

## ORIGINAL ARTICLE

# DYNAMICS OF CHANGES OF C-REACTIVE PROTEIN LEVEL IN BLOOD SERUM IN THE DEVELOPMENT AND COURSE OF EXPERIMENTAL PERIODONTITIS AND THEIR CORRECTION BY FLAVONOL

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## ABSTRACT

**The aim:** To study the value of C-reactive protein in the experimental animals blood serum with bacterial-immune periodontitis and its correction with quercetin.

**Materials and methods:** Modeling of periodontitis was performed by the following method: after thiopental anesthesia (at a dose of 40 mg / kg intramuscularly) rats were fixed. A subcostal injection of 0.01 ml of egg protein with cultures of *Streptococcus hemolytic* and *Staphylococcus aureus* at a dose of 4 CFU was performed in the area of periodontal tissues of the lower incisor as an initiating inflammatory factor. To enhance the immune process, a complete Freund's adjuvant was introduced into the animal's hind limb at the same time.

**Results:** Analysis of the results of the study of the content of C-reactive protein in the blood serum of animals with experimental bacteria and immune periodontitis, receiving injections of quercetin, showed a significant decrease by 1.31 times, compared with animals with this simulated pathology on the 14th day of the experiment without the use of flavonol. When comparing this indicator on the 14th day of development of experimental periodontitis with correction, it was found that it remained slightly higher than the indicators of the intact group of rats.

**Conclusions:** The level of C-reactive protein in the blood serum of experimental animals is an important indicator of the immune-inflammatory response, which increases its activation of the inflammatory system. The administration of flavonoid quercetin for 7 days helps to reduce the level of C-reactive protein in the blood serum of animals with experimental bacterial and immune periodontitis.

**KEY WORDS:** Protein level, Blood serum, Experimental periodontitis, Flavonol

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## INTRODUCTION

Currently, there is an active search for new biomarkers of inflammation in patients with generalized periodontitis, with high prognostic value and assessment of factors, including carbohydrate metabolism disorders that affect the activation of various inflammatory mechanisms. Among the biomarkers known today, C-reactive protein (CRP) is probably one of the most promising indicators of inflammation and its definition occupies a special place among many clinical and laboratory tests. This is due to its ability to reflect the activity of the inflammatory process caused by bacterial infections and immunological diseases [1]. C-reactive protein is a short form of pentraxin that belongs to plasma proteins bound to calcium-dependent ligands, a superfamily of soluble pattern recognition molecules, and is mainly found as a pentameric compound [2]. The role of the protein is to facilitate the removal of microorganisms and necrotic tissues by activating cellular cytotoxic cascades. As a standard for assessing the level of systemic inflammation in patients, a highly sensitive C-reactive protein is used, which is not inferior in prognostic signif-

icance to other markers of inflammatory processes in the body. Microbe-inflammatory and destructive processes occurring in the body are characterized by changes in the concentration of plasma proteins [3].

In recent decades, the world medical community has paid special attention to the treatment and prevention of periodontal disease [4]. Eliminating the development and consequences of inflammatory processes in the periodontal complex means solving one of the global problems of oral health – tooth loss and, as a consequence, reduced masticatory efficiency and quality of life [5]. Therefore, an interesting and promising area is the study of organoprotective capabilities of quercetin, which has antioxidant, antitoxic, antiischemic, anti-inflammatory and membrane-stabilizing properties [6], which will determine new aspects of its use in patients of this category. The bioflavonoid quercetin belongs to the family of polyphenolic phytochemicals with pronounced antioxidant and anti-inflammatory properties. It is a powerful free absorber, antioxidant and anti-inflammatory agent [7]. The therapeutic effect of the drug, which belongs to the class of polyphenolic biofla-

**Table I.** Indices of C-reactive protein in the serum of experimental animals in different periods of development and course of experimental periodontitis and the use of quercetin ( $M \pm m$ )

Conditions and indicator of the experiment	Control group (intact animals)	White rats with experimental periodontitis			
		No correction		With correction	
Experiment duration (days)	-	7	14	30	14
Number of animals	10	8	8	8	8
C-reactive protein, mg / l	0,45 ± 0,01	0,75 ± 0,02 $p_1 < 0,01$	0,67 ± 0,01 $p_1 < 0,01; p_2 < 0,01$	0,64 ± 0,02 $p_1 < 0,01; p_2 < 0,01; p_3 < 0,05$	0,51 ± 0,02 $p_1 < 0,05; p_3 < 0,01$

Marking:  $p_1$  - the significance of the differences with intact animals;  $p_2$  - the significance of the differences with animals with experimental periodontitis on 7th day of the study;  $p_3$  - the significance of the differences with animals with experimental periodontitis on 14th day of the study without correction by quercetin.

vonoids, is due to its anti-inflammatory and antioxidant activity. Quercetin reduces inflammation by inhibiting the production of cytokines such as interleukin-6, interleukin-8 and tumor necrosis factor, as well as by inhibiting the activation of nuclear factor  $\kappa B$  (NF- $\kappa B$ ) [8]. In addition, a study in an animal model of inflammatory pain origin showed that quercetin reduces pain, oxidative stress and cytokine production [9].

## THE AIM

To study the value of C-reactive protein in the experimental animals blood serum with bacterial-immune periodontitis and its correction with quercetin.

## MATERIALS AND METHODS

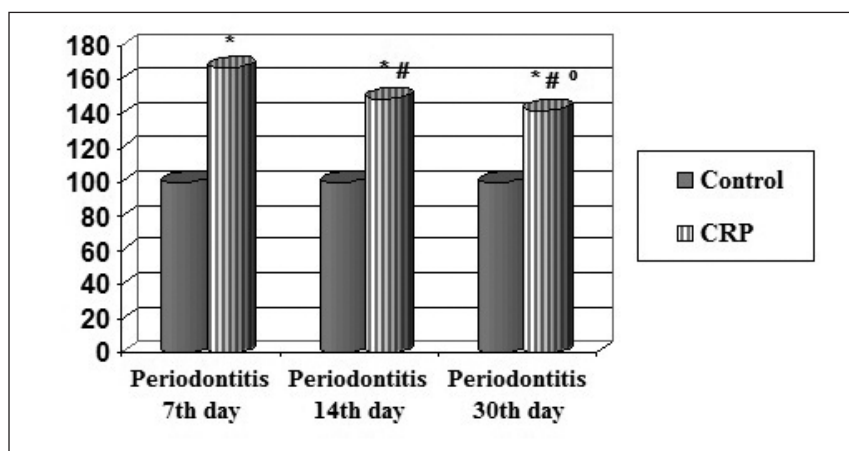
Experiments and research were conducted on the basis of the Central Research Laboratory (certificate of technical competence № 001/18 from 26.09.2018 to 28.12.2023) and the Interdepartmental training and research laboratory (certificate of technical competence № 132/17 from 29.12.2017 to 28.12.2022) of I. Horbachevsky Ternopil National Medical University on 42 nonlinear white mature male rats aged 7-8 months. Animals selected for the study were in the vivarium on a standard diet in accordance with sanitary and hygienic standards and GLP requirements. Operations were carried out in compliance 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 Experiments (Kyiv, 2001).

Modeling of periodontitis was performed by the following method: after thiopental anesthesia (at a dose of 40 mg / kg intramuscularly) rats were fixed. A subcostal injection of 0.01 ml of egg protein with cultures of Streptococcus hemolytic and Staphylococcus aureus at a dose of 4 CFU was performed in the area of periodontal tissues of the lower incisor as an initiating inflammatory factor. To enhance the immune process, a complete Freund's adjuvant was introduced into the animal's hind limb at the same time.

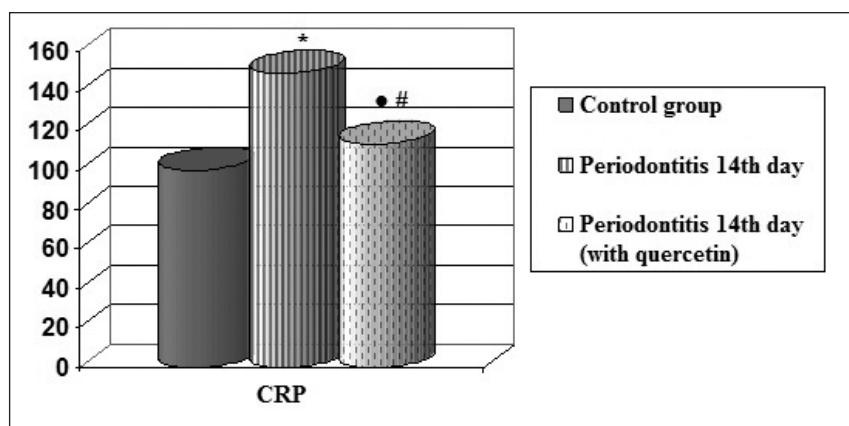
These groups of animals were studied on the 7<sup>th</sup> and 14<sup>th</sup> days (groups II and III). Group IV animals were re-injected with adjuvant pathogen on the 14<sup>th</sup> day of the development of the inflammatory process in the periodontal complex and were examined on the 30<sup>th</sup> day. Due to this, an increase in the reproduction efficiency of bacterial and immune periodontitis was achieved. As established by our previous studies, microbial contamination coincided with that in humans [10].

The level of C-reactive protein in the serum was determined by enzyme-linked immunosorbent assay according to the instructions (High Sensitivity CRP (hs-CRP) Test System «Monobind Inc.», USA). The study was performed as follows: after selecting the required number of wells, added 25 mcl of standards, controls and test samples and 100 mcl of Enzyme Reagent CRP in each well. Carefully stir them for 20-30 seconds. Incubate for 15 minutes at room temperature. The contents of the wells were removed by aspiration. 350 mcl of wash buffer and 100 mcl of substrate working solution were added to each well, incubated for 15 min at room temperature. Stopped the development of color by adding to each well 50 mcl of stop solution and stirred for 15-20 seconds. The absorbance values of the wells at a wavelength of 450 nm were measured (measurements were performed at a reference wavelength of 620-630 nm). During the analysis, Streptavidine sorbed in the cells and biotinylated antibodies to the C-reactive protein interacted on the surface of the microwells. When mixing biotinylated antibodies and serum containing CRP antigen, between CRP antigen and antibodies there was a reaction to form an antibody-antigen complex. Sequentially, the biotin bound to the antibody interacted with Streptavidine deposited in the wells, resulting in immobilization of the complex. The results were displayed in mg / l [11].

Statistical processing of digital data was performed using the software «STATISTICA» 10.0 («Statsoft», USA) using variational-statistical methods of evaluation of the obtained data. The values of the arithmetic mean ( $M$ ), its variance and error of the mean ( $m$ ), the sample size ( $n$ ) were calculated for all indicators. The reliability of the difference between the values of the independent quan-



**Fig. 1.** Dynamics of C-reactive protein content in white rats blood serum with experimental periodontitis (% of control)



**Fig. 2.** The effect of quercetin on the content of C-reactive protein in white rats blood serum with experimental periodontitis (% of control)

titative values was determined at the normal distribution by the Mann-Whitney U-test. The difference in the results of the study was considered probable when the reliability coefficient was less than 0.05 [12].

## RESULTS

Regarding the change in the content of C-reactive protein in the serum of experimental animals with periodontitis, it should be noted that its content on the 7<sup>th</sup> day of the experiment significantly exceeded (by 1.67 times;  $p < 0.01$ ) indicators that were in animals of the control group (Table I, Fig. 1).

In the rats of the experimental group, which were observed on the 14<sup>th</sup> day of the study, compared with the group of animals that were studied on the 7<sup>th</sup> day, there was a statistically significant increase the level of C-reactive protein in blood serum (by 1.12 times;  $p < 0.01$ ) (Fig. 1).

Also in this period there was a probable increase in the values of this indicator of inflammation (by 1.49 times;  $p < 0.01$ ) relative to the corresponding control. Analysis of the results obtained on the 30<sup>th</sup> day of the study showed a similar nature of changes, ie a probable increase in C-reactive protein, relative to the data of the control group (by 1.42 times;  $p < 0.01$ ).

The determination of the level of C-reactive protein in the serum on the 30<sup>th</sup> day of the experiment showed that its content in the blood was significantly lower than the

data on the 7<sup>th</sup> and 14<sup>th</sup> day of observation – by 1.17 times ( $p < 0.01$ ) and by 1.05 times ( $p < 0.05$ ), respectively.

Analysis of the results of the study of the content of C-reactive protein in the blood serum of animals with experimental bacteria and immune periodontitis, receiving injections of quercetin, showed a significant decrease by 1.31 times ( $p < 0.01$ ), compared with animals with this simulated pathology on the 14<sup>th</sup> day of the experiment without the use of flavonol (Table I).

However, when comparing this indicator on the 14<sup>th</sup> day of development of experimental periodontitis with correction, it was found that it remained slightly higher (by 1.13 times;  $p < 0.05$ ) than the indicators of the intact group of rats (Fig. 2).

## DISCUSSION

One of the indicators of tissue damage in inflammatory processes is C-reactive protein, which belongs to the so-called acute phase proteins. C-reactive protein is involved in the interaction of T- and B-lymphocytes, activates complement in the classical way. C-reactive protein stimulates protective reactions, activates immunity [13]. CRP is produced in the liver and in much smaller numbers by peripheral blood lymphocytes, during acute episodes of inflammation or infection. Determination of serum C-reactive protein level is used in the clinic as a non-specific marker for inflammation, infection and tissue damage

associated with the acute phase [14]. It should be noted that CRP is a biomarker of elevated levels of IL-1, IL-6 and TNF- $\alpha$ . In addition to its role in humoral innate immune responses, C-protein recognizes and binds several intrinsic ligands, such as the complement system, leading to a significant increase in infarct size, cell receptors, apoptotic cells, growth factors, and extracellular matrix components, and therefore contributes to the progression of cardiovascular disease [15]. Inflammation releases interleukin-6 and other cytokines that cause CRP and fibrinogen synthesis in the liver. In the process of inflammation, C-reactive protein stimulates the synthesis of pro-inflammatory factors: the release IL-1 $\beta$ , IL-6, TNF- $\alpha$  by monocytes, the expression of human endothelial cells of adhesion molecules and protein chemotaxis of monocytes-1 [16]. It also activates cholesterol synthesis, reduces the expression and activity of nitrogen monoxide (NO) in the vascular endothelium. Thus, CRP is not only a marker of the inflammatory process, but also its inducer. C-reactive protein plays a key role at all stages of this inflammatory process: it stimulates immune responses, including phagocytosis, participates in the interaction of T- and B-lymphocytes and can actively influence the activation of the complement system by inducing apoptosis, vascular cell activation, involvement leukocytes, lipid accumulation, platelet aggregation [17]. On the surface of many bacteria, the C-reactive protein forms compounds with phosphatidylcholine molecules that are strong opsonins, ie antibodies and complement factors that enhance macrophage phagocytosis and stimulate the digestion of microorganisms. C-reactive protein is a very sensitive element of blood, one of the first to respond to tissue damage [18, 19]. The presence or increase in the level of CRP in the serum is a sign of inflammation, damage, penetration of foreign microorganisms, parasites and fungi. Thus, increasing the concentration of CRP is a biochemical marker of the development of both the inflammatory process in the body in general and periodontitis in particular.

## CONCLUSIONS

1. The level of C-reactive protein in the blood serum of experimental animals is an important indicator of the immune-inflammatory response, which increases its activation of the inflammatory system. The development and course of simulated inflammation of bacterial-immune genesis is accompanied by an increase in serum concentrations of CRP throughout the period of formation of the inflammatory focus in the periodontal complex, which is associated with the response of innate humoral immune responses to antigen stimulation.
2. The administration of flavonoid quercetin for 7 days helps to reduce the level of C-reactive protein in the blood serum of animals with experimental bacterial and immune periodontitis, which may be a sign of stabilization and attenuation of the inflammatory process and one of the indicators of its effective influence on this pathogenetic link of inflammatory process in the periodontal complex.

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**Conflict of interest:**

*The Authors declare no conflict of interest.*

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