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LIPID PEROXIDATION AND ANTIOXIDANT PROTECTION IN PERIODONTAL TISSUES UNDER THE ACTION OF LOCAL PATHOGENIC FACTOR ON GUMS IN RATS EXPOSED TO MODELED SYSTEMIC INFLAMMATORY RESPONSE*

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The purpose of the work was to study the state of lipid peroxidation (LPO) and antioxidant system in periodontal tissues under the influence of the local pathogenic factor on the gums in rats against modeled systemic inflammatory response (SIR) reproduction. The study was carried out on 40 white male rats of the Wistar line weighing 180-220 g, divided into 4 groups: the 1st included intact animals, the 2nd was made up by animals subjected to SIR modeling, the 3rd included animals, which were subjected to modeled acute gingivitis by applying 5% sodium hydroxide (NaOH) solution onto their gums, and the 4th group involved rats with acute gingivitis induced by 5% NaOH solution against modeled SIR. SIR was induced by intraperitoneal administration of lipopolysaccharide Salmonella typhi (pyrogenalum) in a dose that stimulated rise in temperature by 1.5 °C. During the following seven weeks of the experiment, rats were given 4 MPD / kg of body weight once a week. To simulate the action of local pathogenic factor affecting the gums we used the modeled acute gingivitis. For this purpose we irrigated the rats' gums with 5% NaOH solution through 10 s. The simulation of SIR is accompanied by the development of decompensated LPO in periodontal tissues, by the decrease in their antioxidant potential as well as the activity of superoxide dismutase and catalase. The application of 5% NaOH solution onto the gums is accompanied by the development of compensated LPO in periodontal tissues with an increase in the activity of superoxide dismutase and catalase. The application of 5% NaOH solution onto the gums against modeled SIR causes an increase in the production of by-products of LPO in periodontal tissues and reduced their antioxidant potential, as well as the activity of superoxide dismutase and catalase compared to separate simulations of SIR and acute gingivitis.

Key words: signal systemic inflammatory response, acute gingivitis, lipid peroxidation, antioxidant system, periodontium.

Метою роботи було з'ясування стану пероксидного окиснення ліпідів (ПОЛ) та антиоксидантної системи у тканинах пародонта щурів за умов дії місцевого патогенного чинника на ясна щурів на тлі відтворення системної запальної відповіді (СЗВ). Дослідження були проведені на 40 білих щурах-самцях лінії Вістар масою 180-220 г, розподілених на 4 групи: 1-ша – інтактні тварини, 2-га – після відтворення СЗВ, 3-тя – після аплікації на ясна 5% розчину гідроксиду натрію (NaOH), 4-та – після аплікації на ясна 5% розчину NaOH на тлі відтвореної СЗВ. Останню відтворювали шляхом внутрішньоочеревинного введення ліпополісахариду Salmonella typhi (пірогенал) у дозі, яка сприяла у щурів підвищенню температури на 1,5°С. Для відтворення дії місцевого патогенного чинника на ясна використовували модель гострого гінгівіту. Щурам на ясна наносили 5% розчин NaOH шляхом зрошення протягом 10 с. Відтворення СЗВ супроводжується розвитком декомпенсованого ПОЛ у м'яких тканинах пародонта, зниженням у них антиоксидантного потенціалу, активності супероксиддисмутази та каталази. Аплікація на ясна 5% розчину NaOH супроводжується розвитком компенсованого ПОЛ у тканинах пародонта зі збільшенням активності супероксиддисмутази та каталази. Нанесення на ясна 5% розчину NaOH на тлі відтвореної СЗВ викликає збільшення вторинних продуктів ПОЛ у гомогенаті м'яких тканин пародонта та зменшення у них антиоксидантного потенціалу, активності супероксиддисмутази та каталази порівняно з окремим відтворенням СЗВ та гострого гінгівіту.

Ключові слова: системна запальна відповідь, гострий гінгівіт, пероксидне окиснення ліпідів, антиоксидантна система, пародонт.

Introduction

Numerous literary reports have convincingly demonstrated the role of derangements of oxidative metabolism in the pathogenesis of stress, toxic, infectious and neurodystrophic affection of periodontal lesions [2, 3].

We have shown an increase in the production of superoxide anion radicals in periodontium tissues by mitochondrial and NADPH-dependent electron transport chains of microsomes and nitric oxide synthase (NOS) as well as by leukocyte NADPH oxidase in systemic inflammatory response (SIR). There has been revealed interfering the mechanism of autoregulation of the physiological nitric oxide concentration in the periodontium that leads to simultaneous increase in nitric oxide formation through the NOS and nitrate / nitrite reductase mechanisms, resulting in the development of oxidative-nitrosative stress with rising peroxynitrite concentration [16].

Reactive oxygen and nitrogen species (ROS / RNS) are known to initiate lipid peroxidation (LPO), which results in the disintegration in the connective tissues of periodontium and the inhibition of collagen synthesis [12].

Thus, free radical processes are a pathogenetic chain that provides the connection between systemic somatic pathology and the state of the oral organs and tissues. Most of manifestations relating to the complex of metabolic disorders caused by systemic inflammation are closely interwoven with the leading pathogenetic links in the development and progression of chronic periodontitis [4, 10, 15].

However, the state of metabolic processes in periodontium under the conditions of combined action of general (systemic inflammation) and local pathogenic factors still remains unexplored.

The purpose of the work was to study the state of LPO and antioxidant system in periodontal tissues of rats

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under the influence of the local pathogenic factor (5% sodium hydroxide solution) on the gums in rats against modeled SIR reproduction.

Materials and methods

The study was carried out on 40 white male rats of the Wistar line weighing 180-220 g, divided into 4 groups: the 1st included intact animals, the 2nd was made up by animals subjected to SIR modeling, the 3rd included animals, which were subjected to modeled acute gingivitis by applying 5% sodium hydroxide (NaOH) solution onto their gums, and the 4th group involved rats with acute gingivitis induced by 5% NaOH solution against modeled SIR.

SIR was induced by intraperitoneal administration of lipopolysaccharide (LPS) *Salmonella typhi* (pyrogenalum) in a dose that stimulated rise in temperature by 1.5°C according to the scheme [16]: during the first week, 4 minimum pyrogenic doses (MPD) of 0.4 μg/kg of rat mass were administered 3 times a week. During the following seven weeks of the experiment, rats were given 4 MPD/kg of body weight once a week.

To simulate the action of local pathogenic factor affecting the gums we used the modeled acute gingivitis [6]. For this purpose we irrigated the rats' gums with 5% NaOH solution through 10 s.

The research was guided by the principles of biomedical ethics. The animals were decapitated with ethereal anesthesia in three days after the simulation of the experimental models had been stopped. Soft tissues of periodontium were the objects of the study.

The level of LPO in the tissues was evaluated by the formation of a stained trimethine complex during the reaction of tiobarbituric acid (TBA) and TBA-active products before and after 1.5-hour incubation of homogenate in the prooxidant iron-ascorbate buffer solution [13]. The activity of the antioxidant system was assessed by increasing in the concentration of TBA active products during 1.5 hour incubation in iron-ascorbate buffer solution, as well as by the activity of antioxidant enzymes – superoxide dismutase (SOD) and catalase [13].

The findings obtained were statistically processed. To verify the normality distribution, the calculation of the Shapiro-Wilk criterion was applied. If they corresponded to the normal distribution, then the Student's t-test was used to compare independent samples. When the results ranges were not subject to normal distribution, statistical processing was performed using a nonparametric method, the Mann-Whitney test. Statistical calculations were performed using the "StatisticSoft 6.0" program.

Results and discussion

SIR modeling led to significant increasing of concentration of compounds reacting with TBA before and after incubation of homogenate in the prooxidant iron-ascorbate buffer solution – by 93.8% ($p<0.01$) and 97.4% ($p<0.001$) respectively (See table). The increment in these compounds for incubation time nearly doubled (in 2.04 times, $p<0.01$) that is the evidence of considerable decrease in antioxidant potential and the development of decompensated LPO reduction in the periodontal soft tissues. This is also confirmed by a decrease in the activity of SOD and catalase – by 34.8% ($p<0.01$) and 46.4% ($p<0.001$) respectively.

Table

Indicators of LPO and antioxidant system in periodontal tissues of rats exposed to the action of the local pathogenic factor against modeled SIR (M±m, n=40)

Groups of the animals studied	Concentration of compounds reacting with thiobarbituric acid, μmol/kg			Antioxidant enzymes activity	
	Before incubation	After incubation	Increment for incubation time	SOD, act. un.	Catalase, μkat/g
Intact animals	20.9±3.8	34.3±2.5	13.4±1.8	0.23±0.02	0.28±0.02
Animals with SIR	40.5±3.0 *	67.7±4.4 *	27.3±2.8 *	0.15±0.01 *	0.15±0.01 *
Animals after NaOH application	34.2±3.0 *	51.7±1.7 *	17.5±3.4	0.32±0.02 *	0.36±0.01 *
Animals with SIR after NaOH application	54.6±3.2 */**/***	86.2±4.7 */**/***	31.5±4.1 */***	0.08±0.01 */**/***	0.12±0.02 */***

*Note: * – p<0.05 compared with values of intact rats,*
*** – p<0.05 compared with values of the 2nd group,*
**** – p<0.05 compared with values of the 3rd group.*

The application of 5% NaOH solution onto the gums was accompanied with the increase in the concentration of compounds reacting with TBA products before and after incubation of homogenate in the prooxidant iron-ascorbate buffer solution – by 63.6% ($p<0.05$) and 50.7% ($p<0.001$) respectively. The increment in these compounds through incubation time was unchanged. This suggests the development of compensated LPO in the soft periodontal tissues. Under this condition the activity of both SOD and catalase was growing by 39.1% ($p<0.02$) and 28.6% ($p<0.01$) respectively.

The application of 5% NaOH solution onto the gums against SIR led to significant increasing of concentration of compounds reacting with TBA products before incubation of homogenate in the prooxidant iron-ascorbate buffer solution that surpassed the results in the 2nd and the 3rd groups by 34.8% ($p<0.02$) and 59.6% ($p<0.01$), respectively. The concentration of compounds reacting with TBA products after incubation grew too by 27.3% ($p<0.05$) and 66.7% ($p<0.001$) compared with the results in the 2nd and the 3rd groups. The increment of these compounds for incubation time by 80.0% ($p<0.05$) surpassed the relevant result in the 3rd group. This points our much higher LPO level in periodontal tissues. This is also proven by the lowering SOD activity by 46.7% ($p<0.01$) and 75.0% ($p<0.001$) compared with the findings in the 2nd and the 3rd groups, as well as by decreased catalase activity by 66.7% ($p<0.001$) compared with the findings of the 3rd group.

In recent years, the development of SIR has been associated with the permanent activation of certain transcription factors (NF-κB, AP-1) [5, 11]. NF-κB activation is also an important causative factor of the pathogenesis of free-radical pathology of periodontium [1, 8] and disintegration of its connective tissues [7]. The consequence is the expression of genes of inflammatory cytokines, inducible nitric oxide synthase, metalloproteinases, cellular molecules adhesion, cyclooxygenase-2, etc., capable of

inducing ROS / RNS generation [14]. The development of oxidative / nitrosative stress in periodontium tissues has also been evidenced by our previous studies [16].

Thus, the preconditioning of the body by the introduction of LPS that is accompanied by the SIR development creates the conditions for more considerable exhaustion of the antioxidant system in the periodontal tissues under local affection with chemical agent (5% NaOH solution). This causes even greater activation of free radical processes, with the formation of a significant concentration of secondary LPO products. Under these conditions, the antioxidant system ceases to respond adequately, resulting in the development of the periodontal involution, the formation of conditions for virtually unobstructed spread of the inflammatory process, destruction of collagen fibers and resorption of the alveolar process of the jaws [7, 9, 12].

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Conclusions

1. The simulation of systemic inflammatory response is accompanied by the development of decompensated lipid peroxidation in periodontal tissues, by the decrease in their antioxidant potential as well as the activity of superoxide dismutase and catalase.

2. The application of 5% NaOH solution onto the gums (model of acute gingivitis) is accompanied by the development of compensated lipid peroxidation in periodontal tissues with an increase in the activity of superoxide dismutase and catalase.

3. The application of 5% NaOH solution onto the gums against modeled systemic inflammatory response causes an increase in the production of by-products of lipid peroxidation in periodontal tissues and reduced their antioxidant potential, as well as the activity of superoxide dismutase and catalase compared to separate simulations of SIR and acute gingivitis.

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