels of Hypoxia-Inducible Factor-1α. Eur J Dent. 2025;19(1):133-43. doi: 10.1055/s-0044-1785530
45. Taylor CT, Scholz CC. The effect of HIF on metabolism and immunity. Nat Rev Nephrol. 2022;18(9):573-87. doi: 10.1038/s41581-022-00587-8
46. Shan C, Xia Y, Wu Z, Zhao J. HIF-1α and periodontitis: Novel insights linking host-environment interplay to periodontal phenotypes. Prog Biophys Mol Biol. 2023;184:50-78. doi: 10.1016/j.pbiomolbio.2023
47. Yan K, Wu C, Ye Y, Li L, Wang X, He W, et al. A20 inhibits osteoclastogenesis via TRAF6-dependent autophagy in human periodontal ligament cells under hypoxia. Cell Prolif. 2020;53(3):e12778. doi: 10.1111/cpr.12778
48. Lin XJ, Yuan Q, Zhou J, Dong YL, Sunchuri D, Guo ZL. Cellular senescence: A new perspective on the suppression of periodontitis (Review). Mol Med Rep. 2024;30(6):238. doi: 10.3892/mmr.2024.13362
49. Bishop EL, Gudgeon N and Dimeloe S. Control of T Cell Metabolism by Cytokines and Hormones. Front. Immunol. 2021;12:653605. doi: 10.3389/fimmu.2021.653605
50. Chaparro A, Lozano M, Gaedechens D, López C, Albers D, Hernández M, et al. Exploring the expression of pro-inflammatory and hypoxia-related microRNA-20a, microRNA-30e, and microRNA-93 in periodontitis and gingival mesenchymal stem cells under hypoxia. Int J Mol Sci. 2022;23(18):10310. doi: 10.3390/ijms231810310
51. Kazmi S, Salehi-Pourmehr H, Sadigh-Eteghad S, Farhoudi M. The efficacy and safety of interleukin-1 receptor antagonist in stroke patients: A systematic review. J Clin Neurosci. 2024;120:120-8. doi: 10.1016/j.jocn.2024.01.009
52. Xu J, Zheng Y, Zhao Y, Zhang Y, Li H, Zhang A, et al. Succinate/IL-1β signaling axis promotes the inflammatory progression of endothelial and exacerbates atherosclerosis. Front Immunol. 2022;13:817572. doi: 10.3389/fimmu.2022.817572
53. Lacher SE, Skon-Hegg C, Ruis BL, Krznarich J, Slattery M. An antioxidant response element regulates the HIF1α axis in breast cancer cells. Free Radic Biol Med. 2023;204:243-251. doi: 10.1016/j.freeradbiomed.2023.05.003