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# Resveratrol reduces the level of chronic systemic inflammation in stable coronary artery disease

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## Abstract

This study aimed to investigate the effects of plant polyphenol of resveratrol on chronic systemic inflammation indicators of stable coronary artery disease. 85 patients with coronary heart disease were recruited and prescribed a standard therapy (β-blockers, statins, aspirin). 30 patients received resveratrol at a dose of 100 mg daily and the other serves as the control group. Cytokines and the expression of mRNA gene of inhibitor of kappa B α (IkBα) of nuclear factor of transcription kappa B (NF-kB) were determined. The results show that patients with coronary artery disease exhibited increased levels of interleukin-1β (IL-1β), tumor necrosis factor (TNFα), and IL-10 in the blood. Resveratrol treatment led to a reliable reduction IL-1β and TNFα, the content of IL-10 tended to reduce. In addition, we failed to notice any significant difference in the inhibitor of kappa B α (IkBα) of nuclear factor of transcription kappa B between groups. In conclusion, in patients with coronary artery disease, resveratrol shows anti-inflammatory properties via reducing the content of proinflammatory cytokines in the blood, such as IL-1β and TNFα.

**Abbreviations and symbols:** CSI: Chronic systemic inflammation; CHD: Coronary heart disease; CK: Cytokines; TNFα: Tumor necrosis factor; IL-1β: Interleukin-1β; IL-10: Interleukin-10; IkBα: Inhibitor of kappa B α; NF-kB: Nuclear factor of transcription kappa B; Real-time PCR: Polymerase chain reaction in real time; ASVD: Atherosclerosis; ET: Vascular endothelium; COX: Cyclooxygenase; LOX: Lipoxygenase; IKKα: IkB-kinase α; eNOS: Endothelial synthetase of nitric oxide; TET: Treadmill exercise test; HF: Heart failure; LVEF: Left ventricular ejection fraction; EQ-5D-3L: Health-related quality of life questionnaire 3 level versions; EQ-5D-VAS: EQ-5D visual analogue scale; SIRT1: Sirtuin 1; NO: Nitric oxide; EMP: Circulating endothelial microparticles; STAT1: Transcription activator transmitting a signal 1; IRF-1: Factor regulating interferon 1

## Introduction

Atherosclerosis (ASVD) has been still relevant and unsolved problem of clinical medicine. Coronary heart disease (CHD), which morphological basis is ASVD, heads the list of the top 10 causes of death in the world and is accounted for 12.8%. In Ukraine, CHD is among leading causes of cardiovascular mortality (68.8%) [1].

Chronic systemic inflammation (CSI) is the pathogenetic basis of ASVD. Under the influence of damaging factors, such as free radicals, endo- and exotoxins, high blood pressure, etc., vascular endothelium (ET) is activated with the development of its systemic dysfunction: functioning molecular signaling cascades with increased synthesis of proinflammatory cytokines (CK) and adhesion molecules is enhanced, the transmembrane transport and regulation of vascular tone are disrupted [2]. These data substantiate the relevance of active search for CSI correction agents at ASVD in order to improve treatment.

Recently, researchers attention has been drawn to the polyphenolic compounds of plant origin. Polyphenols possess hydrophilic properties and play the role of free radical scavengers, which lead to their antioxidant effect. They also activate paraoxonase in the blood promoting hydrolysis of hydroperoxides [3]. Anti-inflammatory, anti-adhesive, angiogenic, vasodilative and many other properties of polyphenols have been found out, which are implemented mainly through effects on molecular targets of intracellular cascades [4].

One of the representatives of the plant polyphenol is phytoalexin resveratrol (3,4,5-trihydroxy-trans-stilbene) found in more than 30 kinds of plants and used in medicine as a natural extract and synthetic drug [5]. Resveratrol has a direct antiradical action by means of three hydroxyl groups in its chemical formula, regulates the activity of enzymes of cyclooxygenase (COX) and lipoxygenase (LOX), has anti-inflammatory activity-it inhibits the pathogenic effect of a key factor of activating process of inflammation of NF-kB through inhibition of IkB-kinase α (IKKα), and has endothelio protective effect through activation of endothelial synthetase of nitric oxide (eNOS) as well [6-8].

The aim of our research was to study the effects of resveratrol on the CSI indicators in stable CHD.

## Materials and methods

The study involved 85 people of both sexes (36 females and 49 males) aged 48–67, diagnosed with CHD, stable angina pectoris, FC II, HF 0-I, the average risk. The selection of patients was carried out using an objective and instrumental examination: Rose angina questionnaires, SCORE table, bicycle ergometer and Doppler echocardiography. Every patient gave a written informed consent to participate in the research, according to the requirements of the Declaration of Helsinki. The criterion for inclusion into the study was signs of coronary

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heart disease: stable angina pectoris, FC II. Exclusion criteria were the presence of Stage 2 heart failure (HF), Stage 2 hypertension, concomitant chronic diseases of bronchopulmonary system, liver and kidney dysfunction, endocrine or allergic disorders, diseases of the musculoskeletal system in the acute stage, cancer, thrombophlebitis. Bicycle ergometer was used with a continuously increasing step-by-step protocol of dosed physical load with duration of one stage of 2 minutes, the test was considered to be "positive" in the case of occurrence of objective evidence of myocardial ischemia during the trial. Every patient completed a load capacity of 75 W, which corresponded to FC II. The presence of heart failure (HF) syndrome was established by clinical data and results of Doppler echocardiography. In the presence of clinical symptoms (shortness of breath with exertion, palpitations, fatigue) and decrease in left ventricular ejection fraction (LVEF), the diagnosis of HF was established. 43% of CHD patients had LVEF values of 45–50%, corresponding to the degree of heart failure of Stage 1 with preserved systolic function, and the rest had ejection fraction greater than 50%. All the patients showed signs of diastolic dysfunction of the left ventricle with impaired relaxation (Type 1). The degree of risk was being determined by the total assessment using SCORE table and LVEF values that in all patients of study groups was less than 3% of annual mortality risk (the average level) [9]. Every patient was prescribed a standard CHD therapy: along with recommendations for lifestyle (diet therapy, dosed physical exertion, smoking cessation), beta-blockers (5 mg of bisoprolol once a day in the morning), statins (10 mg of atorvastatin once a day at bedtime) and 75 mg of aspirin at bedtime were prescribed. After stabilization of the clinical course of CHD in a month after the basic treatment patients were divided into the study group (30 people) and comparison ones (55 people) by random sampling and examining with clinical and laboratory methods.

For objectification of patients' condition the Health-Related Quality of Life Questionnaire 3 level version (EQ-5D-3L) with the estimation of EQ-5D index and visual analogue scale (EQ-5D-VAS) data analysis were used [10].

To assess the level of CSI, the level of cytokines (i.e. tumor necrosis factor (TNFα), interleukin-1β (IL-1β), and IL-10) in the blood was determined via immunoenzymatic method using the test system "Vector-Best" (Novosibirsk) based on the solid-phase "sandwich"–variant of immunoenzymatic analysis with mono- and polyclonal antibodies [11]. The expression of the gene of inhibitor of kappa B α (IkBα) in peripheral blood mononuclear cells was determined by polymerase chain reaction in real time (real-time PCR) using the DT Light DNA amplifier ("DNA Technology", Russia) [12]. To obtain cDNA, a set of reagents for the reaction of inverse transcription (SYNTOL, Russia) was used. The total RNA was isolated from biological sample using the reagent set "RIBO-zol-B" (AmpliSens, Russia). The sequence of primers for determining IkBα gene expression-F: 5'–GGC TGA AGA AGG AGC GGC TA–3', R: 5'–CCA TCT GCT CGT ACT CCT CG–3'. Amplification mode: 95.0–5 minutes–1 cycle; 62.0–40 seconds, 95.0–15 seconds–40 cycles. As a reference gene the "housekeeping" gene GAPDH was used. For data analysis a relative Ct method of calculation by the formula $2^{-\Delta Ct}$ and $2^{-\Delta\Delta Ct}$ was applied.

After the examination of patients of the research group they were additionally prescribed resveratrol at a dose of 100 mg once daily per os on the background of basic therapy; the comparison group continued taking basic therapy. The results of treatment were evaluated after 2 months by re-examination in the above mentioned amount. During the examination and treatment of patients any complications, allergic reactions or hypersensitivity to medicines were not found.

Statistical analysis of the results of the research was carried out using KyPlot program. The hypothesis of normal distribution was checked by Shapiro-Wilk test. When comparing these study groups before and after treatment, paired Student's t-test was used, for inappropriate distribution-Wilcoxon signed-rank test and Steel test for paired observations. When comparing data between groups, unpaired Student's t-test and Steel-Dwass test (nonparametric analogue of Tukey's range test) were used. Search for relations between variables was held using Pearson`s correlation or, subject to maldistribution, Spearman`s and Kendall`s rank correlation. Data of statistical analysis were presented in the form of X ± σ, where X is an average value, σ is an average square deviation. Due to improper distribution and characteristics of discontinuous series the data were given as Me (Q1-Q3), where Me is a median, Q1 and Q3 are the first and third quartiles. Data differences were considered to be significant at a level of $p<0.05$ [13].

## Results and discussion

Mean EQ-5D-index before treatment in patients with coronary artery disease was 0.738 ± 0.061, EQ-5D-VAS–55.63 ± 5.38. After 2 months of treatment patients treated with resveratrol more often than the comparison group patients noted the appearance of vitality, efficiency improving, reducing the number and duration of episodes of pain in the heart. EQ-5D-index ($p<0.001$) and values by EQ-5D-VAS scale ($p<0.001$) decreased. In the comparison group EQ-5D-index also decreased (<0.001) after treatment, EQ-5D-VAS did not significantly change ($p > 0.05$). Data of EQ-5D questionnaire have been shown on Figure 1.

Patients with coronary artery disease have shown an increased content of IL-1β (9.76 ± 3.33 pg/mL) (in healthy people–1.6 (confidence interval–0-11 pg/mL)), TNFα (9.11 ± 2.43 pg/mL) (in healthy people–0.5 (0-6) pg/mL), IL-10 content was 10.97 ± 2.97 pg/mL (in healthy people–5 (0-31) pg/mL), corresponding with modern scientific data on changes in the CK level in terms of atherogenesis [2,14]. IL-1β and TNFα are one of the leading mediators of inflammatory response, inducing production of proinflammatory CK, chemoattractants, adhesion molecules, growth factors through increasing the transcriptional activity of NF-kB.

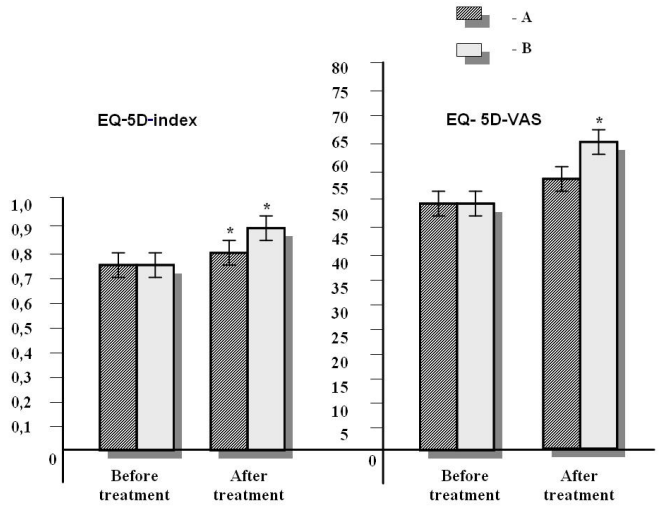


**Figure 1.** Dynamics of subjective status of patients according to the questionnaire EQ-5D-3L [*: Reliable difference between indicators before and after treatment; A: Group of comparison; B: Group of investigation (resveratrol)].

After 2 months of therapy with resveratrol in patients there was a decrease of the level of CK (IL-1β and TNFα), and the tendency to lower levels of IL-10 (0.05>p<0.1) was noted (Table 1). However, in the comparison group presumable changes in the levels of the CK were not found, despite the statements on anti-inflammatory effect of statins [15]. Perhaps for pleiotropic anti-inflammatory effect larger therapeutic doses of statins are necessary, but with building-up a dose the probability of complications increases, especially in chronic forms of coronary artery disease (according to large-scale studies TNT, IDEAL) [16]. Therefore, the combination of statins with resveratrol can afford to get the necessary pathogenetically valid therapeutic effect on the ability to titrate the dose of statins, focusing exclusively on the blood lipid spectrum and levels of transaminases with guaranteed anti-inflammatory effect from the lowest doses due to the combined potential effect of these drugs.

Recent scientific studies have shown that effectiveness of resveratrol to reduce the level of inflammatory CK is implemented by several mechanisms: by direct antioxidant action as free radical scavenger due to the presence of OH-groups, by activation of catalase, superoxide dismutase, increased levels of glutathione transferase, peroxidase and reductase, decreased activity of COX and LOX, regulation of nitric oxide (NO) synthesis, increased expression level of protein sirtuin 1 (SIRT1) providing density of histone backbone and preventing activation of IKKα [17,18]. IKKα subunit has the greatest importance in the structure of the IKK because being activated it destroys IkBα connection with the dimmer NF-kB (p50/p65). NF-kB p65 (Rel A) subunit, which is the gene transcription factor of molecules of inflammatory response, is translocated into the nucleus [19]. Each of these mechanisms can contribute to our results of reducing IL-1β and TNFα under the influence of resveratrol. It has been proven that inactivation of NF-kB does not affect IL-10 expression processes, which can be an explanation for no reduction of CK in our study [20].

In patients treated with resveratrol, as in the comparison group, the expression of mRNA IkBα in blood mononuclear cells did not change significantly (p=0.441 and p=0.570 respectively). IkBα retains NF-kB in an inactive state in the cytosol of cells, preventing the transcription of inflammatory molecules. Reduced synthesis of proinflammatory CK in our study could be achieved by other mechanisms of resveratrol action, such as antioxidant one or activation of SIRT1 (Table 2).

The revealed ability of resveratrol to reduce the content of EMP $CD32^{+}CD40^{+}$ demonstrates its anti-inflammatory and endothelioprotective properties [21]. It is known that increasing the number of EMP is the evidence of ET dysfunction. Predominant cause of this increasing may be determined by definition of the expression of appropriate molecules. Thus, for EMP of apoptotic endotheliocytes typical are CD31 and CD105 markers, for EMP, forming activated endotheliocytes,-CD32 (FcγRII), CD40 (TNFRSF5), CD54 (ICAM-1), CD62 (E та P), CD64 (FCGR1B), CD146 markers [22]. Inflammatory activation of ET, which marker is EMP $CD32^{+}CD40^{+}$ studied, is considered to be an essential component of atherogenesis [23].

Arguably, resveratrol acts on one of the above-mentioned mechanisms inhibiting proinflammatory signal transduction. According to scientific data, inhibition of STAT1 (transcription activator transmitting a signal–1) or IRF-1 (factor regulating interferon 1), but not NF-kB, inhibits the expression of CD40 molecule at mRNA and protein level [24,25]. Despite the lack of probable reduction of mRNA IkBα expression under resveratrol in our study, a positive effect of resveratrol on levels of proinflammatory CK has been found out. In our opinion, inhibition of STAT1 or IRF-1 may also be one of the possible mechanisms of its action [26].

## Conclusions

The intake of resveratrol in patients with stable coronary heart disease reveals a positive impact on the level of systemic inflammation during two-month treatment, unlike statins (atorvastatin) as a means of basic therapy. Along with improved clinical course, according to the EQ-5D questionnaire, resveratrol reduces the levels of proinflammatory CK IL-1β and TNFα, but the impact of resveratrol on the IkBα expression at mRNA level has not been revealed. Mechanisms of anti-inflammatory activity of resveratrol are multimodal and cause CSI correction involving different signaling cascades. Taking into account proven anti-inflammatory effect by endpoints of assessment of the inflammatory response level, resveratrol should be considered as effective pathogenetically reasonable treatment for ASVD and coronary artery disease.

**Table 1.** Cytokines 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 |
| Group of comparison | X | 8.53 | 8.34 | 9.46 | 7.16 | 10.51 | 8.72 |
| | σ | ±3.24 | ±2.17 | ±2.98 | ±2.98 | ±3.33 | ±3.51 |
| | | p=0.866 | | p=0.127 | | p=0.134 | |
| Group of investigation (resveratrol) | X | 9.69 | 7.28 | 10.05 | 6.98 | 11.41 | 9.39 |
| | σ | ±1.63 | ±2.18 | ±3.67 | ±2.52 | ±2.61 | ±3.06 |
| | | p=0.013 | | p=0.002 | | p=0.055 | |

X: The sample mean; σ: Standard deviation; p: The probability

**Table 2.** Level of mRNA IkBα expression in peripheral blood mononuclear cells of the subjects of the study.

| Group / Mark | Statistical index | Group of comparison | | Group of investigation (resveratrol) | |
|---|---|---|---|---|---|
| | | Before treatment | After treatment | Before treatment | After treatment |
| Expression mRNA IkBα. $2^{-\Delta\Delta Ct}$ | X | 0.0234 | 0.0253 | 0.0246 | 0.0220 |
| | σ | ±0.0198 | ±0.0155 | ±0.0131 | ±0.0092 |
| | p | 0.570 | | 0.441 | |
| $2^{-\Delta\Delta Ct}$ | X | 0.120 | | -0.142 | |
| | (min:max) | (-2.64:+2.83) | | (-2.0:+1.87) | |

X: The sample mean, σ: Standard quadratic deviation, (min:max): Extreme value variation series; p: The probability; *: Significant differences with the data of all groups before and after treatment (p<0.01)

## Conflict of interests

Authors have accepted full responsibility for the conduct of the study, had access to the data, and controlled the decision to publish it. All authors have made a significant contribution to the preparation of the manuscript, acquisition and analysis of the study data. The study was funded by the authors. The results do not reflect the interests of any organizations and personalities. Our scientific work has no competing interest.

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