ORIGINAL ARTICLE

CYTOKINOGENESIS DISORDERS IN MECHANISMS OF THE EXPERIMENTAL PERODONTITIS DEVELOPMENT AND THEIR CORRECTION BY FLAVONOL

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ABSTRACT

The aim: Evaluation of changes in proinflammatory and anti-inflammatory units of the cytokine profile in the mechanisms of development of experimental bacterial-immune periodontitis and elucidation of the effect of flavonol quercetin on its parameters.

Materials and methods: Experimental periodontitis was caused by introducing into the tissues of the periodontal complex a mixture of microorganisms diluted with egg protein. In order to enhance the immune response, a complete Freund's adjuvant was injected into the rat's paw at the same time. For correction, intramuscular injections of water-soluble quercetin at a dose of 100 mg / kg body weight were performed for 7 days (7th to 14th day).

Results: The use of flavonol quercetin led to a decrease in the serum content of experimental animals with pro-inflammatory cytokines. With regard to anti-inflammatory cytokines, their content in the blood of animals during the development of this simulated inflammatory process changed in the opposite direction. Quercetin effectively eliminated the imbalance of the immune system and increased the level of IL-10 and IL-4 in the serum. After injections of quercetin, the relation of pro- and anti-inflammatory cytokines in the serum of animals on 14th day of the study was reduced compared to their content in rats that did not receive correction.

Conclusions: The formation and course of experimental bacterial-immune periodontitis is accompanied by a complex of pathological changes in immunocytokinogenesis, which is manifested by a progressive increase in serum concentrations of proinflammatory cytokines and a decrease in anti-inflammatory cytokines. Flavonol quercetin reduces the concentration of pro-inflammatory and increases the content of anti-inflammatory cytokines.

KEY WORDS: periodontitis, proinflammatory cytokines, immune system, quercetin, Inflammatory process, antiinflammatory cytokines

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INTRODUCTION

Periodontal disease is important problems of modern medicine that prevail among people of different age groups, with predisposition to progression and diverse influence on the tooth-jaw system and organism as a whole, as well as the difficulties in treatment [1]. Generalized periodontitis develops in a result of the influence local and general factors, the leading of which is considered bacterial [2]. Parodontopathogens affecting on the tissues of the periodontal complex triggers a number corresponding of reactions, leading to the development of inflammation [3]. At present there is considerable increase of the inflammatory diseases of periodontium and "rejuvenation" of the patients contingent with this pathology. To day periodontitis is considered as a complex autoimmune and neurotrophic process. The large number of scientific researches devoted to study of this problem [4]. However, there are not of significant changes in real dentist practical work that could give reliable results in the short term (recovery or achievement of long-term remission).

Well-known methods of treatment require new approaches to their solve on the base of pathogenetically grounded elaborations and improvements. It is necessity more deeply and detailed research essence of immunological processes disorders as important link of the pathogenesis of this pathology [5]. In particular, it is generally recognized that one of the key components in the immune system is cytokinogenesis, which is considered as a universal effector that responds to numerous signals about destabilization of the organism internal environment and is one of key pathogenetic chain in the control of the immune-inflammatory reaction [6].

However, the relationship between clinical morphological indices and mediators of the inflammatory-destructive process in periodontium is insufficiently studied. The determination of the mechanisms of the relationship between destructive processes in the development of the inflammation in the periodontal tissues and the immune system will allow elaborate and introduction of new methods of treatment in the periodontology based on the modulating properties of cytokines in the regulation of immune response of organism [7].

It is known that some polyphenols and flavonoids of plant origin can exhibit potent antioxidant properties in many inflammatory and degenerative diseases [8, 9], which may be expedient to study their properties in the presence of periodontitis. In this case,

the results of studies of the flavonol quercetin properties are noteworthy. It is a potent antioxidant that also exhibits antiischemic, make membranestabilizing, immunomodulating effect, regulates effectively energy metabolism in the myocardium, while reducing its oxygen requirement, exhibits antiarrhythmic and anabolic effects, has a significant redox potential [7]. Antiinflammatory and antiallergic effects of quercetin are associated with the ability to suppress calcium ATP-ase and leukotriene synthesis. On the other hand, flavonol suppresses the activity of hyaluronidase and the content of immune cells (phagocytes, T-lymphocytes, B-lymphocytes) in the blood, resulting in decreased manifestations of secondary immunosuppression. Consequently, it may be outlook for studying its properties in the condition of periodontitis.

THE AIM

The aim of this study was to determinate the role of cytokinogenesis and effect of flavonol in the pathogenesis, development and course of experimental periodontitis.

MATERIALS AND METHODS

The research was performed with use of white clinically healthy rats with weight of 150-200 g, in vivarium conditions accordance to the sanitary standards and GLP. The investigations were performed according to 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).

In this experiment animals (rats) were selected and randomise divided into three groups: I group (n = 10) – control animals; II group (n = 8) – animals with bacterial-immune inflammation in the periodontium on the 14th day of the study; III group (n = 8) – animals with bacterial-immune inflammation in the periodontium on the 14th day of the study, which was introduced flavonol quercetin. Experimental modelled bacterial-immune periodontitis in rats was caused by injection into the periodontal tissue a mixture of bacteria diluted with protein (egg white) [10]. In order to strengthen the immune response, an injection into the rat's paw of the full Freund's adjuvant was performed at the same time. 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). They were sacrificed by bleeding after thiopental anaesthesia on the 14th day. Blood serum was selected for determination the content of tumour necrosis factor alpha (TNF-α), interleukin-1 beta (IL-1β), interleukin-4 (IL-4), interleukin-10 (IL-10) by the method Solid-phase enzyme immunoassay using a set of RayBio Rat Cytokine Antibody Array reagents (RayBiotech, Norcross, USA) [11]. The content of TNF-α, IL-1β, IL-4 and IL-10 was expressed in ng / l. The results were statistically processed using the software STATISTICA Version 10.0 ("Statsoft", USA). The reliability of the difference in values between independent quantitative values was determined with the normal distribution by criterion U-criterion Mann-Whitney [12].

RESULTS

In a result of our studies, it was found that in the white rat's blood serum with experimental bacterial-immune inflamation the indicators of proinflammatory cytokines of the first line: IL-1β, TNF-α was significantly higher as compared to intact.

Table I. Indices of proinflammatory cytokines in the animal's blood serum with experimental bacterial-immune periodontitis and with correction (M ± m)

Experiment conditions and indices	Control, intact rats	Rats with periodontal inflammation	
		Without correction	After quercetin correction
Duration of the experiment (days)	-	14	14
Number of the rats	10	8	8
IL-1β (ng / l)	8.40±0.51	25.70±0.59 p_1<0.01	20.65±0.66 p_1<0.01; p_2<0.01
TNF-α (ng / l)	25.80±1.48	37.97±0.93 p_1<0.01	31.17±1.15 p_1<0.05; p_2<0.01

Note 1: p_1 – indicator of differences relative to intact animals.
Note 2: p_2 – indicator of differences relative to animals with bacterial-immune periodontital inflammation on the 14th day of the experiment without correction.

Table II. Indices of antiinflammatory cytokines in the animal's blood serum with experimental bacterial-immune periodontitis and with correction (M ± m)

Experiment conditions and indices	Control, intact rats	Rats with periodontal inflammation	
		Without correction	After quercetin correction
Duration of the experiment (days)	-	14	14
Number of the rats	10	8	8
IL-10 (ng / l)	71.06±2.96	44.30±2.87 p_1<0.01	56,30±3,38 p_1<0.05; p_2<0.05
IL-4 (ng / l)	20.05±1.04	14.92±0.65 p_1<0.01	16.48±0.44 p_1<0.05; p_2<0.05

Note 1: p_1 – indicator of differences relative to intact animals.
Note 2: p_2 – indicator of differences relative to animals with bacterial-immune periodontital inflammation on the 14th day of the experiment without correction.

Table III. The relation of proinflammatory and antiinflammatory cytokines in the animal's blood serum with modelled bacterial-immune periodontitis and with correction (M ± m)

Experiment conditions and indices	Control, intact rats	Rats with periodontal inflammation	
		Without correction	After quercetin correction
Duration of the experiment (days)	-	14	14
Number of the rats	10	8	8
IL-1β / IL-10	0.12±0.01	0.59±0.03 p_1<0.01	0.38±0.03 p_1<0.01; p_2<0.01

Note 1: p_1 – indicator of differences relative to intact animals.
Note 2: p_2 – indicator of differences relative to animals with bacterial-immune periodontital inflammation on the 14th day of the experiment without correction.

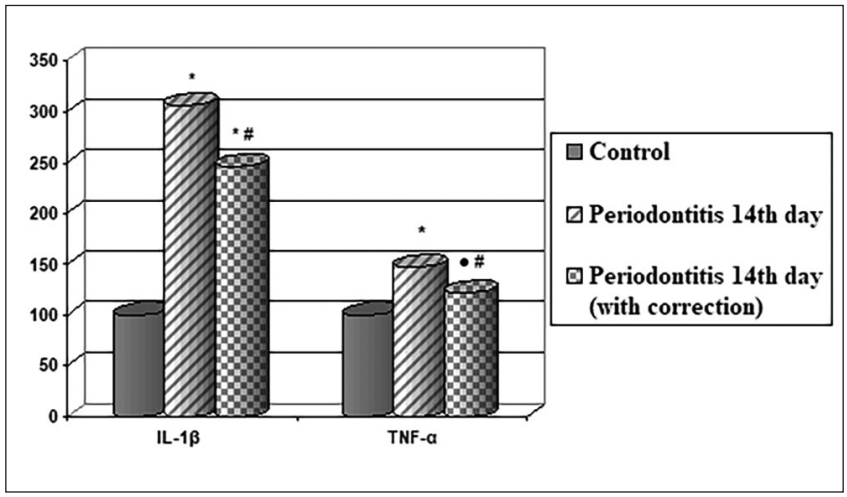

Fig. 1. Changes of interleukin-1β and tumour necrosis factor-α parameters in the animal's blood on the 14th day of the experiment in conditions of modelled bacterial-immune periodontital inflammation development and correction quercetin (% of control).
Note 1: * – differences between the control rats (p<0.01). Note 2: • – differences between control rats (p<0.05). Note 3: # – differences between rats with periodontital inflammation on the 14th day of the research without quercetin correction (p<0.01).

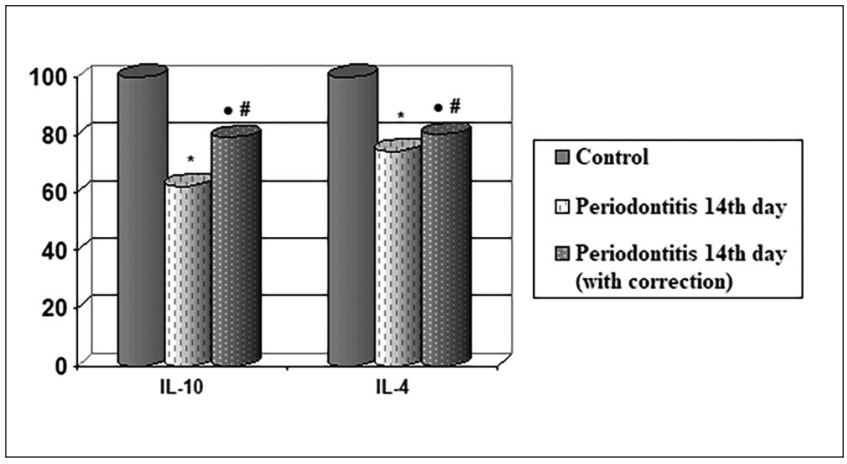

Fig. 2. Changes of interleukin-10 and interleukin-4 parameters in the animal's blood on the 14th day of the experiment in conditions of modelled bacterial-immune periodontital inflammation development and correction quercetin (% of control).
Note 1: * – differences between the control rats (p<0.01). Note 2: • – differences between control rats (p<0.05). Note 3: # – differences between rats with periodontital inflammation on the 14th day of the research without quercetin correction (p<0.01).

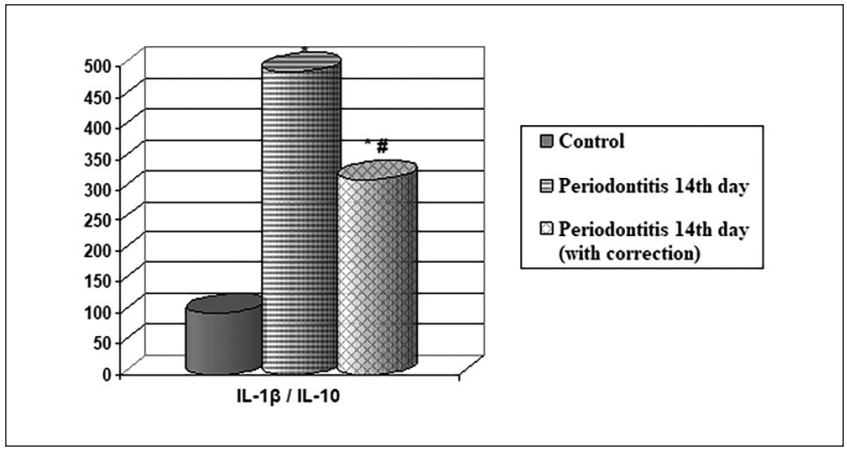

Fig. 3. Changes relation of proinflammatory and antiinflammatory cytokines in the animal's blood on the 14th day of the experiment in conditions of modelled bacterial-immune periodontital inflammation development and correction quercetin (% of control).
Note 1: * – differences between control rats (p<0.01).
Note 2: # – differences between rats with periodontital inflammation on the 14th day of the research without quercetin correction (p<0.01).

IL-1β is one of the main proinflammatory cytokine and is an activator of T-cells, NK-cells, NKT-cells, stimulates the formation of T-cell cytokines [9]. The development of inflammatory process in the experimental animals with bacterial-immune periodontitis on the 14th day is accompanied by significant increase (by 3.06 times; p<0.01) of interleukin-1β contents in the serum in relation to intact group of the rats.

In respect of the TNF-α, which stimulates the activity of leukocytes, the production with cells IL-1β, IL-6 and has a destructive effect to the tissue, then under these conditions, it was also found to increase its content as compared with the control values (by 1.47 times p<0.01) (Table I).

The introduction of the flavonol quercetin resulted reduce of proinflammatory cytokines, in particularly IL-1β (by 1.25 times; p<0.01) in the rat's blood serum, as compared with such indices of the animals with experimental periodontitis on 14th day which did not cure this substance (table I, fig. 1). However, for these experimental conditions, the indices were remained still slightly higher than indicators of the control rats (by 2.50 times; p<0.01).

Regarding the influence of this flavonol on the indices of TNF-α content in experimental animals with periodontitis, it should be noted that quercetin reduced it in blood serum (by 1.22 times; p<0.01), as compared to rats on the 14th day, which did not treatment this medicine drug (Figure 1).

At the same time, in comparing of this proinflammatory cytokine content which was observed after administration of quercetin on the 14th day of madelled experimental periodontitis, it remained slightly higher relative to the control animals (by 1.18 times; p<0.01).

With regard to the above, antiinflammatory cytokines, particulary, IL-10 and IL-4, their content in the blood of animals with experimental periodontitis was changed in the opposite direction.

IL-10 belongs to the link of antiinflammatory cytokines and is an important endogenous regulator of immune and inflammatory processes that can suppress the activation and function of T-cells, Natural killer-cells, macrophages, and their proinflammatory cytokines. As shown results of the study indices of antiinflammatory cytokine IL-10 on the 14th day of the periodontitis was decreased (by 1.61 times (p<0.01) relative to the control animals.

Regarding the parameters of antiinflammatory cytokine IL-4 in blood serum of experimental animals with periodontal inflammation the study showed decrease of this interleukin (1.34 times; p<0.01) in relation to the corresponding control (Table II).

The introduction of the flavonol for 7 days resulted significant changes of the antiinflammatory cytokines indicators in the animal's blood serum with periodontitis. The quercetin effectively removed imbalance of the immune system and reduced the effects of oxidative stress with an inflammatory process in periodontal tissues, elevated the level of IL-10 in blood (by 1.27 times; p<0.05), in rats with an experimental periodontitis on the 14th day without correction. So, dynamic equilibrium between the spectrum of proinflammatory and antiinflammatory cytokines was restored. At the same time, this index did not reach the means of the control animals and was higher by 1.26 times (p<0.05).

In a detailed analysis of the effects of this flavonol about antiinflammatory interleukin-4 indices in the blood serum of animals with bacterial-immune periodontitis and comparison of obtained a results on the 14th day in rats that were not treated quercetin (Figure 2), given index exceeded control means by 1.11 times (p<0,05). However, its level was lower comparative to the group of intact animals (by 1.22 time; p<0.05).

As a result of a decrease parameters of antiinflammatory and an increase indices of proinflammatory cytokines in the experimental animals blood serum, their correlation (IL-1β / IL-10) was disturbed and thus an imbalance of regulatory mechanisms arose that determine the nature of the course and end of the inflammatory process. In comparison of the above meant the relation of IL-1β / IL-10 in blood serum of animals with inflammatory process in periodontal complex it was found decreased in relation to the intact animals (by 4.92 times; p<0.01). Dynamic increase of the corelation of IL-1β / IL-10 indicates about progressive development of inflammatory reaction in periodontum (Table III).

The ratio of pro- and antiinflammatory cytokines indices in blood of rats on the 14th day after treatment by quercetin was reduced (by 1.55 times, p<0.01), as compared to their means in the rats which had not been given therapeutic agent (Table III).

At the same time, the relation of IL-1β / IL-10 in serum of this animal group remained at a rather high level (Figure 3), exceeding the control value by 3.17 times (p<0.01).

DISCUSSION

One of the important factors of jaw alveolar processes resorption is disturbance of the balance between activity of osteoblasts and osteoclasts under action of bacterial products (e.g., lipopolysaccharides), as well as stimulation of osteoclasts with the participation of IL-1β, IL-6, and TNF-α [13]. These same cytokines can activate osteoclast formation and bone resorption. Strengthened migration of macrophages under the influence of cytokines and their constant presence in tissues enhances destructive processes in periodontium [14]. In this case, the degree of the alveolar bone resorption depends on expression of the immune response of the organism to bacterial invasion. Antiinflammatory cytokines (IL-4, IL-10, IL-12) inhibit this process and proinflammatory cytokines, opposite, inhibit osteoporosis and macrophage activity, contributing to normal course of inflammation in the periodontuim [15].

Thus, the results were obtained in that research suggest that introduction of the flavonol quercetin within 7 days (intramuscular at a dose of 100 mg / kg) to rats during expressed experimental bacterial-immune periodontitis promote to the normalization of the cytokine spectrum and inhibits the further development of the inflammatory process. This evidence significant activation of the protective processes of the organism in opposition to elevated production of the proinflammatory cytokines in the modelled inflammation formation. These results may be promising in the context of further experimental studies in order to be found other properties of quercetin in inflammatory processes of the maxillofacial area, in particular bacterial-immune periodontitis.

CONCLUSIONS

1. Formation and progress of the bacterial-immune inflammation in periodontum are accompanied by a complex of patho-

logical changes immunocytokinogenesis, which manifests as a progressive increase of proinflammatory cytokines content in the blood and depends on the presence the initiating inflammation antigens of bacterial and protein origin.
2. In condition of the experimental periodontitis the indications of antiinflammatory cytokines, particularly IL-10 and IL-4, occur decrease in blood serum of animals, that evidence about weakening of protective mechanisms of immune system for development of inflammatory process and formation of this pathology.
3. Flavonol quercetin reduces the content of proinflammatory cytokines (TNF-α and IL-1β) and increases the parameters of antiinflammatory cytokines (IL-10 and IL-4) in blood serum of rats with bacterial-immune periodontitis, promotes to stabilize and recover of the inflammatory process.

REFERENCES

1. Cekici A., Kantarci A., Hasturk H. et al. Inflammatory and immune pathways in the pathogenesis of periodontal disease. Periodontol 2000. 2014;64(1):57-80.
2. Hajishengallis G., Abe T., Maekawa T. et al. Role of complement in host-microbe homeostasis of the periodontium. Semin Immunol. 2013;25(1):65-72.
3. Thumbigere-Math V., Michalowicz B.S., Hodges J.S. et al. Periodontal disease as a risk factor for bisphosphonate-related osteonecrosis of the jaw. J Periodontol. 2014;85(2):226-233.
4. Gao H., Li B., Zhao L. et al. Influence of nanotopography on periodontal ligament stem cell functions and cell sheet based periodontal regeneration. Int. J. Nanomedicine. 2015;10:4009-4027.
5. Demkovych A., Bondarenko Yu., Hasiuk P. A. Oxidative modification of proteins in the process of experimental periodontitis development. Interventional Medicine and Applied Science. 2017;9(4):218-221.
6. Grygorovych E.Sh. Osobennosti klinicheskogo techeniya hronicheskogo generalizovannogo parodontita i polimorfizm genov klyuchevuh vospalitelnuh cytokinov il-1β, il-10, tnfα, il-4 [Features of the clinical course of chronic generalized periodontitis and gene polymorphism of key inflammatory cytokines IL-1beta, IL-1RN, TNFa, IL-4]. Ural Medical Journal. 2015;6:26-32. (in Russian)
7. Shang H.S., Lu H.F., Lee C.H. et al. Quercetin induced cell apoptosis and altered gene expression in AGS human gastric cancer cells. Environ Toxicol. 2018;33(11):1168-1181.
8. Mikhailova Y.A., Kravchun P.H., Petyunina O.V. et al. The influence of cardioprotection on the state of hemodyncmics at treatment of cardiorenal syndrome in patients with chronic heart failure and chronic kidney disease. International Medical Journal. 2013;19(1):44-49.
9. Costa L.G., Garrick J.M., Roquè P.J. et al. Mechanisms of neuroprotection by Quercetin: counteracting oxidative stress and more. Oxid Med Cell. Longev. 2016;7:1-10.
10. Demkovych A. Effects of flavonol quercetin on activity of lipid peroxide oxidation in experimental bacterial-immune periodontitis. Interventional Medicine and Applied Science. 2019;11(1):55-59.
11. Sennikov S.V., Silkov A.N. Metody opredileniya cytokinov [Methods for the determination of cytokines]. Cytokines and inflammation. 2005;4(1):22-27. (in Russian)
12. Berger R.L., Casella C. Statistical Inference. 2nd ed. Florida: Duxbury Press. 2001, 374 p.
13. Genco R.J., Garcia W.S., Compton R. Risk factors for periodontal disease. Risk assessment and periodontal prevention in primary care. Periodontol 2000. 2016;71(1):10-21.
14. Mkhize N.V.P., Qulu L., Mabandla M.V. The Effect of Quercetin on Pro- and Anti-Inflammatory Cytokines in a Prenatally Stressed Rat Model of Febrile Seizures. J Exp Neurosci. 2017;11:1179069517704668.
15. Patel R.V., Mistry B.M., Shinde S.K. et al. Therapeutic potential of quercetin as a cardiovascular agent. Eur J Med Chem. 2018;155:889-904.

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Conflict of interest:
The Authors declare no conflict of interest.

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