ураженнями жувальних поверхонь зубів. Отже, ураження пульпи зуба при карієсі залежить не лише від ступеня каріозного процесу, але й від його локалізації, що потрібно враховувати при виборі оптимального методу лікування карієсу, що також попередить розвиток його ускладнень.

Ключові слова: експериментальний карієс, пульпа, морфологія.

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глубокими поражениями жевательных поверхностей зубов. Итак, поражения пульпы зуба при кариесе зависит не только от степени кариозного процесса, но и от его локализации, что нужно учитывать при выборе оптимального метода лечения кариеса, также предупредит развитие его осложнений.

Ключевые слова: экспериментальный кариес, пульпа, морфология.

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INFLUENCE OF INHIBITORS OF TRANSCRIPTION FACTOR KAPPA B ON DEPOLIMERIZATION OF BIOPOLYMERS IN PERIODONTAL CONNECTIVE TISSUE UNDER SYSTEMIC INFLAMMATORY RESPONSE IN RATS

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The article presents the results obtained in the experiment on 40 white rats aimed at investigating the influence of ammonium pyrrolidine dithiocarbamate (APDTC), an inhibitor of the nuclear translocation of the transcription factor κB (NF-κB), on the depolymerization of collagen, proteoglycans and sialoglycoproteins in extracellular matrix of soft and osseous tissues under modeled systemic inflammatory response (SIR). The latter was induced by intraperitoneal administration of lipopolysaccharide Salmonella typhi (pyrogenalum) in a dose of 0.4 μg/kg of weight 3 times during the first week, and once a week for the following 7 weeks. It has been shown the SIR simulation is accompanied by activation of the collagenolysis as well as depolymerization of proteoglycans and sialoglycoproteins in the tissues of the gum, periodontal ligament and alveolar process of the jaws as evidenced by a considerable increase in the concentration of free hydroxyproline, glycosaminoglycans and N-acetylneuraminic acid. The application of APDTC during SIR significantly reduces the depolymerization of collagen, proteoglycans and sialoglycoproteins in soft and bone periodontal tissues, and limits the degree of resorption of the jaw alveolar process. This allows us to conclude the use of this compound during SIR is an effective means to correct disruption the periodontal connective tissue in rats.

Key words: nuclear factor kappa B, systemic inflammatory response, connective tissue biopolymers, collagen, proteoglycans, glycoproteins, periodontium.

The work is a part of the research project "The role of reactive oxygen species, nitric oxide system and transcriptional factors in the mechanisms of pathological systemogenesis", state registration No. 0114U004941.

Depolymerization of periodontal connective tissue biopolymers is considered to be an important link in the pathogenesis of chronic periodontitis under the influence of general (emotional and pain stress) [14] and local factors [10] that impedes its treatment by means of regenerative therapy [5]. Previously, we have shown that the modeling of lipopolysaccharide (LPS)-induced systemic inflammatory response (SIR) in periodontal tissues is accompanied by an increase in production of reactive oxygen and nitrogen species by mitochondria, endoplasmic reticulum and NO synthase (NOS), as well as NADPN-oxidase of white blood cells. The progression of oxidative-nitrosative stress results in the collagenolysis and the depolymerization of proteoglycans and sialoglycoproteins, both in the connective tissue of the gums and the periodontal ligament and in the bone tissue of the alveolar process [1]. It is noteworthy that most of the activators of NF-κB as bacterial LPS, proinflammatory cytokines, E2 prostaglandins, stress, viruses, etc. are involved in the pathogenesis of chronic periodontitis [3].

However, the relevant literature presents contradictory information regarding the effect of NF-κB on connective tissue status during inflammation [2, 6]. Solving this problem is of exceptional importance in searching new approaches towards the pathogenetic therapy of inflammatory and dystrophic periodontal diseases.

The purpose of the study was to investigate the effect of ammonium pyrrolidine dithiocarbamate (APDTC), an inhibitor of the nuclear translocation of the transcription factor κB (NF-κB), on the depolymerization of collagen, proteoglycans and sialoglycoproteins in extracellular matrix of soft and osseous tissues in periodontium of rats under modeled systemic inflammatory response induced with LPS Salmonella typhi.

Materials and methods. The series of the experiment were performed on 30 white male Wistar rats weighing 180-220 g, which were divided into 3 groups (with 10 animals in each group): the 1st group included intact animals, the 2nd group included rats, which were subjected to the systemic administration of LPS (pyrogen, Medgamal, Russia), and the 3rd group consisted of the animals, which received APDTC (manufactured by Sigma-Aldrich, Inc., USA) intraperitoneally in a dose of 76 mg/kg 3 times a week,

starting with the 30th day of the LPS experiment [12]. The latter was administered in a dose of 0.4 μg/kg of body weight 3 times through the 1st week, and then for the next 7 weeks once a week [15]. The animals were decapitated under light ether anesthesia, following the principles of biomedical ethics.

The level of collagenolysis was assessed by the content of free hydroxyproline (FHP) [13]. The level of depolymerization of proteoglycans and sialoglycoproteins was evaluated by determining their monomers, glycosaminoglycans (GAGs) [11] and N-acetylneuraminic acid (NANA) [9], respectively. Using the light microscope with an eyepiece micrometer, we measured the distance from the edge of the dental alveolar socket to the lower edge of the crown of the third molar (L_0) and the distance from the edge of the dental alveolar socket to the upper edge of the dental crown (L_1), followed by the calculation of the molar root exposure coefficient (C) using the formula $(C) = L_0 / L_1$.

Statistical calculations were performed using "StatisticSoft 6.0". To check the distribution for normality, the calculation of the Shapiro-Wilk test was applied. If the series corresponded to the normal distribution, then the Student's t test for independent samples was used to compare them. When the data were not subject to normal distribution, statistical processing was performed using a non-parametric method, the Mann-Whitney test.

Results of the study and their discussion. The LPS administration led to a significant increase in the concentration of collagen monomers, proteoglycans and sialoglycoproteins of the connective tissue of the gums and periodontal ligament (table 1). FHP content increased by 66.2% ($p<0.01$), GAGs by 66.8% ($p<0.05$), and NANA by 62.9% ($p<0.001$) that indicates the activation of the processes of collagenolysis and depolymerization of proteoglycans and glycoproteins in soft tissues of periodontium under this condition.

Table 1

Effect of APDTC on the indices of biopolymer depolymerization in connective tissues of gingival and periodontal ligament under SIR conditions (M±m, n = 30)

Experimental groups	FHP, μmol /g	GAGs, μmol /g	NANA, μmol /g
Intact animals	4.08±0.48	1.93±0.34	4.56±0.17
Systemic LPS administration	6.78±0.35 *	3.22±0.34 *	7.43±0.33 *
Applying APDTC under systemic LPS administration	4.89±0.30 **	2.03±0.26 **	5.67±0.17 */**

Notes (in Table 1-2): * - p <0.05 compared with the results of the intact group, ** - p <0.05 compared with the results of the second group.

The APDTC administration reduced the FHP concentration by 27.9% ($p<0.01$), GAGs by 37.0% ($p<0.05$), and NANA by 23.7% ($p<0, 01$) in the soft tissues of periodontium compared to the values of the 2nd group.

Systemic LPS administration also resulted in the marked changes in the biopolymer composition of extracellular matrix in bone tissues of the alveolar process (table 2): the FHP content increased by 69.9% ($p <0.001$), the GAGs increased by 72.4% ($p<0.02$), and NANA by 115.0% ($p<0.01$).

Using APDTC led to the lowering of FHP concentration by 29.2% ($p<0.001$), GAGs by 42.3% ($p<0.01$), and NANA by 50.3% ($p<0.01$) compared with the values in the 2nd group.

Table 2

Effect of APDTC on the indices of depolymerization of periodontal bone biopolymers and the molar root exposure coefficient (C) under SIR condition (M±m, n = 30)

Experimental groups	FHP, μmol /g	GAGs, μmol /g	NANA, μmol /g	C
Intact animals	3.06±0.28	1.70±0.30	2.01±0.35	25.0±1.4
Systemic LPS administration	5.20±0.19 *	2.93±0.22 *	4.33±0.37 *	37.5±2.2 *
Using APDTC under systemic LPS administration	3.68±0.22 **	1.69±0.16 **	2.15±0.32 **	27.8±1.6 **

As it has been demonstrated previously, NF-κB activation enhances the expression of collagenase 3 (matrix metalloproteinase 13) genes [8]. The activation of NF-κB has been found to be an important link in the pathogenesis of free radical lesions of periodontium, collagenolysis and depolymerization of proteoglycans of its connective tissue in experimental metabolic syndrome, where SIR is an inseparable component. And the administration of a nuclear translocation inhibitor NF-κB 4-methyl-N- (3-phenylpropyl) benzene-1, 2-diamine under these conditions lowers the amount of FHP and GAGs in periodontium tissues [7].

According to our data obtained, depolymerization of periodontal connective tissue biopolymers caused the changes in the dental root exposure coefficient (C), characterizes the intensity of the alveolar process resorption (see Table 2). Under SIR conditions, the C value increased by 50.0% ($p<0.01$). APDTC administration decreased this index by 25.9% ($p<0.01$).

The study has revealed that in the osseous tissues of periodontium, NF-κB-dependent processes associated with NF-κB receptor activator (RANK), its ligand (RANKL), and an erroneous receptor, osteoprotegerin, play an important role in regulating osteoclast resorptive activity [4]. This can be considered as an additional mechanism of destructive changes in osseous tissue of periodontium.

Conclusion

The use of ammonium pyrrolidine dithiocarbamate (APDTC), an inhibitor of the nuclear translocation of the transcription factor κB (NF-κB), under the conditions of systemic administration of Salmonella typhi lipopolysaccharide is an effective means in correction of periodontium connective tissue disorganization of the periodontium in rats, reduces depolymerization of collagen, proteoglicans and sialoglycoproteins in soft and osseous tissues of periodontium, as well as decreases alveolar process resorption.

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Реферати

ВПЛИВ ІНГІБІТОРА ТРАНСКРИПЦІЙНОГО ФАКТОРА КАППА В НА ДЕПОЛІМЕРИЗАЦІЮ БІОПОЛІМЕРІВ СПОЛУЧНОЇ ТКАНИНИ ПАРОДОНТА ЩУРІВ ЗА УМОВ СИСТЕМНОЇ ЗАПАЛЬНОЇ ВІДПОВІДІ

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В експерименті на 40 білих щурах досліджено вплив інгібітора ядерної транслокації транскрипційного фактора каппа В (NF-κB) амонію піролідиндітіокарбамату (APDTC – ammonium pyrrolidinedithiocarbamate) на деполімеризацію колагену, протеогліканів та сіалоглікопротеїнів позаклітинного матриксу м'яких і кісткової тканин пародонта за умов експериментальної системної запальної відповіді (СЗВ). Останню моделювали шляхом внутрішньочеревного введення ліпополісахариду Salmonella typhi (пірогенал) у дозі 0,4 мкг/кг маси протягом 1-го тижня 3 рази, протягом наступних 7-ми тижнів – 1 раз у тиждень. Показано, що моделювання СЗВ супроводжується активацією процесів колагенолізу, а також деполімеризації протеогліканів та сіалоглікопротеїнів у тканинах ясен, періодонтальної

ВЛИЯНИЕ ИНГИБИТОРА ТРАНСКРИПЦИОННОГО ФАКТОРА КАППА В НА ДЕПОЛИМЕРИЗАЦИЮ БИОПОЛИМЕРОВ СОЕДИНИТЕЛЬНОЙ ТКАНИ ПАРОДОНТА КРЫС ПРИ СИСТЕМНОМ ВОСПАЛИТЕЛЬНОМ ОТВЕТЕ

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В эксперименте на 40 белых крысах исследовано влияние ингибитора ядерной транслокации транскрипционного фактора каппа В (NF-κB) аммония пирролидиндитиокарбамата (APDTC - ammonium pyrrolidinedithiocarbamate) на деполимеризацию коллагена, протеогликанов и сиалогликопротеинов внеклеточного матрикса мягких и костной тканей пародонта в условиях экспериментального системного воспалительного ответа (СВО). Последний моделировали путем внутрибрюшного введения липополисахарида Salmonella typhi (пирогенала) в дозе 0,4 мкг/кг в течение 1-й недели 3 раза, в течение следующих 7-ми недель - 1 раз в неделю. Показано, что моделирование СВО сопровождается активацией процессов коллагенолиза и деполимеризации протеогликанов и сиалогликопротеинов в тканях десны, периодонтальной

зв'язки та альвеолярного відростку щелеп, що підтверджується суттєвим збільшенням концентрації вільного оксипроліну, глікозаміногліканів та N-ацетилнейрамінової кислоти. Застосування APDTC за умов СЗВ істотно зменшує у м'яких і кістковій тканинах пародонта деполімеризацію колагену, протеогліканів та сіалоглікопротеїнів, обмежує ступінь резорбції альвеолярного відростка щелеп. Зроблено висновок, що застосування цієї сполуки при СЗВ є ефективним засобом корекції дезорганізації сполучної тканини пародонта щурів.

Ключові слова: ядерний фактор каппа В, системна запальна відповідь, біополімери сполучної тканини, колаген, протеоглікани, глікопротеїни, пародонт.

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связки и альвеолярного отростка челюстей, что подтверждается существенным увеличением концентрации свободного оксипролина, гликозаминогликанов и N-ацетилнейраминовой кислоты. Применение APDTC при СВО существенно уменьшает в мягких и костной тканях пародонта деполимеризацию коллагена, протеогликанов и сиалогликопротеинов, ограничивает степень резорбции альвеолярного отростка челюстей. Сделан вывод, что применение этого соединения при СЗВ является эффективным средством коррекции дезорганизации соединительной ткани пародонта крыс.

Ключевые слова: ядерный фактор каппа В, системный воспалительный ответ, биополимеры соединительной ткани, коллаген, протеогликаны, гликопротеины, пародонт.

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EFFECTIVENESS OF THE PLATELET-RICH PLASMA APPLICATION AT DIFFERENT SIMULATION PERIODS OF DEGENERATIVE DISC DISEASE IN RATS

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The article describes the study of the platelet-rich plasma application effectiveness for tissue regeneration of intervertebral discs in rats with simulated degenerative disk disease of the caudal vertebrae for 60 and 90 days. The experiment involved 80 rats, which were divided into four groups: Group I – rats with a simulated pathology without correction for 60 days, Group II – rats with a simulated pathology without correction for 90 days, Group III – rats with a pathology and its correction for 60 days, Group IV – rats with a pathology and its correction for 90 days. The described morphological changes in the invertebral disk tissue suggest that the application of platelet-rich plasma in the pathology simulation for 60 days leads to the degenerative process inhibition and restores the invertebral disk structure. The application of platelet-rich plasma for 90-day pathology simulation is less effective.

Key words: degenerative disk disease, intervertebral disc, fibrous ring, nucleus pulposus, platelet-rich plasma.

The work is a fragment of the research project "To develop and substantiate methods of correction of liver fibrous changes at chronic hepatitis and cirrhosis", state registration No. 0116U008927.

Spinal diseases take the second place among the causes of temporary disability, and, eventually, often lead to permanent disability [2]. In 90% of cases, spinal diseases are based on degenerative disk disease (DDD) [3]. At the age of 30 years, signs of DDD are detected in 57% of cases, and at the age of 60 years and older – in 100% [3]. In Ukraine, annually about 1 million patients with DDD seek medical help, more than 16 thousand of them subsequently become disabled. Thus, this pathology is of great importance not only for the medical, but also for the socio-economic sphere.

Currently, the study of the effectiveness of tissue therapy application to regenerate the intervertebral disc (IVD) structure after DDD is a promising area. The literature data indicate the effectiveness of using some growth factors for the intervertebral disk morphology regeneration after DDD in the experiment [9]. The use of platelet-rich plasma (PRP) on IVD tissues in laboratory rats after the acute IVD injury in the early stages of the DDD formation has a positive effect [7, 8]. However, often DDD-associated visits to medical institutions for qualified care occur after the first clinical manifestations, when the pathological process is already expressed.

The purpose of work was to study the efficiency of using PRP in the DDD simulation for 60 and 90 days.

Materials and methods. The study was carried out on Whistar rats of both sexes aged 4-6 months (80 animals), which were divided into four groups: Group I – animals with DDD for 60 days, without correction; Group II – animals with DDD for 90 days, without correction; Group III – animals with DDD for 60 days, which were injected with PRP; Group IV – animals with DDD for 90 days, which were injected with PRP. A separate group (10 animals) studied as intact animals. Animal preparation, anesthesia, surgery, postoperative care and terminal sacrifice were carried out in accordance with the Law of Ukraine "On protection animals from brutal treatment" No. 27, Art. 230 of 2006, and the general principles of ethics of experiments on animals and the Code of Ethics for Ukrainian Scientists.