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Original Article

Quercetin reduces the transcriptional activity of NF-kB in stable coronary artery disease☆

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ABSTRACT

Objective: The aim of this study was to determine the effect of quercetin on the indicators of chronic systemic inflammation (CSI) in stable coronary artery disease (CAD).
Methods: This study included 85 patients with CAD, stable angina pectoris, functional class (FC) II, and heart failure (HF) 0-I. Each patient was prescribed beta-blockers, statins, and aspirin. In addition, a total of 30 patients, forming the study group received quercetin at a daily dose of 120 mg for two months, while the remaining 55 patients made up the control group. The levels of cytokines, such as tumor necrosis factor (TNF-α), interleukin-1β (IL-1β), and interleukin-10 (IL-10) in serum and the expression of the inhibitor of kappa B α (IkBα) gene in blood mononuclear cells, were determined.
Results: The increased levels of IL-1β and TNF-α, as well as a moderate increase in IL-10 levels, were detected in the serum of patients with CAD. The expression of the IkBα gene ($2^{-\delta Ct}$) did not differ significantly between the groups. Under the influence of quercetin, levels of IL-1β and TNF-α were reduced and IL-10 levels tended to decrease. In contrast, the serum levels of these cytokines did not change significantly in the control group. The administration of quercetin decreased the expression of the IkBα gene (0.0092 ± 0.0033 against 0.0261 ± 0.0166, $p = 0.003$; $2^{-\delta\delta Ct}$, 2.82 ± 1.39 times) in contrast to the control group.
Conclusion: Quercetin showed anti-inflammatory properties in patients with CAD, indicating a decrease in transcriptional activity of the nuclear factor of transcription kappa B (NF-kB).

1. Introduction

Chronic systemic inflammation (CSI) is an underlying cause of the development and progression of atherosclerotic vascular lesions.[1] Given the steady increase in the prevalence of coronary artery disease (CAD) which is a leading cause of mortality in the world, searching for new pathogenetically grounded therapeutic approaches that impact the inflammatory component of atherogenesis is important.

According to recent research, the polyphenolic compounds of plant origin exhibit anti-inflammatory, antioxidant, endotheliprotective, vasodilative, and many other propertiesare believed to be promising vasoprotectives.[2] One polyphenol is the flavonol 3,5,7,3′,4′-pentahydroxyflavone or quercetin, which is the aglycone of the glycoside rutin (Fig. 1). Quercetin has antiradical activity due to the presence of reactive hydroxyl groups in its structure (Fig. 2). Therefore, it reduces the formation of reactive oxygen species (ROS) by inhibiting nicotinamide adenine dinucleotide phosphate oxidase (NADPH oxidase) and xanthine oxidase, decreases the activity of cyclooxygenase (COX) and lipoxygenase (LOX), and regulates the activity of intracellular signaling cascades involved in inflammatory reactions.[3] The effectiveness of a soluble form of quercetin (Corvitin) via the i.v. the route in reducing the size of necrosis in acute myocardial infarction (AMI) and improving left ventricular systolic function has been proven in patients with congestive heart failure.[4,5]

Abbreviations: Akt, protein kinase B α; AMPK, protein kinase activated by adenosine monophosphate; AP-1, activating protein 1; ASVD, atherosclerosis; BMI, body mass index; CAD, coronary artery disease; CK, cytokines; COX, cyclooxygenase; CSI, chronic systemic inflammation; CVD, cardiovascular diseases; Echo, Doppler echocardiography; ET, vascular endothelium; FC II, functional class; HF, heart failure; IkBα, inhibitor of kappa B α; IKKα, IkB-kinase α; IL-10, interleukin-10; IL-1β, interleukin-1β; LOX, lipoxygenase; LVEF, left ventricular ejection fraction; MAP3K, protein kinase activated by mitogen 3; NF-kB, nuclear factor of transcription kappa B; Real-time PCR, real-time polymerase chain reaction; SCORE, Systematic COronary Risk Evaluation; SIRT1, sirtuin 1; TNFα, tumor necrosis factor; TRAF6, TNF-α-associated factor 6; W, Watt standard unit of power measurement.

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Table 1
Demographic and clinical characteristics of patients.

Indicator	Patients with stable CAD				p
	Control Group		Study Group		
	Abs. number	%	Abs. number	%	
Men	32	58.2	17	56.7	$p > 0.05$
Women	23	41.8	13	43.3	$p > 0.05$
Age, $X \pm \sigma$	57 ± 8.4	–	54 ± 6.2	–	$p > 0.05$
Duration of CAD	8 ± 5.6	–	7 ± 3.2	–	$p > 0.05$
Smokers	21	38.2	6	20	$p < 0.05$
Duration of smoking in years, $X \pm \sigma$	29 ± 11.7	–	27 ± 10.4	–	$p > 0.05$
Weighed heredity of CVD	42	76.4	21	70	$p > 0.05$
Presence of HF I stage	28	50.9	17	56.7	$p > 0.05$
Presence of arterial hypertension I stage low-moderate risk	38	69.1	14	46.7	$p < 0.05$
Overweight (BMI: 25–29.9 kg/m^2)	31	56.3	19	63.3	$p > 0.05$
Obesity I degree (BMI: 30–34.9 kg/m^2)	3	5.5	2	6.7	$p > 0.05$

Note – CVD: cardiovascular diseases, BMI: body mass index; X: sample mean, σ: standard deviation, p: probability.

Therefore, the aim of our study was to determine the effect of quercetin on the indicators of CSI in stable coronary artery disease (CAD).

2. Materials and methods

The study included 85 patients of both sexes (36 females and 49 males) aged between 48 and 67 years (Table 1). The patients were selected on the based on the Rose Angina Questionnaire (RAQ), the SCORE (Systematic COronary Risk Evaluation) scale, the bicycle ergometer test, and the Doppler echocardiography examination (echo). According to the requirements of the Declaration of Helsinki, a written informed consent was obtained from all study participants.

Significant differences between groups of patients were observed in attitude to smoking and the presence of arterial hypertension of the first stage. Such differences arose as a result of randomization and did not have a significant effect on the studied indicators. The inclusion criteria were the signs of CAD such as stable angina pectoris, FC II, etc. The exclusion criteria were the presence of stage 2 heart failure (HF), stage 2 hypertension, concomitant chronic diseases of the bronchopulmonary system, liver and kidney dysfunction, endocrine or allergic disorders, acute musculoskeletal system diseases, cancer, and thrombophlebitis. The bicycle ergometer test was performed using a step-by-step protocol of continuously increasing doses of the physical load with duration of 2 min in each stage. The test was considered to be "positive" if the occurrence of myocardial ischemia was objectively evidenced during the test. Each patient completed a load capacity of 75 W (Watt), corresponding to FC (functional class) II. The Doppler echocardiography examination revealed that all patients showed the signs of stage 1 left ventricular diastolic dysfunction (violation of relaxation type). The diagnosis of HF was established by the presence of clinical symptoms such as shortness of breath with exertion, palpitations, and fatigue as well as a decrease in the left ventricular ejection fraction (LVEF) within the specified range. About 39% of the patients had LVEF values of 45–50%, corresponding to stage 1 HF with preserved systolic function, while the remaining 61% of the patients had LVEF values greater than 50%. The average annual mortality risk, determined by using the combined assessment of the SCORE table and the LVEF values, was found to be less than 3% in selected patients.[6] In order to stabilize the clinical course of CAD, each patient enrolled in the study was prescribed a standard medication therapy comprising of beta-blockers (5 mg of bisoprolol once daily in the morning), statins (10 mg of atorvastatin once daily at bedtime), and 75 mg of aspirin at bedtime together with lifestyle recommendations such as diet therapy, dosed physical exertion, and smoking cessation. After a month of this standardized treatment, the patients were randomly divided into a study group of 30 patients and a control group of 55 patients and were examined using clinical and laboratory methods.

In order to assess the level of CSI, the levels of the cytokines (CKs) such as tumor necrosis factor (TNF-α), interleukin-1β (IL-1β), and interleukin-10 (IL-10) were immunoenzymatically determined in blood, using the test system "Vector-Best" (Novosibirsk, Russia), which is based on a solid-phase "sandwich"variant of the immunoenzymatic analysis with monoclonal and polyclonal antibodies.[7] Using the DT Light DNA amplifier (DNA Technology, Russia), the expression of the inhibitor of kappa B α (IkBα) gene in peripheral blood mononuclear cells was determined by the real-time polymerase chain reaction (real-time PCR).[8] In order to obtain cDNA, a set of reagents was used for the reverse transcription reaction (syntol, Russia). Total RNA was isolated from biological samples, using the reagent set "RIBO-zol-B" (AmpliSens, Russia).The sequences of the primers used for determining IkBα gene expression were F: 5′-GGC TGA AGA AGG AGC GGC TA-3′ and R: 5′-CCA TCT GCT CGT ACT CCT CG-3′. The amplification conditions were 95.0 °C, 5 min for the first cycle, followed 62.0 °C for 40 s and 95.0 °C for 15 s for 40 cycles. The "housekeeping" gene, glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was used as a reference gene (internal control) for normalizing the expression level of the target gene. Using the

Table 2
Cytokine levels in the blood serum of patients with CAD of both study groups and healthy people.

Group/Mark	TNFα, pg/mL	IL-1β, pg/mL	IL-10, pg/mL
Patients with CAD of both groups, $X \pm \sigma$	8.53 ± 3.24	9.46 ± 2.98	10.51 ± 3.33
Healthy people, Me (confidence interval)	1.6 (0–11)	0.5 (0–6)	5 (0–31)

Note – X: sample mean, σ: standard deviation, Me: median.

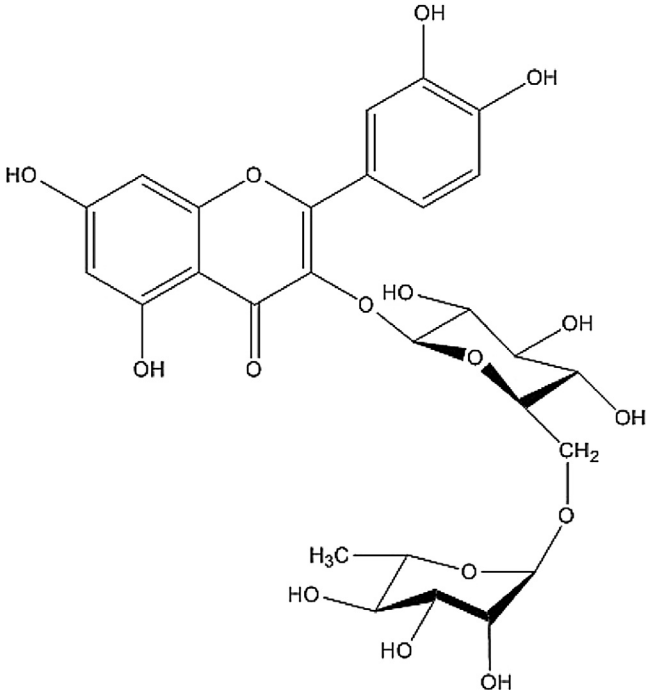

Fig. 1. Chemical structure of rutin(quercetin-3 rutinoside).

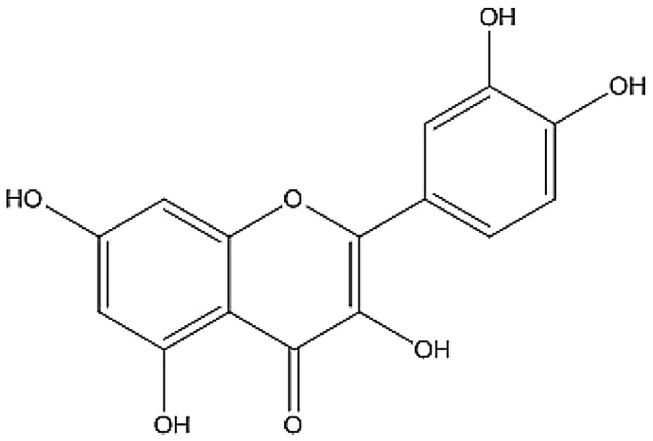

Fig. 2. Chemical structure of quercetin.

formulae $20^{-\Delta Ct}$ and $2^{-\Delta\Delta Ct}$, the relative Ct method of calculation was applied for data analysis.

Following the baseline examination, the patients from the study group were additionally prescribed quercetin at a daily dose of 120 mg that was divided into three doses per day along with the basic therapy. On the other hand, the control group continued taking the basic therapy. The patients did not keep to a special diet. The consumption of flavonoids as a part of the plant component of the diet was the same in both groups. The results of this treatment were evaluated after two months by a similar re-examination. During the examination and treatment of patients with quercetin, complications, side effects, allergic reactions or hypersensitivity to medicines were not found.

All statistical analyses were carried out using the KyPlot program. The hypothesis of normal distribution was checked by the Shapiro–Wilk test. Fisher's Criterion was used for comparing the samples according to the frequency of occurrence of the trait. Paired Student's *t*-test was used for comparing the study groups before and after the treatment. For inappropriate distribution, the Wilcoxon Signed Rank test and the Steel test for paired observations were used. Unpaired Student's *t*-test and the Steel–Dwass test (nonparametric analog of Tukey's range test) were used for comparing the data between the study groups.

The relationship between the variables was determined using Pearson's correlation test or Spearman's and Kendall's rank correlation test, subject to maldistribution. The data of statistical analysis are presented in the form of $X \pm \sigma$, where X is the average value and σ is the standard deviation. Due to improper distribution and characteristics of discontinuous variables, the data were represented as Me (Q1–Q3), where Me is the median and Q1 and Q3 are the first and the third quartiles, respectively. Statistical data were considered to be different at a significance level of $p < 0.05$.[9]

3. Results

The analysis of CKs in patients with CAD of both groups showed increased serum levels of IL-1β, TNF-α, and IL-10 (Table 2).[10]

After two months of the therapy with quercetin, there was a decrease in the level of IL–1β in the blood of the patient and also a decreasing trend for TNFα and IL-10 ($p = 0.060$ and $p = 0.064$, respectively) was noted (Table 3). On the other hand, significant changes in serum CK levels were not found in the control group.

Under the influence of quercetin, the expression of the IkBα gene in blood mononuclear cells decreased significantly in the study group relative to the control group ($p = 0.003$, Table 4).

The results of a correlation analysis, conducted on the markers of inflammation, studied in the patients with CAD, are shown in Table 5.

Moderate direct correlations between the mRNA levels of the IkBα gene and the serum levels of TNF-α and IL-10, including a weak direct correlation between the mRNA levels of the IkBα gene and the serum levels of IL-1β were revealed. The above data confirmed the consistency of CK dynamics with the activation of respective signal transduction cascades. Furthermore, the serum levels of IL-1β and IL-10, as well as those of TNF-α and IL-10, were found to be moderately correlated. However, no correlation was found between the levels of IL-1β and TNF-α in our study.

Table 3
Cytokine levels in the blood serum of the subjects of the study.

Group/Mark	Statistical index	TNFα, pg/mL		IL-1β, pg/mL		IL-10, pg/mL	
		Before therapy	After therapy	Before therapy	After therapy	Before therapy	After therapy
Control Group	X σ	8.53 ± 3.24 p = 0.866	8.34 ± 2.17	9.46 ± 2.98 p = 0.127	7.16 ± 2.98	10.51 ± 3.33 p = 0.134	8.72 ± 3.51
Study Group (**quercetin**)	X σ	7.32 ± 2.50 p = 0.060	5.74 ± 1.77	8.46 ± 3.12 p = 0.008	6.14 ± 2.44	9.92 ± 4.08 p = 0.064	6.16 ± 3.98

Note – X: sample mean, σ: standard deviation, p: probability.

Table 4
The level of mRNA IkBα expression in peripheral blood mononuclear cells of the subjects of the study.

Group/Mark	Statistical index	Control Group		Study Group (quercetin)	
		Before treatment	After treatment	Before treatment	After treatment
Expression mRNA IkBα, $2^{-\delta Ct}$	X σ	0.0234 ± 0.0198	0.0253 ± 0.0155	0.0261 ± 0.0166	0.0092* ± 0.0033
	p	0.570		0.003	
$2^{-\delta\delta Ct}$	X (min-max)	0.120 (−2.64 to +2.83)		−2.817* (−5.66 to −1.07)	

Note – X: sample mean, σ: standard quadratic deviation, (min-max): extreme value variation series, p: probability.
* Significant differences between the groups before and after the treatment ($p < 0.01$).

4. Discussion

The increased levels of proinflammatory CKs in terms of atherogenesis result from the activation of immunocompetent cells and the endothelium (ET), as evidenced by numerous scientific studies.[1,11] IL-1β and TNF-α, the two leading mediators of inflammatory response, are synthesized under the influence of pathogenic stimuli due to an increase in the transcriptional activity of the NF-kB gene.[1,11] In turn, these CKs support the functioning of proinflammatory signal transduction cascades, involving NF-kB and activating protein-1 (AP-1) by modulating the activity of various protein kinases and phosphatases.[11,12] IL-1β also activates the expression of the anti-inflammatory CK, IL-10, by the AP-1 signaling pathway that explains the slightly elevated levels of IL-10 observed in patients with CAD.[13]

Under the influence of standard medication, no changes were found in the levels of CKs in the comparison group. Arguably, in our study, the selected dosage regimen and the period of using statins were not sufficient to exert their anti-inflammatory effects, as previously found.[14] There was no effect of statins on the expression level of the IkBα gene, which justifies the lack of significant dynamics of CK levels.

NF-kB-dependent mechanisms are the basis of chronic diseases with constantly high levels of CKs.[15] The reduction in the expression of the IkBα gene under the treatment of quercetin, which is associated with the decreased transcriptional activity of NF-kB, is accompanied by the reduced levels of IL-1β in blood. Taken together, these effects confirm the anti-inflammatory activity of this polyphenol at different levels of signal transduction. IL-1β is of immense pathogenetic importance in the inflammatory activation of ET. IL-1β is related to TNF receptor-associated factor 6 (TRAF6) action on IkB kinase-α (IKKα), which destroys the relationship of the dimer NF-kB p50/p65 with IkBα, thereby leading to the translocation of the p65 subunit into the nucleus with the transcription of the genes of various inflammatory molecules, including the very same IL-1β. Similar effects characterize the activation of the mitogen-activated protein kinase (MAP3K, protein kinase activated by mitogen 3)/AP-1 signaling pathway, involving IL-1β).[11,16,17]

As signal transduction by NF-kB stimulates the simultaneous expression of IkBα, these indicators change unidirectionally. Brown et al found that the activation of NF-kB is regulated by a feedback mechanism through its interaction with IkBα, which is saturated with free NF-kB subunits to form dimers. Following the restoration of this balance, excess IkBα is quickly degraded.[18] Therefore, in our study, the decrease in the expression of the IkBα gene as well as the serum levels of IL-1β, as determined by the effects of quercetin, confirm its role as an inhibitor of inflammatory transduction involving NF-kB.

The process of IL-10 expression is associated with the AP-1 signaling pathway in which, activating molecules such as IL-1β and CD40 ligand from the TNF family can act through their respective receptors. The activation of NF-kB may be accompanied by an increase in the anti-inflammatory CK, IL-10, as TRAF6 is involved in all these signaling pathways. However, the inactivation of NF-kB does not affect the process of IL-10 expression through the AP-1 signaling pathway, which could explain the lack of significant dynamics of CK levels in our study.[13]

The recent scientific studies have shown the effectiveness of quercetin in activating the protein sirtuin 1 (SIRT1), which makes the histone backbone denser and prevents the transcription of genes; this results in different pathogenic effects. Quercetin inhibits atherosclerotic vascular disease (ASVD) induced by oxidized low-density lipoprotein mediated endothelial damage by activating the SIRT1 protein and modulating the AMP-activated protein kinase (AMPK—activated by adenosine monophosphate)/NADPH oxidase/Akt1 (protein kinase B α)/endothelial nitric oxide synthase (eNOS) signaling pathways.[19,20] In addition to the proven positive effects of quercetin, such as antioxidant activity, protection of membranes from oxidative damage by preventing lipid peroxidation, and enhancing membrane rigidity, perhaps this mechanism has also influenced the results of our study.[21] All the above effects support the cardioprotective action of quercetin.

Of the seven large observational studies conducted between the years 1961 and 2000, six revealed a reduction in the risk of CAD when a diet rich in flavonols was consumed.[22] The effects of quercetin, observed in our study at the molecular and epigenetic levels, can explain some of the mechanisms of its protective action.

Table 5
Correlation coefficients between the inflammatory mediators in patients with stable coronary artery disease.

mRNA IkBα	**0.360****	**0.270***	**0.382****
IL-10	**0.491****	**0.377****	
IL-1β	–		
TNFα			
	TNFα	IL-1β	IL-10

Note.
* $p < 0.05$.
** $p < 0.01$.

5. Conclusion

Thus, unlike statins (atorvastatin), the application of quercetin basic therapy in patients with stable coronary artery disease reveals a positive impact on the indicators of chronic systemic inflammation during a two-month treatment period. Quercetin reduces the levels of IL-1β and, to a lesser extent, TNF-α in blood, in addition to reducing the levels of IkBα mRNA, thereby indicating a decrease in the transcriptional activity of NF-kB. Arguably, the anti-inflammatory effects of quercetin may be due to its impact on the different levels of signal transduction. The results obtained in our study substantiate the relevance of further studies on the molecular mechanisms underlying the anti-inflammatory activities of quercetin and its widespread use in the treatment of coronary artery disease.

Conflict of interest

None.

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