Effects of quercetin on antioxidant potential in the experimental periodontitis development

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(Received: October 17, 2017; Revised manuscript received: October 11, 2018; Accepted: February 2, 2019)

Abstract: The results of experimental research of antioxidant system are presented in this article. Superoxide dismutase activity, catalase, and ceruloplasmin have been determined on the 7th and 14th days of experimental periodontitis development both without correction and with the injection of a water-soluble quercetin drug (corvutin). Hence, there was a decrease in superoxide dismutase activity, intensive increase in catalase activity, and ceruloplasmin maintenance in the blood serum during acute period of inflammatory process. The usage of flavonoid for 7 days resulted in stabilization of radical oxidation due to reduction of superoxide dismutase activity, maintenance at the high-level catalase activity, and ceruloplasmin concentration in the rat's blood plasma with experimental bacterial-immune periodontitis.

Keywords: periodontitis, catalase, superoxide dismutase, ceruloplasmin, antioxidant protection, quercetin

Introduction

The improvement of the existing and the creation of new methods of generalized periodontitis treatment are among the critical problems of modern dentistry, because the frequency of periodontal disease worldwide is currently within of 5%–20% and increases with age to 75% [1, 2]. The elucidation of the mechanisms of their development on the base study of metabolic processes, whose disorders are caused by the damage of the periodontal complex structures, leads to the formation of inflammatory process of various degrees and intensity [3, 4]. Activation of lipid peroxidation (LPO) is one of the trigger mechanisms of stress damage with disorder of the cellular metabolism, which is first of all associated with damage of cellular and subcellular membranes [5–8]. There are systems of antioxidant protection to neutralize excess of LPO and maintain a steady intracellular concentration of free radicals and lipoperoxide in the body [9, 10]. Flavonoid corvutin has an antioxidant, anti-ischemic, membrane-stabilizing, and immunomodulating

effect [11, 12]. The active substance of this drug is a complex of quercetin with polyvinylpyrrolidone. Quercetin is a classical antioxidant that effectively influences energetic metabolism in the myocardium, reduces its requirement of oxygen, stabilizes the cytoplasmic membrane, and displays antiarrhythmic and anabolic effects. Antioxidant activity of the medicine is associated with its ability to suppress LPO. The drug is able to reduce the concentration of free radicals and toxic products of peroxidation [13].

The aim of this study was to determine effectiveness of water-soluble liposomal form of quercetin in correction of the antioxidant system disorders in the experimental periodontitis development.

Materials and Methods

The investigation was performed with use of white clinically healthy rats weighing 150–200 g in the conditions of vivarium. Animals were kept under a standard diet

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balanced with nutritional elements. 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 Experimental 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 research ($n=8$); (3) animals with experimental periodontitis on the 14th day of the research treated by quercetin ($n=8$). Experimental bacterial-immune periodontitis was produced in the experimental animals by introducing complex mixtures of microorganisms (*Staphylococcus aureus* and *Streptococcus hemolytic* in the dose of four colonies forming units) diluted in egg protein into periodontal tissues [14]. Simultaneously with the injection of the pathogen, to enhance the immune response, a complete Freund's adjuvant was injected into the rat's paw. In the third group of rats, corvutin (PJSC Borshchahivskiy CPP, Ukraine) was used as water-soluble quercetin drug by intramuscular injection (100 mg/kg) for 7 days (from 7th to 14th day). On the 14th day, experimental animals were exsanguinated under thiopental anesthesia. For further research, the blood serum was selected. Superoxide dismutase (SOD), catalase activity, and ceruloplasmin maintenance were determined in the serum.

The determination of SOD activity was carried out by a technique based on the ability of the enzyme to inhibit the reduction of nitroterazolium blue [15]. In this study, 1 ml of blood was taken, which was prepared on phosphate buffer (pH = 7.4). Preliminary treatment of the material with chloroform-alcohol mixture and KH_2PO_4 was carried out, followed by centrifugation. To the supernatant were added 1.3 ml of pyrophosphate buffer (pH 8.3), 1 ml of nitroterazolium blue solution, 0.3 ml of phenazinmetasulfate solution, and 2 ml of nicotinamide adenine dinucleotide phosphate (NADPH_2) solution. The samples were kept in the dark and photometricals on a SF-46 spectrophotometer (540 nm) in a 1-cm cuvette against samples to which NADPH_2 was not added. The control was a phosphate buffer. The activity of the enzyme, which is able to inhibit the reduction of nitroterazolium blue by 50%, was taken as 1 conditioned unit. Determination of catalase activity [16] is based on the ability of hydrogen peroxide to form a stable colored complex with ammonium molybdate, the intensity of which is inversely proportional to the activity of catalase in the test substrate. Blood serum from which 10% homogenate of Tris buffer (pH = 7.8) was prepared in the cold. The reaction was initiated by adding 0.1 ml of plasma or homogenate to 2 ml of a 0.03% hydrogen peroxide solution. After 10 min, the reaction was stopped by the addition of 1 ml of 4% ammonium molybdate. The color intensity was measured on a SF-46 spectrophotometer at 410 nm. The activity of catalase was expressed in microcatal per liter.

The serum level of ceruloplasmin was determined by a method that is based on the study of the optical density of oxidation products of n-phenylenediamine in the presence of ceruloplasmin [17]. Its amount is proportional to the intensity of the color. The samples were held for 30 min at 4 °C and then their optical density was determined against the control on a SF-46 spectrophotometer at 530 nm and expressed in milligrams per liter.

The results were statistically analyzed by means of non-parametric indices [18]. The data were presented in the arithmetic mean $\pm$ standard deviation of the mean value (m) for a specific number of the animals (n). Changes were considered statistically significant at $p < 0.05$. Statistica 10.0 (StatSoft, USA) software was used for analyses of the results.

Results and Discussion

After injection of complex microbial mixtures diluted in egg protein into the periodontal tissue, hyperergic inflammatory process with expressed changes in the soft tissue of the lower jaw had been occurred that is accompanied by edema and hyperemia of the mucous membrane, and the characteristics of the symptoms were the same as the changes in humans [19, 20].

The systemic character of the changes in a result of periodontal inflammatory formation was determined on the basis of biochemical investigation of antioxidant system according to SOD, catalase activity, and ceruloplasmin maintenance in rat's blood serum of I–III groups of experimental animals (Table I) as indices of depth of alternative processes in tissues. Simultaneously, the determination of bioflavonoid quercetin effectiveness in the processes of antioxidant protection and experimental periodontitis development on the base of testing activity of these enzymes is effectively carried out.

In the expressed period of experimental periodontitis, which was manifested on the 14th day of the research, SOD activity in the blood serum was observed to have decreased (by 1.25 times; $p = 0.0005$) in comparison with the animals of the control group. That is, insufficiency of the first line of antioxidant protection is associated with reduced SOD expression in response to an elevated level of superoxide anion radical in the inflammatory area formed at this stage of inflammatory process development.

The intramuscular injections of antioxidant quercetin at a dose 100 mg/kg for 7 days increased the activity of SOD in the blood serum by 1.13 times ($p = 0.001$) as compared to animals group with experimental periodontitis on the 14th day but not receive this substance. These tests indicated its effect in relation to enzymic link of antioxidant protection (Table I, Fig. 1). However, they were still lower than in the rats of control group (by 1.11 times; $p = 0.02$).

The study of one of the key enzymes of antioxidant protection – catalase in the blood serum with

Table I Indices of the antioxidant protection system in blood serum of the rats with experimental periodontitis and their correction ($M \pm m$)

Form of the experiment	Control, intact animals	White rats with periodontitis	
		Without correction	With correction by quercetin
Experiment duration (days)	–	14	14
Number of animals	10	8	8
Superoxide dismutase, conditioned (units/ml)	2.294 ± 0.066	1.840 ± 0.040 $p_1 = 0.0005$	2.071 ± 0.019 $p_1 = 0.02, p_2 = 0.001$
Catalase (mccat/L)	0.118 ± 0.001	0.382 ± 0.008 $p_1 = 0.0005$	0.590 ± 0.019 $p_1 = 0.0005, p_2 = 0.0009$
Ceruloplasmin (mg/L)	1.09 ± 0.01	2.70 ± 0.07 $p_1 = 0.0005$	1.36 ± 0.08 $p_1 = 0.001, p_2 = 0.0009$

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

Fig. 1. SOD activity changes in rat's blood serum for experimental periodontitis and its correction by quercetin (% of control). * – significant differences in relation to the intact animals ($p = 0.0005$); • – significant differences in relation to the intact animals ($p < 0.02$); # – significant differences in relation to the animals with periodontitis on the 14th day of the experiment ($p = 0.001$)

experimental periodontitis – showed an opposite trend of changes as compared to the values of the SOD activity. In addition, the degree of expression was somewhat higher. In particular, in the expressed manifestation period of experimental periodontitis, which included the 14th day of the experiment, increase in catalase activity in the blood serum was observed as compared to the control group (by 3.24 times; $p = 0.0005$).

In regard to this, the effect of flavonol on catalase activity was investigated in experimental animals with

periodontitis. It is worth noting that quercetin increased its activity in blood serum (by 1.54 times; $p = 0.0009$) as compared to the group animals on the 14th day, which did not receive the drug (Table I, Fig. 2). At the same time, it was established that after correction by quercetin in animals on the 14th day of experiment, the changes in antioxidant system indices remained at a high level in relation to index in control group (by 5.00 times; $p = 0.0005$).

The increase in catalase activity evidenced about initiation of antioxidant system for complete neutralization

Fig. 2. Catalase activity changes in rat's blood serum for experimental periodontitis and its correction by quercetin (% of control). * – significant differences in relation to the intact animals ($p = 0.0005$); # – significant differences in relation to the animals with periodontitis on the 14th day of the experiment ($p = 0.0009$)

Table II | Superoxide dismutase and catalase activity correlation in rat's blood serum with experimental periodontitis and their correction ($M \pm m$)

Form of the experiment	Control, intact animals	Animals with periodontitis	
		Without correction	With correction by quercetin
Experiment duration (days)	–	14	14
Number of animals	10	8	8
SOD/catalase	19.56 ± 0.50	4.84 ± 0.18 $p_1 = 0.0005$	3.54 ± 0.12 $p_1 = 0.0005, p_2 = 0.0009$

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

Fig. 3. | Changes of ceruloplasmin maintenance in the rat's blood serum with experimental periodontitis and correction by quercetin (% of control). * – significant differences in relation to the intact animals ($p = 0.0005$); • – significant differences in relation to the intact animals ($p = 0.001$); # – significant differences in relation to the animals with periodontitis on the 14th day of the experiment ($p = 0.0009$)

of hydroxylated products of LPO, which are formed excessively for inflammatory process development in periodontal tissues, and simultaneously prevent formation of oxidative stress, weak alternative changes, and transition of inflammatory process into chronic process with possible complications on the system level.

The correlation of SOD/catalase activity (Table II) in the animal blood serum with experimental bacterial-immune periodontitis was found significantly lower in the animals, examined on the 14th day (4.04 times; $p = 0.0005$) as compared with the parameters of the control group. The data obtained in this stage of the experimental periodontitis evidenced about more considerable tension of one link and weakening of the other, leading to violation of coordination in the work of antioxidant enzymes and decrease in the level of antiradical protection [21].

The correlation of SOD/catalase after injections of quercetin was even lower (by 1.37 times; $p = 0.0009$) as compared to that found in the rats on the 14th day of the experiment without correction and less by 5.53 times ($p = 0.0005$) in relation to indices of intact animals. This fact may evidence about different correlative role of tested enzymes in the system of antioxidant protection.

Properly from the data in Table I, quercetin did not decrease the activity of the catalase during the studied period of the inflammatory process, and also continued to stimulate it in contrast with SOD.

An important element of antioxidant protection is also ceruloplasmin (ferroxidase) – copper-containing protein, which similar to SOD takes part in dismutation reaction; however, unlike from SOD that protects intracellular structures, it functions and neutralizes oxygen-active species in the blood, preventing LPO of cellular membranes [22].

The animals of the second experimental group are investigated on the 14th day in which the ceruloplasmin content in the blood serum was higher (by 2.48 times; $p = 0.0005$) in relation to the control group (Table I, Fig. 3).

The experimental animals are treated by quercetin, which showed significant reduction of ceruloplasmin in blood serum starting from the 7th day and ending on the 14th day of the experiment, as compared to the 14th day in animals without correction (by 1.99 times; $p = 0.0009$). However, its level in blood serum in the animals group with experimental periodontitis remained higher of the control values (by 1.25 times; $p = 0.001$).

Conclusions

1. The development of experimental bacterial-immune periodontitis is accompanied by systemic changes in the antioxidant protective system that is manifested as decrease in SOD activity, intensive increase of

catalase activity, and ceruloplasmin maintenance in the blood serum during acute period of inflammatory process, which evidenced about disorder of separate links of oxidant and antioxidant processes and its manifestation of antioxidant potential destabilization in the animal blood.

2. Flavonol quercetin promotes stabilization of free-radical oxidation due to reduction of SOD activity, maintenance at the high-level catalase activity, and ceruloplasmin concentration in the blood plasma by applying for 7 days to the animals with experimental bacterial-immune periodontitis.

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Funding sources: No financial support was received for this study.

Authors' contribution: AD contributed in the gathering and listing data, the data analysis and interpretation, writing of the article, and the idea and the planning of the study. PH contributed in critical review of the article. YB contributed in final approval of the manuscript. All the authors have full access to all data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

Conflict of interest: The authors declares no conflict of interest.

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