

Effects of flavonol quercetin on syndrome of endogenous intoxication in experimental periodontitis

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Abstract

The article presents the results of study effect of quercetin on the indicators of endogenous intoxication syndrome under conditions of experimental periodontitis. One of the key links in the pathogenesis of any inflammatory disease, including maxillofacial area, is endogenous intoxication, an important pathophysiological mechanism of which is the activation of lipid peroxidation. The experimental animals were treated by intramuscular injections with water-soluble quercetin for 7 days. For further research the blood serum was selected in which was determined the content of middle molecular weight molecules and erythrocyte intoxication index. Excessive lipoperoxidation was accompanied by accumulation of peroxidation products and depletion of antioxidant reserves, which led to accumulation of toxic substances. That is why the study of the level of middle molecular weight molecules and erythrocyte intoxication index in simulated bacterial-immune periodontitis is a reliable criterion. Quercetin was able to reduce the expression of endogenous intoxication syndrome in experimental bacterial-immune periodontitis.

Keywords

Periodontitis, endogenous intoxication, middle molecular weight molecules, erythrocyte intoxication index, lipid peroxidation, quercetine

Introduction

The periodontal complex is the tissue that surrounds the tooth and ensures its stable position relative to the alveolar hole. It consists of gums, periodontal ligament, root cementum, bone tissue of alveolar processes. Periodontal disease affects either any of these components, or the entire periodontium. By nature, the process of change is dystrophic, tumor or inflammatory, and the latter type accounts for 90–93% of all diseases in this area. According to the WHO, 98% of the adult population of the planet has problems associated with periodontal tissue pathology.

For many years there has been a tendency to earlier onset of this disease and its aggressive course (Milosavljevic et al. 2021). With periodontitis, the connection between the teeth and gums weakens, and the alveolar processes of the jaw bones are destroyed. Chronic periodontitis can occur in the background of periodontal pathogenic microflora, which is present in plaque. These microorganisms produce toxins that are detrimental to the gum epithelium. As a result, chronic hypertrophic or catarrhal gingivitis develops. Pathogens further penetrate the gingival sulcus, deepening it and forming gingival pockets. Sub- and supragingival plaque, which contains many bacteria,

gradually contributes to the destruction of connective tissue. This effect extends to the periodontal ligament, which forms the gingival connections (Pavlenkova et al. 2018). The development of periodontitis is accompanied by oxidative stress, which is characterized by an increase in the intensity of lipid peroxidation (LPO), which leads to disruption of lipid metabolism. These are the main structural components of cell membranes, accompanied by membrane destruction and impaired metabolism and the mucous membrane of the oral cavity, which lead to the development of endogenous intoxication (Tripathi et al. 2018).

Endogenous intoxication is the accumulation of endogenous toxic products in the body (Pinelis et al. 2017; Eldzharov et al. 2021). Since recently, a special priority for the objective assessment of endotoxemia has been the method of middle molecular weight molecules and their fractions, as these markers of endogenous intoxication are not only integral but also an objective indicator of toxicity of a particular environment regardless of etiopathogenetic features of a disease (Gnid and Dyrk 2016). Literature data indicates that important indicators of endogenous intoxication syndrome are the level of middle molecular weight molecules (MMWM) and erythrocyte intoxication index (EII) (Díaz et al. 2020). Among the criteria of endogenous intoxication, special attention is paid to molecules of average mass. Some scientists believe that the level of MMWM is one of the most sensitive signs of endogenous intoxication (Synyachenko et al. 2021). In order to reduce the intensity of lipoperoxidation and endogenous intoxication, we used the drug quercetin, which in our previous studies were found to have antioxidant properties (Demkovych 2019; Demkovych et al. 2019). In the available literature we have not found any information on the use of flavonol quercetin for the correction of endogenous intoxication in chronic generalized periodontitis. It is also known that it has membrane-stabilizing, anti-ischemic and anti-inflammatory properties (Massi et al. 2017). Because of this, the correction of pathological processes of the oral mucosa with antioxidant drugs allows to optimize the intensity of inflammation, reduce the period of the catabolic phase and accelerate the onset of regeneration (Sculean et al. 2019).

In this regard, the problem of timely diagnosis and monitoring of endogenous intoxication syndrome and correction of its main indicators in periodontology today is relevant and promising.

Objective. To study the indicators of endogenous intoxication syndrome and the effect of quercetin on them under the conditions of experimental periodontitis.

Materials and methods

The experiment was conducted on 26 white outbred rats weighing 180–200 g, which were contained in the usual diet of vivarium. The research was performed according to the general rules and regulations of the European

Convention for the Protection of vertebrate animals that use for experimental and other scientific purposes (Strasbourg 1986), the General ethical animal experimentation (Kyiv 2001). The animals were divided into three groups: 1st group – intact (10 rats); 2nd group – animals in which the model periodontitis on the 14th day (8 rats); 3rd group – animals in which the model periodontitis on the 14th day which was introduced flavonol quercetin (8 rats). The experimental bacterial-immune periodontitis in the experimental animals was caused by injection into the tissue of the periodontal complex of the microorganisms mixture diluted with egg's albumin (Demkovych et al. 2017). To enhance the immune response, an injection of complete Freund's adjuvant was simultaneously injected into the rat's paw. The experimental animals (rats) of the 3rd group were treated by intramuscular injections with water-soluble quercetin in a dose 100 mg / kg of animal weight for 7 days (from the 7th to the 14th day). For further research the blood serum was selected.

To determine the content of middle molecular weight molecules from the serum was isolated acid-soluble fraction, which was obtained by adding 1.8 ml of 10% solution of trichloroacetic acid to 0.2 ml of serum. Subsequent centrifugation was performed at 3000 rpm for 30 minutes. The isolated 0.5 ml fraction was diluted 1:10 with distilled water and the optical density was determined at 254 nm (chain amino acids) and 280 nm (aromatic amino acids) against distilled water on a spectrophotometer. The results were expressed in conventional units, numerically equal to the extinction (Krynytska et al. 2018).

The method of determining the erythrocyte intoxication index (EII) was based on information about erythrocytes as a universal adsorbent, which allows to estimate the level of EI by changing the sorption capacity of erythrocytes polar, almost impermeable to their methylene blue membrane. In a test tube, containing 1 ml of 3.8% sodium citrate solution, 4 ml of blood was taken, mixed and the erythrocytes were separated by centrifugation for 10 min at 3000 rpm. Plasma was removed. Transferred 1 ml of erythrocyte mass in a test tube containing 3 ml of a solution of methylene blue (0.025%), prepared in saline. The samples were stirred and incubated for 10–12 min at room temperature, then centrifuged again for 10 min at 3000 rpm. The supernatant was transferred to a cuvette and the optical density relative to saline was determined at a wavelength of 630 nm on a spectrophotometer. The amount of absorbed dye (as a percentage) was calculated from the difference between the optical density of the initial dye solution and the dye solution after incubation with erythrocytes (Shcherba and Korda 2019).

The results were statistically analyzed by means of non-parametric indices methods in the STATISTICA 10.0 software (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 (Orlov 2015).

Results

The level of endotoxigenesis is determined by the content of hydrophilic and hydrophobic products in the blood. Middle molecular weight molecules belong to the hydrophilic components of endogenous intoxication. They are formed due to increased proteolysis and have an alternative effect on the body due to high functional activity (Vining et al. 2019).

As it can be seen from the table, the content of middle molecular weight molecules, which has been determined at a wavelength of 254 nm in the serum (chain amino acids), has increased on the 14th day – by 1.28 times ($p < 0.01$) compared with the intact group. There has also been an increase in serum middle molecular weight molecules, which has been determined at a wavelength of 280 nm (aromatic amino acids). On the 14th day, this indicator has retained this vector of direction of change, and was higher relative to the intact group of animals (by 1.46 times; $p < 0.01$).

Table 1. The content of middle molecular weight molecules in the serum and erythrocyte index of intoxication of experimental animals under the condition of experimental periodontitis with the use of quercetin ($M \pm m$).

Conditions and indicators of the experiment	Control. Intact group	White rats with experimental periodontitis	
		No correction	With quercetin correction
Experiment duration (days)	-	14	14
Number of animals	10	8	8
MMWM ₂₅₄ , condit. units	353.80 ± 4.53	454.00 ± 10.39 $p_1 < 0.01$	419.00 ± 10.23 $p_1 < 0.01; p_2 < 0.01$
MMWM ₂₈₀ , condit. units	144.30 ± 4.67	210.75 ± 10.93 $p_1 < 0.01$	185.75 ± 7.73 $p_1 < 0.01; p_2 < 0.05$
Erythrocyte intoxication index, %	31.06 ± 1.88	54.30 ± 1.66 $p_1 < 0.01$	49.55 ± 1.41 $p_1 < 0.01; p_2 < 0.05$

Notes. p_1 - the significance of differences relative to intact animals; p_2 - significance of differences in animals with experimental periodontitis on the 14th day of the study without correction.

Increased permeability of erythrocyte membranes is a reflection of disorders of the functions and structure of plasma membranes of all cells of the body. The erythrocyte intoxication index characterizes the inhibition of sorption activity (including methylene blue), which is a consequence of the rearrangement of lipid components of cell membranes and a decrease in the functional capacity of erythrocytes due to exposure to toxic substances (Chemych et al. 2020).

As a result of the study of the total toxic effect on the erythrocyte membranes of the blood of experimental animals with experimental bacterial-immune periodontitis – the level of erythrocyte intoxication index (EII), significant changes were also found (Table). In particular, it was found, that on the 14th day of this experimental model of periodontitis, there has been a further gradual increase of EII (by 1.75 times; $p < 0.01$) compared with the intact group of animals, indicating a decrease in the adsorption capacity of erythrocytes throughout period of inflammation.

Analyzing the results of the study of endogenous intoxication in the serum of animals with experimental

bacterial-immune periodontitis receiving quercetin, it should be noted, that there has been a significant decrease in the hydrophilic components of endogenous intoxication, namely middle molecular weight molecules, which were determined at a wavelength of 254 nm – by 1.08 times ($p < 0.05$), compared with data from animals with our simulated pathology on the 14th day of the experiment without the introduction of flavonol (Fig. 1).

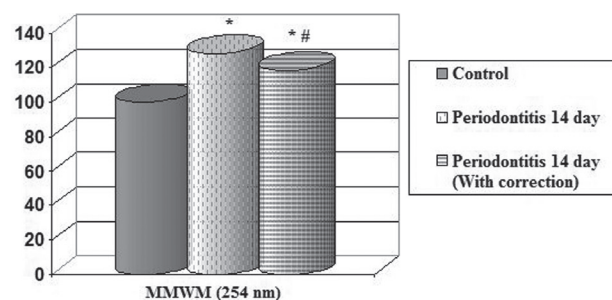


Figure 1. Dynamics of content middle molecular weight molecules (254 nm) in the blood serum of white rats with experimental periodontitis with quercetin correction (in % of control). Notes: * – significant of differences in relation to the intact animals ($p < 0.01$); # – # – significant of differences in relation to the animals with periodontitis on the 7th day of experiment without correction ($p < 0.05$).

However, when comparing the indicator $MMWM_{254}$, which took place on the 14th day of development of experimental periodontitis with correction, and the indicators of the control group of animals, it was found that it remained significantly higher (by 1.18 times; $p < 0.01$) than animals comparison groups.

Analyzing the changes in the level of middle molecular weight molecules, which were determined on a spectrophotometer at a wavelength of 280 nm, in the blood serum of experimental animals with periodontitis which received quercetin during the development of the pathological process, it should be noted that the therapeutic efficacy of this tool is confirmed by lower serum $MMWM_{280}$ (by 1.14 times, $p < 0.05$), compared with animals studied on the 14th day, and have not received this medicine (Fig. 2).

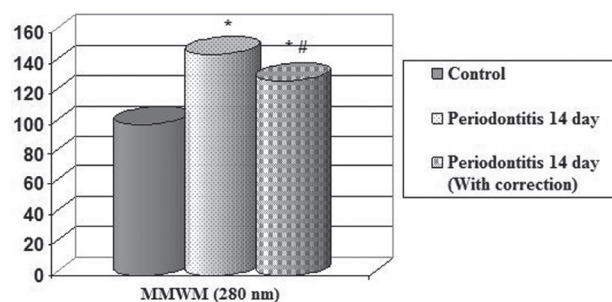


Figure 2. Dynamics of content middle molecular weight molecules (280 nm) in the blood serum of white rats with experimental periodontitis with quercetin correction (in % of control). Notes: * – significant of differences in relation to the intact animals ($p < 0.01$); # – # – significant of differences in relation to the animals with periodontitis on the 7th day of experiment without correction ($p < 0.05$).

It should be noted that when comparing the above levels of aromatic amino acids on the 14th day of development of experimental periodontitis with correction, we found that they remained slightly higher than the control group of animals – by 1.29 times ($p < 0.01$).

According to the results of the study, it was found that there has been a significantly reduced erythrocyte intoxication index in animals treated with quercetin. The effectiveness of flavonol on the course of the inflammatory process in this simulated pathology is confirmed by a decrease of EII (by 1.10 times; $p < 0.05$), compared with animals with bacterial-immune periodontitis on the 14th day which did not receive the medicine (Fig. 3).

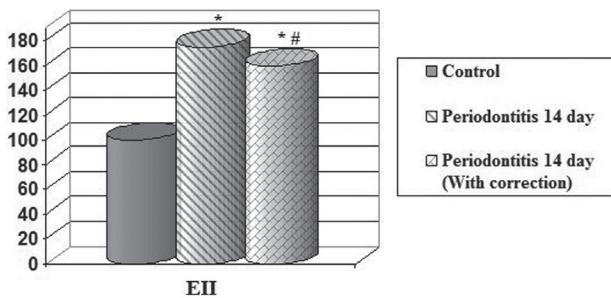


Figure 3. Dynamics of erythrocyte intoxication index in the blood serum of white rats with experimental periodontitis with quercetin correction (in % of control). Notes: * – significant of differences in relation to the intact animals ($p < 0.01$); # – # – significant of differences in relation to the animals with periodontitis on the 7th day of experiment without correction ($p < 0.05$).

However, when comparing the changes in the studied index of endogenous intoxication observed on the 14th day of experimental periodontitis after quercetin injections, it was found that after correction it remained significantly higher relative to the control group of animals (by 1.60 times; $p < 0.01$).

Discussion

One of the difficult problems of modern dentistry is periodontal disease. In recent years, along with the known concepts of the pathogenesis of inflammatory and inflammatory-dystrophic periodontal diseases, much attention has been paid to the activation of lipid peroxidation. In violation of antioxidant protection, free radical oxidation in the periodontium develops avalanche-like. The level of peroxidation of phospholipids of cell membranes increases with the destruction of the latter and the death of periodontal cells with the release of endogenous toxins. Cell division is disturbed and inert products of peroxide denaturation of lipids and proteins accumulate. It is known, that uncontrolled LPO reactions can not only lead to metabolic disorders, but also cause structural changes in tissues, suppress the body's defense mechanisms, which in turn promotes the activation of microbes that colonize the gums and periodontal pockets.

There is evidence that the activation of LPO processes is an important pathophysiological mechanism of endogenous intoxication (Gulyaeva et al. 2019). Excessive lipoperoxidation is accompanied by the accumulation of products of peroxidation and depletion of reserves of antioxidant systems. LPO products damage cell membranes and intracellular organelles, which is then accompanied by destructive tissue changes, hyperenzymemia and accumulation of toxic substances. Thus, the periodontium becomes a center of formation of toxic metabolites, in excess concentrations of which not only have a toxic effect on periodontal tissues, impairing cell metabolism, but also provoke significant disorders of metabolic processes in the body (Narendra et al. 2018; Kilmukhametova et al. 2021).

Endogenous intoxication not only accompanies most diseases, but is an important factor in their pathogenesis and in many cases determines the possible adverse effects, because an important feature of MMWM is their high biological activity. They have neurotoxic activity, inhibit protein synthesis, promote hemolysis of erythrocytes, inhibit erythropoiesis and enzyme activity, cause a state of secondary immunosuppression (Ying et al. 2020). Since middle molecular weight molecules are markers of endotoxicosis, a sharp increase in their content indicates the development and generalization of endogenous intoxication. MMWM are also able to block cell receptors by binding to the active centers of the albumin molecule, competing with regulatory peptides, and thus disrupting the process of humoral regulation (Kolesnikova et al. 2020). Therefore, the formation of endogenous intoxication syndrome is an important part of the pathogenesis of periodontitis.

The syndrome of endogenous intoxication is accompanied by increased tissue breakdown, increased catabolic processes due to the accumulation of excessive amounts of biologically active substances, deformed protein metabolites and other toxic substances of endogenous origin (Yavtushenko et al. 2020). The increase in the level of eretric acid intoxication index is explained by the toxic effect on erythrocyte membranes, which was manifested by a significant increase on the 14th day of the experiment. Intramuscular administration of quercetin for therapeutic purposes for 7 days in the period from the 7th to the 14th day at a dose of 100 mg / kg body weight of animals per day helped to reduce the level of MMWM and EII in the blood compared to the group of animals not exposed flavonol. Therefore, quercetin was able to consequently reduce the increased manifestation of the immune response and endogenous intoxication syndrome in experimental bacterial-immune periodontitis.

Conclusion

1. In rats with modeled bacterial-immune periodontitis, an increase in the level of endogenous intoxication was

found, which indicates an increase in the permeability of erythrocyte membranes and an increase in the level of middle molecular weight molecules associated with direct damage, destructive changes, inflammation, hypoxia and peroxia activation. lipid oxidation.

2. The use of flavonol quercetin under conditions of experimental bacterial-immune periodontitis helps to reduce the level of endogenous intoxication of the

organism and the attenuation of the inflammatory process in the periodontal complex.

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