Effects of flavonol quercetin on activity of lipid peroxide oxidation in experimental bacterial-immune periodontitis

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Abstract: The article presents the results of lipid peroxidation activity in blood serum, which were assessed on the base changes in the level of oxidative modification of proteins, TBA-active products, and total amount of nitrogen (II) oxide metabolites ($\text{NO}_2^- + \text{NO}_3^-$). Indices were determined on the 14th day of development in experimental periodontitis both without correction, and in condition treatment with a water-soluble flavonol quercetin (corvutin). The treated animals take intramuscular injections of corvutin in a dose 100 mg/kg weight for 7 days. For further testing, blood serum was selected. The results were statistically analyzed using parametric and non-parametric indices. In this investigation, characterized dynamics of changes in the indices of oxidative processes activity were revealed both during the period of formation and the course of periodontitis. In particular, it was found that, for the period of the most expressed inflammatory reaction in the periodontal complex, intensive increase in the level of reactive forms of oxygen. The treatment with flavonol quercetin for 7 days resulted in stabilization of free radical processes and the suppression of the inflammatory reaction.

Keywords: periodontitis, quercetin, TBA-active products, nitrogen (II) oxide, oxidative modification of proteins

Introduction

Improvement and innovation of new methods in periodontitis treatment are one of the essential tasks in the field of modern dentistry [1, 2]. Inflammatory process in the periodontal complex tissues is most common among diseases of the maxillofacial site [3, 4]. The leading role inhibits oxidation processes (as initiating factors) and pathogenic microflora, their associations, in particular *Staphylococcus* and *Streptococcus*, which result in reduced resistance of the oral tissues to infectious agents [5, 6]. Activation of lipid peroxidation (LPO) is one of the triggering mechanisms of stress damage with the disorder of the cellular metabolism, which is primarily associated with the damage to cellular and subcellular membranes [7–10]. To neutralize excess LPO and maintain a steady intracellular concentration of free radicals and

lipoperoxidum, there is an antioxidant defense system in the body [11, 12]. In recent years, much attention has been paid to the substances of plant origin polyphenols as antioxidants; in particular, it is known that the flavonol quercetin has an antioxidant, anti-ischemic, membrane-stabilizing, and immunomodulating effect [13, 14]. The active substance of this drug is a complex of quercetin with polyvinylpyrrolidone. Quercetin is an antioxidant that effectively regulates energetic metabolism in the myocardium, reduces its requirement in oxygen, stabilizes the cytoplasmic membrane, and causes anti-arrhythmic and anabolic effects. The drug is able to reduce the concentration of free radicals and toxic peroxidation products [15].

The purpose of the research was to determinate the pathogenetic role of LPO and effectiveness of flavonol quercetin in the development and course of experimental bacterial-immune periodontitis.

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Materials and Methods

The investigation was performed using white clinically healthy rats weighing 150–200 g in the conditions of vivarium. The experiments were carried out in accordance with the general rules and provisions of the European Convention for the Protection of Vertebrate animals used for research and other scientific purposes (Strasbourg, 1986), the General Ethical Principles of animal experimentation (Kiev, 2001). The experimental animals were randomly selected and divided into three groups: (1) intact animals, control ($n = 10$); (2) animals with experimental periodontitis on the 14th day of the study ($n = 8$); and (3) animals with experimental periodontitis on the 14th day of the study, which was introduced with quercetin (corvutin) ($n = 8$). Experimental periodontitis in the experimental rats was made to cause using injection of a mixture of microorganisms diluted with egg protein into the tissue of the periodontal complex [16]. To enhance the immune response, a complete Freund's adjuvant was injected into the rat's paw at the same time. The experimental rats of the third group were treated with water-soluble quercetin (corvutin) in a dose 100 mg/kg of animal weight intramuscularly for 7 days (from the 7th to the 14th day). On the 14th day, the experimental rats were sacrificed by bleeding under thiopental anesthesia. For further testing, blood serum was selected. The level of oxidative modification of proteins (OMPs; neutral and basic characters), the content of TBA-active products, and the total amount of metabolites of nitrogen (II) oxide were determined in the serum. The results were statistically analyzed using parametric and non-parametric indices [17] in the Excel software (Microsoft, USA) and STATISTICA 10.0 (StatSoft, USA). The reliability of the differences in values between independent quantitative values was determined using a normal distribution according to the Mann–Whitney U criterion [18].

Results

According to the results of the research, it was established that the contents of OMP of neutral proteins (OMP₃₇₀) increased by 1.35 times ($p < 0.01$) on the 14th day of the experiment as compared to intact group of rats (Table I).

The treatment of experimental rats with antioxidant quercetin, in a dose of 100 mg/kg for 7 days intramuscularly, led to a decrease in the level of aldehyde and ketone derivatives in the blood serum by 1.07 times ($p < 0.05$) as compared to this group of rats with experimental periodontitis on the 14th day, without correction with flavonol, which contributed to decrease of oxidative stress (Fig. 1). However, indices were higher than the control group (by 1.27 times; $p < 0.01$).

The OMP of the main character (OMP₄₃₀) was increased in blood serum on the 14th day of the study (by 1.41 times; $p < 0.01$), as compared to the control group (Fig. 1). In comparison of this indicator with that was found in the rats on the 14th day without correction, it was decreased by 1.15 times ($p < 0.01$). However, its

Fig. 1. Changes of OMP₃₇₀ and OMP₄₃₀ indices in the rat's serum on the 14th day of the experimental periodontitis and with correction by quercetin (% of control). *Significant differences in relation to intact rats ($p < 0.01$). #Significant differences in relation to rats with periodontitis on the 14th day of the experiment without correction ($p < 0.01$). •Significant differences in relation to the rats with periodontitis on the 14th day of the experiment with correction ($p < 0.05$)

Table I The content of OMP₃₇₀ and OMP₄₃₀ in the rat's serum with experimental bacterial-immune periodontitis and correction by quercetin ($M \pm m$)

Experimental form	Control and intact animals	Animals with experimental periodontitis	
		Without correction	After correction with quercetin
Experiment duration (days)	–	14	14
Number of rats	10	8	8
OMP ₃₇₀ (mol/L)	0.48 ± 0.02	0.65 ± 0.01 $p_1 < 0.01$	0.61 ± 0.02 $p_1 < 0.01$; $p_2 < 0.05$
OMP ₄₃₀ (mol/L)	0.61 ± 0.01	0.86 ± 0.01 $p_1 < 0.01$	0.75 ± 0.01 $p_1 < 0.01$; $p_2 < 0.01$

p_1 : significant differences in relation to intact rats; p_2 : significant differences in relation to the rats with experimental periodontitis on the 14th day of the research without correction

Table II The content of TBA-active products and nitrogen (II) oxide metabolites in the rat's blood serum with experimental bacterial-immune periodontitis and correction by quercetin ($M \pm m$)

Experimental form	Control and intact animals	Animals with experimental periodontitis	
		Without correction	After correction with quercetin
Experiment duration (days)	–	14	14
Number of the rats	10	8	8
TBA-active products (mcmol/L)	2.555 ± 0.092	8.066 ± 0.143 $p_1 < 0.01$	6.374 ± 0.045 $p_1 < 0.01; p_2 < 0.01$
$\text{NO}_2^- + \text{NO}_3^-$ (mcmol/L)	0.028 ± 0.001	0.147 ± 0.003 $p_1 < 0.01$	0.081 ± 0.003 $p_1 < 0.01; p_2 < 0.01$

p_1 : significant differences in relation to intact rats; p_2 : significant differences in relation to the rats with experimental periodontitis on the 14th day of the research without correction

level was higher in relation to the control group of rats (by 1.23 times; $p < 0.01$).

During investigation, one of the main indicators of LPO activity – the contents of TBA-active products – showed the corresponding changes (Table II). In particular, the formation level of the experimental bacterial-immune periodontitis was higher on the 14th day (by 3.16 times; $p < 0.01$) as compared to the control group.

Treatment with quercetin of the experimental animals from the 7th day to the 14th day of the experiment significantly decreased the content of TBA-active products in the blood serum, as compared to the same period without injections of that drug (by 1.27 times; $p < 0.01$). Simultaneously, its content in blood serum of researched group remained at high level (Fig. 2), as compared to control values, and was higher (by 2.49 times; $p < 0.01$).

Contents of unstable products of free radical oxidation and metabolites of nitrogen (II) oxide ($\text{NO}_2^- + \text{NO}_3^-$) in blood serum were significantly increased (by 5.25 times; $p < 0.01$) on the 14th day of the inflammatory process development in the periodontal complex in relation to the control rats (Table II; Fig. 3).

In addition, quercetin effectively reduced the level of NO in the blood serum (by 1.82 times; $p < 0.01$) than the

Fig. 2. Changes of TBA-active products indices in the rat's blood serum on the 14th day of the research in conditions of development of experimental periodontitis and with correction by quercetin (% of control). *Significant differences in relation to the intact rats ($p < 0.01$). #Significant differences in relation to the rats with periodontitis on the 14th day of the experiment without correction ($p < 0.01$)

Fig. 3. Changes of metabolites of nitrogen (II) oxide indices in the rat's blood serum on the 14th day of the research in conditions of experimental periodontitis development and with correction by quercetin (% of control). *Significant differences in relation to the intact rats ($p < 0.01$). #Significant differences in relation to the rats with periodontitis on the 14th day of the experiment without correction ($p < 0.01$)

rats with experimental periodontitis on the 14th day without correction (Fig. 3). At the same time, this indicator did not reach the values of the control group and was higher by 2.89 times ($p < 0.01$).

Discussion

The introduction of microorganism mixture in the periodontal tissue with diluted egg protein caused hyperergic inflammatory process with expressive changes in the lower jaw soft tissues, which were accompanied by edema and hyperemia of the mucous membrane and cellular infiltration of surrounding tissues, and manifestations were the same as changes occur in humans [19]. At the same time, reliable changes of LPO activity were established.

An important indicator of free radical processes is the OMPs, in a result of which proteolysis in proteosomes is activated, and alternative processes in the inflammatory area are intensified. OMP products, in comparison with lipid peroxides, are more stable and have the ability to be rapidly metabolized by low-molecular antioxidants and

peroxidases [20]. The level products of OMPs of main and neutral character in blood serum on the 14th day of the study were increased, as compared to the control group of rats. Taking the effects of this flavonol into account with regard to the indices of the aldehyde and ketone derivatives concentration, this study found that their level was changed in the opposite direction.

The experimental rats treated with quercetin showed a significant increase in the contents of TBA-active products in the blood serum, as compared to the rats without correction at the same period. Simultaneously, their contents in blood serum of the experimental rats remained at the high level, as compared to the control values. This result proved significant activation of lipid peroxide oxidation and accumulation of their products in the inflammatory process development.

The content of unstable metabolites of nitrogen (II) oxide was also significantly increased on the 14th day of research. This provides grounds to state that the inflammatory reaction in the acute period of development is a source of active NO metabolites formation, which are capable to trigger a cascade of free radical processes with their participation. The treatment of experimental animals with the flavonol quercetin resulted in a significant decrease of metabolic product contents of nitrogen (II) oxide in the blood serum. Quercetin effectively influenced not only the course of the inflammatory process in the periodontal tissues, but also reduced the level of NO in the blood serum and thus contributed to the improvement of dynamic balance between the antioxidant defense system and the free radical processes.

Conclusions

1. In the dynamics of inflammatory process development in the tissues of the periodontal complex with the bacterial-immune component, active products of LPO accumulate that affect the character of the formation, course, and completion.
2. The contents of OMPs of neutral and basic character, metabolites of nitrogen (II) oxide, and TBA-active products in the blood serum increase on the 14th day of the experiment, which indicates an increased activity of free radical LPO.
3. The flavonol quercetin stabilizes the prooxidant-antioxidant system for the course of experimental bacterial-immune periodontitis, which is manifested by a decrease in the neutral and basic OMP content, nitrogen (II) oxide metabolites, and the contents of TBA-active products in the blood serum of experimental animals, thus promoting steady course of inflammatory process.

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