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## THE EFFECTS OF QUERCETIN TO GLUTATHIONE SYSTEM IN THE BACTERIAL-IMMUNE PERIODONTITIS DEVELOPMENT

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Perfecting the known and elaboration of new methods of periodontitis treatment is one of the important tasks and requires extraordinary approaches to their solutions. *The purpose of the study* was to determine effectiveness of water-soluble liposomal form of quercetin in correction of the glutathione system disorders in the experimental periodontitis development. The article presents the results of glutathione system researches in antioxidant defense, in particularity, glutathione peroxidase, glutathione reductase activity and content of reduced glutathione in the rat's blood serum on 14<sup>th</sup> days of the experimental periodontitis development, and after correction with flavonol quercetin, that was injected in doze 100 mg / kg for 7 days (from the 7<sup>th</sup> to the 14<sup>th</sup> day). The results were statistically processed using parametric and nonparametric statistical methods. The reliability of the differences in values between independent quantitative values was determined with a normal distribution according to the Mann-Whitney U criterion. The article shows a decrease enzymic activity of the glutathione system (glutathione peroxidase and reductase) observed at early stage of the inflammatory process with following preservation of their activity at reduced level. At the same time, content of reduced glutathione was decreased. The introduction of quercetin resulted in the increase of enzymes in the serum, and reduced glutathione and enzymes activity in the blood serum of experimental animal with periodontitis as compared to the indices of animals that were not administered this medicine. In addition, quercetin also contributed to the preservation of a pool of reduced glutathione in the animal blood. The development of the inflammatory process of the periodontal complex due to the combined effect of bacterial and immune factors is accompanied by dynamic changes in the activity of the glutathione system. Quercetin effectively stabilizes the prooxidant-antioxidant system during the course of experimental periodontitis, which is manifested by the increased activity of glutathione peroxidase and glutathione reductase and the increase in the content of reduced glutathione.

**Keywords:** Periodontitis, antioxidant defense, glutathione reductase, glutathione peroxidase, reduced glutathione, quercetin.

**Research relation to the plans, programs and department research themes.** This work is an integral part of the complex inter-departmental research work "Systemic and organic disturbances due to the actions of extraordinary factors on the body, mechanisms of their development and pathogenetic correction" (registration number 0116 U003390) and research work of the department of Prosthetic Dentistry: "Pathogenetic approaches to treatment the main dental diseases on the basis of studying the mechanisms of damage of the oral cavity tissues against the background of accompanying somatic pathology"(state registration number 0116 U005076).

**Introduction.** Investigation of development of inflammatory processes in periodontal tissues remains an urgent task at present. It is first of all connected to a relatively high spreading and unfavorable prognosis of them, as well as prevention [10] and treatment imperfection [12, 17].

Unusual approaches are required to solutions of improving the existing and developing new pathogenetically grounded methods of periodontitis treatment.

It is known, that active forms of Oxygen/Nitrogen, which are primarily generated by phagocytes, create unfavorable conditions for infectious agents. Therefore, these active forms of Oxygen/Nitrogen have a very important role in the development of inflammatory processes.

However, their surplus production leads to exhaustion of the antioxidant system and the inability to neutralize toxic peroxidation products, and misbalancing protective mechanisms. As a result, this leads to oxidative stress and damage of DNA molecules, as well as damage to proteins, carbohydrates and lipids [8]. At the same time, active forms of oxygen are able to be included in the regulatory processes of cellular homeostasis and act as signaling factors of transduction, genetic expression and activation of receptors.

Active forms of oxygen, peroxides, and nitrogen monoxide in case of ischemia / hypoxia, are also formed during the process of cellular respiration [14].

It was noted that some polyphenols and flavonoids of plant origin are capable to display potent antioxidant properties in many inflammatory and degenerative diseases [6, 18], that let us focus on the prospect of their studies in the experimental periodontitis. Great attention is paid to the properties of flavanol quercetin. It is a classical antioxidant that has anti-ischemic, membrane stabilizing and immunomodulating effects, all at the same time. This has a strong influence to energetic metabolism in the myocardium, reduces its oxygen starvation, exhibits anti-arrhythmic and anabolic effects, and has a significant reduction potential [20]. The antioxidant activity of the substance is related to its ability to suppress lipid peroxidation, to reduce the concentration of free radicals and toxic peroxidation products. It exhibits anti-inflammatory, anabolic and anti-apoptotic properties [2].

**The purpose of the study** was to determine effectiveness of water-soluble liposomal form of quercetin in correction of the glutathione system disorders in the experimental periodontitis development.

**Material and methods.** The research was performed with use of 26 white clinically healthy unline rats, each weighing 150–200 g, that were in conditions of vivarium in accordance to the sanitary standards and GLP. The investigations were performed according to the general rules and regulations of the “European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes” (Strasbourg, 1986) and the “General Ethical Principles of Animal Experimentation” (Kyiv, 2001).

The animals were random and divided into groups: I – intact animals, control ( $n = 10$ ); II – animals with experimental periodontitis on the 14<sup>th</sup> day of the research ( $n = 8$ ); III – animals with experimental periodontitis on the 14<sup>th</sup> day of the research, having been administered quercetin (corvitin) ( $n = 8$ ). Experimental periodontitis was produced in the experimental animals by introducing complex mixtures of microorganisms diluted in egg protein into periodontal tissue [1]. Simultaneously, together with injecting the pathogen, a complete Freund's adjuvant was injected in the rat's paw to enhance the immune response. Rats of the third group were injected with quercetin in doze of 100 mg / kg for 7 days (from the 7<sup>th</sup> to the 14<sup>th</sup> day). On the 14<sup>th</sup> day experimental animals were exsanguinated under thiopental anesthesia. Blood serum was then taken for further testing, which showed reduced levels of glutathione, glutathione reductase, and glutathione peroxidase activity.

The principle of the method for determining of the reduced glutathione concentration [19] is in the inter-

action of 5,5-dithiobis (2-nitrobenzoic acid) (Elman reagent) with the SH groups of the substrate for study. In this case, a thionitrophenyl anion is formed, the amount of which is directly proportional to the content of the SH groups. Up to 0.2 ml of blood serum (1: 4), 1.6 ml of 3% H<sub>2</sub>O<sub>2</sub> and 0.2 ml of 25 % sulfosalicylic acid were added and centrifuged. Then 2.5 ml of 0.2 mol / l Tris buffer (pH = 8.4) and 0.05 ml of a 0.04% solution of the Elman reagent were added to 0.5 ml of the centrifuged solution. Instead of the test material, 0.2 ml of water was added to the control tube. After 10 minutes, the photometric test samples were run on a SF-46 spectrophotometer at 412 nm against the control. The concentration of the reduced glutathione was calculated from the molar extinction coefficient for the thionitrophenyl anion of 11400 mol / l<sup>-1</sup> · cm<sup>-1</sup>, expressed in millimoles per liter (mmol / l). The glutathione reductase activity in the control and test samples was calculated by subtracting the amount of spectrophotometrically centrifuged NADPH<sub>2</sub> from the amount of NADPH<sub>2</sub>, consumed in the enzymatic reduction of oxidized glutathione (SF-46, 340 nm), and was expressed in mmol / min · l [13]. Glutathione peroxidase activity was calculated by the difference between the amount of reduced glutathione in the control (without H<sub>2</sub>O<sub>2</sub>) and test samples [13], expressed in mmol / min · l.

The results were statistically processed using parametric and nonparametric statistical methods using programs Excel (Microsoft, USA) and STATISTICA 10.0 (Statsoft, USA). The reliability of the differences in values between independent quantitative values was determined with a normal distribution according to the Mann-Whitney U criterion [4].

**Results and discussion.** Introducing protein infused pathogen with a complete Freund's adjuvant to the periodontal tissue caused a development of hyperenergetic inflammatory process, accompanied by edema and hyperemia – characteristics of the human-like symptoms [7]. The key link of antioxidant defense especially the one of reduced glutathione, which provides the function of other elements of antioxidant status [3], may take great part in the mechanisms of inflammatory process development.

In base analysis of changes indices of antioxidant system (**Table 1**), it was established that on the 14<sup>th</sup> day of the experimental periodontitis content of reduced glutathione in blood serum was significantly lower than intact animals by 23.30% ( $p < 0.01$ ).

The intramuscular introduction of antioxidant quercetin for 7 days (from the 7<sup>th</sup> to the 14<sup>th</sup> day) at a dose of 100 mg / kg resulted an increase of reduced glutathione content in the blood serum by 22.82% ( $p < 0.01$ ), as compared with the indices, which occurred in the group of animals with experimental

**Table 1** – Glutathione system activity in the rat's blood serum for dynamics of experimental periodontitis development and the use of quercetin (M ± m)

The form of the experiment	Experiment duration (days)	Number of the animals	Reduced glutathione, mmol / l	Glutathione reductase, mmol / minxl	Glutathione peroxidase, mmol / minxl
Control, intact animals	–	10	4.120 ± 0.002	0.490 ± 0.005	0.594 ± 0.003
Experimental periodontitis	14	8	3.160 ± 0.004 p <sub>1</sub> < 0.01	0.384 ± 0.005 p <sub>1</sub> < 0.01	0.535 ± 0.006 p <sub>1</sub> < 0.01
Experimental periodontitis + quercetin	14	8	3.881 ± 0.002 p <sub>1</sub> < 0.01; p <sub>2</sub> < 0.01	0.445 ± 0.003 p <sub>1</sub> < 0.01; p <sub>2</sub> < 0.01	0.582 ± 0.004 p <sub>1</sub> < 0.05; p <sub>2</sub> < 0.01

Notes: p<sub>1</sub> – significant of differences in relation to intact animals; p<sub>2</sub> – significant of differences in relation to animals with experimental periodontitis on the 14<sup>th</sup> day of the study without correction.

periodontitis, but did not receive this substance (on the 14<sup>th</sup> day of the research). These indicates evidence that flavonoid is able to effectively influence on indices of oxidative stress for acute period of the pathological process (**Table 1**). However, complete reduction of reduced glutathione did not occur and its level remained by 5.80% (p < 0.01) lower than in the control group.

At the same time, glutathione peroxidase activity in animals with periodontal complex inflammation had wavy character and was statistically significantly lower on the 14<sup>th</sup> day of the experiment as compared to the control values (**Table 1**).

Thus, in the animals group that were examined on the 14<sup>th</sup> day of experimental periodontitis development decrease of glutathione peroxidase activity by 9.93% (p < 0.01) was observed in comparison with the control.

Glutathione reductase activity was observed decreased by 21.63% (p < 0.01) on the 14<sup>th</sup> day of the research, as compared with the control.

Thus, the obtained data evidence to disturbance of reducing processes in the glutathione system for investigated periods of development experimental periodontitis.

The introduction of the flavonoid antioxidant quercetin significantly stabilized activity of glutathione link of antioxidant defense in the animal's blood serum with experimental periodontitis. Quercetin effectively suspended the inflammatory process in the periodontal complex due to increase of glutathione peroxidase and glutathione reductase activity in the blood serum (by 8.79%; p < 0.01 and 15.89%; p < 0.01), as compared to animals with experimental periodontitis on the 14<sup>th</sup> day without correction (**Table 1**).

It should be noted that the indices of enzymatic activity of glutathione peroxidase did not reach the level of the control group (lower by 2.02%, p < 0.05). A similar character of changes was observed in relation to glutathione reductase activity for introduction of the quercetin. By comparison with the intact group animals, the activity of this enzyme remained lower by 9.18% (p < 0.01).

So, the values obtained in our investigation evidence that quercetin as a flavonoid antioxidant contributes to the stabilization of glutathione system activity in the experimental periodontitis.

Quercetin affects to enzymes that control various cellular functions, including the secretions of histamine from mast cells [11], extends to phospholipases which catalyses the release of arachidonic acid from phospholipids stored in cell membranes. Arachidonic acid serves as a key substrate for substances such as thromboxane, inflammatory prostaglandins and leukotrienes, also inhibits the enzymes cyclooxygenase and 5-lipoxygenase which catalyses the conversion of arachidonic acid to its metabolites [5, 9]. It has also been shown to limit the function of adhesion molecules on endothelial cells [16]. Quercetin also chelates ions of transition metals such as iron which can initiate the formation of oxygen free radicals [15].

### Conclusion

1. The inflammatory process in the periodontal complex tissues is produced combined action of bacterial and immune factors. It is accompanied by dynamic changes of glutathione system activity, which is displayed by inhibition of glutathione peroxidase and glutathione reductase activity and decrease of reduced glutathione content at the stage of early periods (14 days).
2. Quercetin effectively stabilizes prooxidant-antioxidant system for the experimental periodontitis, which is manifested by an increase of glutathione peroxidase and glutathione reductase activity and an increase of reduced glutathione content in the blood serum of experimental animals.

**The prospect for further scientific research** in this direction is to study immune changes in animals with experimental bacterial-immune periodontitis and their corrections by quercetin.

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### ВПЛИВ КВЕРЦЕТИНУ НА ГЛУТАТІОНОВУ СИСТЕМУ ПРИ РОЗВИТКУ БАКТЕРІАЛЬНО-ІМУННОГО ПАРОДОНТИТУ

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**Резюме.** У статті наведено результати досліджень показників стану глутатіонової системи антиоксидантного захисту, що визначали за активністю глутатіопероксидази, глутатіонредуктази та вмістом відновленого глутатіону на 14-ту добу розвитку експериментального пародонтиту та після корекції кверцетином (на 14-ту добу). При цьому звертається увага на характерну динаміку змін показників системи антиоксидантного захисту в процесі розвитку експериментального пародонтиту. Показано зниження активності ферментів системи глутатіону (глутатіонпероксидази, глутатіонредуктази), що відбувалось в ранні терміни запальної реакції. Разом з тим за даних умов експерименту знижувався вміст відновленого глутатіону.

Застосування кверцетину за умови експериментального пародонтиту приводило до підвищення активності ферментів у сироватці крові відносно показників тварин, яким не вводили даний препарат, проте активність їх не досягала величин контрольної групи. При цьому кверцетин сприяв також збереженню пулу відновленого глутатіону у сироватці крові тварин.

**Ключові слова:** пародонтит, антиоксидантний захист, глутатіонредуктаза, глутатіонпероксидаза, відновлений глутатіон.

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**ВЛИЯНИЕ КВЕРЦЕТИНА НА ГЛУТАТИОНОВУЮ СИСТЕМУ  
ПРИ РАЗВИТИИ БАКТЕРИАЛЬНО-ИММУННОГО ПАРОДОНТИТА**

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**Резюме.** В статье приведены результаты исследований показателей состояния глутатионовой системы антиоксидантной защиты, которые определяли по активности глутатиопероксидазы, глутатионредуктазы и содержанию восстановленного глутатиона на 14-е сутки развития экспериментального пародонтита и после коррекции кверцетином (на 14-е сутки). При этом обращается внимание на характерную динамику изменений показателей системы антиоксидантной защиты в процессе развития экспериментального пародонтита. Показано снижение активности ферментов системы глутатиона (глутатионпероксидазы, глутатионредуктазы), что происходило в ранние сроки воспалительной реакции. Вместе с тем при данных условиях эксперимента снижалось содержание восстановленного глутатиона. Применение кверцетина при экспериментальном пародонтите приводило к повышению активности ферментов в сыворотке крови относительно показателей животных, которым не вводили этот препарат, однако активность их достигала величин контрольной группы. При этом кверцетин способствовал также сохранению пула восстановленного глутатиона в сыворотке крови животных.

**Ключевые слова:** пародонтит, антиоксидантная защита, глутатионредуктаза, глутатионпероксидаза, восстановленный глутатион.

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