

ANTI-INFLAMMATORY EFFECTS OF RESVERATROL IN STABLE CORONARY ARTERY DISEASE AND CONCOMITANT AUTOIMMUNE THYROIDITIS

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Summary

This study aims to examine the effect produced by resveratrol on markers of chronic systemic inflammation in patients with coronary artery disease (CAD) and concomitant autoimmune thyroiditis (Hashimoto's thyroiditis). The study enrolled 85 patients with coronary artery disease: stable exertional angina, class II, 30 of them were found out to have diagnosis of autoimmune thyroiditis (AIT) in its euthyroid state (experimental group), 55 patients made up the control group. In a month of CAD stabilization therapy (β -blockers, statins, aspirin), the levels of interleukin-1 β (IL-1 β), IL-10, tumor necrosis factor (TNF- α) and expression of mRNA inhibitor of kappa B gene (IkB) nuclear factor kB (NF-kB) transcription were measured in the blood of the patients. Then, the experimental group was additionally prescribed to take resveratrol in a dose of 100 mg per day. In 2 months since the beginning of the therapy including resveratrol the patients underwent re-examination. The patients with CAD and AIT comorbidity were found to have increased levels of IL-1 β and TNF- α , moderate increase in IL-10. The findings obtained did not significantly differ from the data from those in the control group. Expression of mRNA IkB was identical in both groups. Under the influence of resveratrol in the patients of the experimental group the levels IL-1 β and TNF- α were significantly decreased, the level of IL-10 was unchanged, and the expression of mRNA IkB α decreased ($p < 0,05$). All these findings of the control group stayed unchanged. The results of the study demonstrate the anti-inflammatory properties of resveratrol in cases of CAD and concomitant AIT that are associated with reduced transcriptional activity of NF-kB.

Keywords: resveratrol, coronary artery disease, autoimmune thyroiditis, chronic systemic inflammation, NF-kB.

ПРОТИВОВЪЗПАЛИТЕЛНИТЕ ЕФЕКТИ НА РЕСВЕРАТРОЛ СЪС СТАБИЛНА ИСХЕМИЧНА БОЛЕСТ НА СЪРЦЕТО В СЪЧЕТАНИЕ С АВТОИМУНЕН ТИРЕОИДИТ

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Резюме

Целта на проучването е да се изследва ефекта на ресвератрол върху маркери на хронично системно възпаление при пациенти с исхемична болест на сърцето (CHD) във връзка с автоимунно тиреоидит (тиреоидит на Хашимото). В изследването са участвали 85 пациенти с исхемична болест на сърцето: ангина, стабилно напрежение, FC II, 30 от които имаше допълнителна диагностика на тиреоидит (проучвателна група) на Хашимото, тиреоиден опция, 55 пациенти, направени от групата сравнение. След един месец за стабилизиране CHD терапия (бета-блокери, статини, аспирин), при пациенти определяне нивата на интерлевкин-1 β кръвта (IL-1 β), IL-10, тумор некрозен фактор (TNF- α) и експресия на ген мРНК инхибитор капа В (I κ B) ядрен транскрипционен фактор κ B (NF- κ B). След това групата на проучване допълнително назначен ресвератрол в доза от 100 мг на ден и 2 месеца са повтори проучвания. ИБС пациенти в комбинация с повишени нива на АИТ намерени IL-1 β , TNF- α , IL- умерено повишаване на 10, данните не са значително различни от резултатите на контролната група. Експресията на мРНК I κ B е идентичен и в двете групи. Под влиянието на ресвератрол при пациенти с нива на изследването IL-1 β , TNF- α значително намалява нивото на IL-10 не се променя експресията на мРНК I κ B α намалява ($p < 0,05$), в сравнение, тези показатели не са се променили група. Тези резултати показват, противовъзпалителните свойства на ресвератрол в комбинация с CHD АИТ, свързани с намалена транскрипционна активност на NF- κ B.

Ключови думи: ресвератрол, исхемична болест на сърцето, автоимунен тиреоидит, хроничен системно възпаление, NF- κ B.

Introduction

Coronary artery disease (CAD) has remained one of the leading causes of death throughout the world [3]. The incidence of endocrine disorders is also reported to be increasing significantly, and thyroid diseases make up their largest part. In Ukraine, the prevalence of autoimmune thyroiditis (AIT) known as the underlying cause of hypothyroidism has increased by 68% over the past decade [4]. Rising stress level, environmental pollution, poor nutrition is considered as primary causes of the disease and underlies the doleful statistics. Increasingly, there is a problem of controlling and managing the comorbidities.

According to relevant research data, the development and progression of atherosclerosis regarded as the morphological basis of CAD is strongly associated with chronic systemic inflammation (CSI), which is also known to play a leading role in the pathogenesis of autoimmune thyroiditis.

A lack of CD4+CD25+ regulatory T cells described in both pathological conditions above mentioned is the main cause resulting in insufficient suppression of inflammation. Both processes are characterized by presence of dendritic cells in the affected area of the antigen forming molecules of major histocompatibility complex, by impaired selection of CD4 + T killer cells, which then develop autoaggressive characteristics and B-cell response with the synthesis of autoantibodies [8, 9]. However, the main factor resulting in the impairment in both CAD and AIT is cytokines (CK) released during the activation of immune and other cells involved in the disease process that mediate the cellular interactions. Cytokines are the main transmitters of inflammation signal transduction, activating cascades that mediate the formation of adhesion molecules, chemoattractant compounds, growth factors, and closing the "vicious circle" of inflammation [6, 8]. Cytokines can also potentiate apoptosis of activated cells. It is well established that in the condition of AIT 30% of thyrocytes undergo apoptosis. The antibodies in the pathogenesis of AIT according to recent reports are of less pathogenic significance, being described mostly as markers of the disease they do not determine the intensity of its course and its prognosis [15].

The thoughts hereinabove explained require elaborating general pathogenetically reasonable approaches to the management of CAD and AIT, and particularly by influencing CSI.

The aim of this research was to study the influence produced by resveratrol, a polyphenol antioxidant, on the level of systemic inflammation in patients with coronary artery disease and concomitant autoimmune thyroiditis.

Materials and methods

The study involved 85 patients aged 48-67 with CAD, stable exertional angina class II 0 CH-I, 30 of whom diagnosed to have AIT, its euthyroid state, made up the experimental group, the rest 55 patients were assigned to the control group. Diagnosis of CAD was established in accordance with the standards of the European Society of Cardiologists, 2013, the diagnosis of AIT was made based on standards set by European Thyroid Association, 2013 [11, 16]. Exclusion criteria were hypertension stage II above, rheumatism, diabetes, chronic liver and kidney diseases, musculoskeletal inflammatory diseases in the acute stage, cancerous diseases. All the patients gave written informed consent to participate in the study, as required by the Helsinki Declaration, 1975. According to laboratory investigations, 5 patients were diagnosed to have subclinical hypothyroidism, and 2 patients had hypothyroidism. The day prior the inclusion in the test group the patients achieved euthyroid state by taking proper doses of L-thyroxine (25-75 mg daily).

In order to stabilize the clinical course all the patients were prescribed the standard CAD therapy: beta-blockers (bisoprolol, 5-10 mg per day), statins (atorvastatin, 10 mg daily), aspirin (100 mg per day). Short-acting nitrates (isosorbide dinitrate) were taken by the patients when necessary. According to the clinical guidelines, in cases of euthyroid AIT no treatment was carried out.

One month since the beginning of stabilization therapy all the participants passed through laboratory studies including evaluation of blood serum cytokines: interleukin-1 β (IL-1 β), tumour necrosis factor α (TNF- α) and IL-10 by ELISA using test-system "Vector-Best" (Novosibirsk, Russia) that was based on sandwich solid-phase ELISA with using mono- and polyclonal antibodies [1]. We also assessed the gene expression inhibitor of nuclear factor-kappa B α (I κ B α) nuclear factor

transcription kB (NF-kB) in peripheral blood mononuclear cells by polymerase chain reaction in real time (Real-time PCR) using a thermocyclers DT Light ("DNA Technology", Russia) [12]. To obtain cDNA we used a set of reagents for the reaction of inverse transcription (SYNTHOL, Russia). The total RNA was isolated from biological sample using a reagents kit "Rybo-sol-B» (AmpliSens, Russia). The sequence of primers for determining gene expression I κ B α - 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 we used a housekeeping gene GAPDH. The $2^{-\Delta\Delta CT}$ and $2^{-\Delta Ct}$ methods were used as a relative quantification strategy for quantitative real-time polymerase chain reaction data analysis.

Having passed the examination described above, the patients of the experimental group were prescribed to take resveratrol in a single oral dose of 100 mg per day in addition to conventional therapy. The control group took the course of conventional treatment. The results of treatment were evaluated in 2 months by re-examining the patients of both groups. During the examination and treatment patients had no complications, allergic reactions and hypersensitive to medicines.

Statistical processing of the findings obtained was carried out by the licensed program KyPlot. The assumption that the data followed a normal distribution was checked by Shapiro–Wilk test. To compare the findings of patients' examinations before and after the course of therapy paired Student t-test was used, in cases of abnormal distribution non-parametric paired Wilcoxon test was performed as well as the Still-test was used for paired observations. To compare data between the groups we performed unpaired Student t-test and Steel-Dwass test (nonparametric analogue of Tukey's test). Data of statistical analysis were presented in the form of $X \pm \sigma$, where X was the average value, σ was the standard deviation. On the assumption of misdistribution and signs of discontinuous series the data were given as Me (Q1-Q3), where Me – median, Q1 and Q3 - upper and lower quartiles. Differences between the results were regarded as significant when the significance threshold was kept at $p < 0.05$.

Results

The patients with CAD and comorbid AIT demonstrated elevated levels of cytokines ($p < 0.05$) (Table 1). In the healthy individuals IL-1 β equalled 1.6 (percentile interval - 0-11) pg/ml, TNF- α — 0,5 (0-6) pg/ml, IL-10 — 5 (0-31) pg/ml [5]. The control group also demonstrated increased cytokines under the study (Table 1). No significant difference between the values of the groups compared was found ($p > 0.05$).

In 2 months after the completion of the therapy including resveratrol the patients with CAD and concomitant AIT were observed to have significantly decreased levels of IL-1 β and TNF- α , while there were no significant changes in the level of IL-10. The control group tended to decrease IL-1 β and IL-10, but differences in values before and after the therapy were not significant ($p > 0.05$) (Table 1).

Table 1

Cytokines levels in the blood serum of the subjects of the study

Group/ Parameter	Statistical indicator	TNF α , pg/ml		IL-1 β , pg/ml		IL-10, pg/ml	
		Before therapy	After therapy	Before therapy	After therapy	Before therapy	After therapy
Control group CAD	X	8,53	8,34	9,46	7,16	10,51 \pm	8,72
	σ	$\pm 3,24$	$\pm 2,17$	$\pm 2,98$	$\pm 2,98$	3,33	$\pm 3,51$
		p = 0,866		p = 0,127		p = 0,134	
Experimental group CAD+AIT (resveratrol)	X	10,54	7,94	10,06 \pm	6,87	10,08 \pm	9,19
	σ	$\pm 2,42$	$\pm 3,43$	2,79	$\pm 2,13$	3,99	$\pm 2,68$
		p = 0,0005		p = 0,0011		p = 0,455	

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

The patients with CAD and those who had CAD and concomitant AIT demonstrated no significant difference ($p > 0.05$) in the levels of mRNA I κ B α gene expression in blood mononuclear cells. Presumably due to the effect of resveratrol the patients showed significantly decreased ($p = 0.003$) mRNA I κ B α gene expression in contrast to the results obtained in the control group (Table 2).

Level of mRNA I κ B α expression in peripheral blood mononuclear cells of the subjects of the study

Group/ Parameter	Statistical indicator	Control group CAD			Experimental group CAD+AIT (resveratrol)		
		Before therapy	After therapy	$2^{-\Delta\Delta Ct}$ (min-max)	Before therapy	After therapy	$2^{-\Delta\Delta Ct}$ (min-max)
Expression mRNA I κ B α , $2^{-\Delta Ct}$	X	0,0234	0,0253	0,120 (-2,64 - -2,83)	0,0207	0,0101*	- 2,016* (-4,5 - +2,83)
	σ	$\pm 0,0198$	$\pm 0,0155$		$\pm 0,0153$	$\pm 0,0062$	
		p=0,570			p=0,031		

Note: X – the sample mean, σ – standard quadratic deviation,

* – Wilcoxon (U), significant differences with the data of all groups.

Discussion

The results obtained reported the intensity of chronic systemic inflammation in stable CAD and concomitant AIT in its euthyroid state did not differ significantly from that in separate stable CAD that was assessed by the level of NF- κ B transcriptional activity and by the direct result of its increase, i.e. cytokines production.

However, we found out the fact of increase in chronic systemic inflammation. Perhaps, the lack of prevalence was determined by the moderate systemic inflammation in stable CAD. In turn, euthyroid state indirectly reflects moderate autoimmune thyroid inflammation as at high rate chronic systemic inflammation apoptosis of thyrocytes becomes more intense followed by loss of functional activity of the thyroid gland.

It is known that the increase in signal transduction involving NF- κ B occurs via appropriate ligand receptors IL-1 β , TNF- α , Toll-like receptors, CD 32, CD 40, CD 64 and others [2]. Moreover, the enhancement of pro-inflammatory signalling may result in the production of inflammatory molecules by means of activating protein-1 (AP-1). Signaling pathways involve numerous proteases and cofactors required for their

functioning and are pluripotent. Signalling by MAP kinase pathway (MAPK - protein kinase that is activated by myogenes) results in the increase of expression of c-Fos and c-Myc genes that mediate the induction of apoptosis [10].

Reduction in the level of pro-inflammatory cytokines as IL-1 β and TNF- α due to resveratrol is the evidence of its effect produced on NF- κ B, the main factor responsible for their production [14]. This conclusion is supported in our study by decreased mRNA I κ B α expression in blood mononuclear cells. The lack of effect produced by resveratrol on IL-10 can be explained by analyzing the signaling pathways of its formation. The same inflammatory stimuli acting on the intermediate transcription TNF- α -associated factor 6 (TRAF6) activate both NF- κ B and AP-1 signaling [7]. This leads to increased transcription of IL-10 that is demonstrated in our study. However, inactivation of NF- κ B does not exclude the AP-1 transcriptional activity that, in our opinion, has caused permanent moderately elevated levels of IL-10, which have anti-inflammatory properties of projective value.

Despite a lot of reports supporting information on the anti-inflammatory efficacy of statins that the patients took as a component of the standard therapy, we found out no evidence of this in our study. We can assume that statins should be taken over longer period of time to implement their pleiotropic effects, and a dose of atorvastatin (10 mg per day) we chose needs further titration to achieve optimal efficacy.

Anti-inflammatory activity of resveratrol we observed in our study can be explained by its effect on different molecular levels of cell organization. With three hydroxyl groups, resveratrol inactivates free radicals, which play a significant role in cell activation. Resveratrol enhances the activity of superoxide dismutase, catalase and glutathione enzyme systems, inhibits cyclooxygenase and lipoxygenase at post-transcriptional level [17]. Our findings on the ability of resveratrol to block transcriptional activity of NF- κ B agree with available relevant research data [14].

Resveratrol blocks I κ B-kinase, which got activated by various pro-inflammatory stimuli, destroys the relationship between NF- κ B (p50 / R65) and I κ B α that leads to the translocation of subunit R65 in the nucleus and activates the transcription of pro-inflammatory genes. It has also been demonstrated that

resveratrol acts as an activator gene sirtuin-1 (SIRT1), which supports mitochondrial metabolism and piston framework density preventing the pathogenic gene transcription. Recent scientific studies have found that SIRT1 diacetylates subunit p65 by Lys 310 residue by weakening the NF- κ B-signaling [13].

Conclusions

Thus, the patients with stable coronary artery disease as well as the patients with coronary artery disease and concomitant autoimmune thyroiditis in its euthyroid state demonstrate marked increase in the level of chronic systemic inflammation without any significant prevalence.

Two month course of resveratrol therapy significantly reduced the level of systemic inflammation in the patients with coronary artery disease and comorbid autoimmune thyroiditis by reducing the transcriptional activity of NF- κ B, and presumably by getting involved other numerous mechanisms of its projective action. Atorvastatin in a dose of 10 mg per day for two month course was found out to produce no pleiotropic anti-inflammatory effect in coronary artery disease.

The anti-inflammatory activity of resveratrol regarded as a component of standard statin therapy of coronary artery disease determines the expediency of combining these agents in the condition studied that increases the effectiveness and safety profile of medication in cases when high doses of statins can be administered.

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Таблица 1

Концентрацията на цитокини в серума на пациенти с изследователски групи

Забележки: X - средна стойност, σ - стандартното отклонение,
p - вероятност.

Таблица 2

Нивото на експресия на мРНК IкVa в периферни кръвни мононуклеарни клетки на пациенти с изследователски групи

Забележки: X - средна стойност, σ - стандартното отклонение,
p – вероятност, * - съществена разлика с тези на всички групи
изследвания преди и след лечението ($p < 0,01$).

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