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The Effects of Resveratrol on Oxidant/Antioxidant Systems and Their Cofactors in Rats*

Wpływ resweratrolu na układ antyoksydacyjny i jego kofaktory u szczurów

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A – research concept and design; **B** – collection and/or assembly of data; **C** – data analysis and interpretation; **D** – writing the article; **E** – critical revision of the article; **F** – final approval of article; **G** – other

Abstract

Background. Resveratrol, which belongs to a class of polyphenolic compounds called stilbenes, is proposed to have anti-aging effects.
Objectives. This research has focused on the effects of resveratrol on oxidant/antioxidant systems and also copper and zinc concentrations in rats.
Material and Methods. The control group consisted of 12 male Wistar albino rats, 10–12 weeks of age, weighing approximately 279 g. The experimental group consisted of 12 male Wistar albino rats, in the same age, weighing 285 g. Before the experimental procedure, a training period was performed for control and experimental groups, systolic arterial blood pressures and heart rates were recorded. Body weights were measured also. The experimental group was administered resveratrol (20 mg/kg) in drinking water for six weeks. At the end of the study, blood glucose levels, red cell superoxide dismutase (SOD), catalase activities, copper, zinc concentrations in plasma and red cells, also, malondialdehyde (MDA) concentrations in plasma were determined in both groups.
Results. At the end of the study, statistically significant increases in SOD and catalase activities, and also in plasma copper and red cell zinc concentrations and statistically significant decreases in body weight, blood glucose, blood pressure, lipid peroxidation product MDA concentration and plasma zinc, red cell copper concentrations were found in resveratrol administered group.
Conclusions. Resveratrol administration affected oxidant/antioxidant systems and their cofactors significantly (**Adv Clin Exp Med 2013, 22, 2, 151–155**).

Key words: rat, resveratrol, oxidant/antioxidant systems, trace elements.

Streszczenie

Wprowadzenie. Resweratrol, który należy do związków polifenolowych zwanych stylbenami, może wywierać działanie przeciwstarzeniowe.
Cel pracy. Badania miały na celu ocenę wpływu resweratrolu na układ utleniacz/przeciwutleniacz, a także na stężenie miedzi i cynku u szczurów.
Materiał i metody. Grupa kontrolna składała się z 12 szczurów albinosów samców szczepu Wistar, w wieku 10–12 tygodni, o wadze około 279 g. Grupa doświadczalna składała się z 12 szczurów albinosów płci męskiej szczepu Wistar, w tym samym wieku, o wadze 285 g. Przed rozpoczęciem doświadczenia w grupie kontrolnej i badanej zmierzono skurczowe ciśnienie krwi i tętno, a także masę ciała. W grupie badanej podawano resweratrol (20 mg / kg) w wodzie do picia przez sześć tygodni. Na koniec badania w obu grupach oznaczono stężenie glukozy we krwi, dysmutazę ponadtlenkową (SOD) czerwonych krwinek, aktywność katalazy, stężenie miedzi i cynku w osoczu i czerwonych krwinkach, a także stężenie aldehydu malonowego (MDA) w osoczu.

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Wyniki. Na koniec badania wykryto statystycznie istotne zwiększenie aktywności katalazy i SOD, a także zwiększone stężenie miedzi w osoczu i cynku w czerwonych krwinkach oraz statystycznie istotne zmniejszenie masy ciała, stężenia glukozy we krwi, ciśnienia krwi, stężenia produktu peroksydacji lipidów MDA, cynku w osoczu, miedzi w czerwonych krwinkach w grupie, w której podawano resweratrol.
Wnioski. Podawanie resweratrolu wpływa znacząco na układ utleniacz/przeciwutleniacz i ich kofaktory (**Adv Clin Exp Med 2013, 22, 2, 151–155**).

Słowa kluczowe: szczur, resweratrol, układ utleniacz/przeciwutleniacz, pierwiastki śladowe.

Resveratrol (3,5,4'-trihydroxystilbene) is a natural polyphenol present in various foodstuffs, mainly in grapes where it is synthesized in response to environmental stress conditions [1, 2]. Many studies have demonstrated that this molecule exhibits a wide range of biological and pharmacological activities both *in vivo* and *in vitro* [2, 3]. A series of studies showed that resveratrol has anti-oxidant properties, anti-inflammatory properties, and anticancer activity.

Some studies have demonstrated that resveratrol modulates lipid and lipoprotein metabolism [4]. Additionally, it was shown that supplemental doses of resveratrol prevented nearly all detrimental changes due to high-calorie intake [5]. In some studies, it was shown that polyphenolic substances may increase the metabolic rate and also fat oxidation, implicating a potential use as an antiobesity treatment. The results of some animal studies suggested that oral doses of resveratrol could decrease the risk of cardiovascular diseases [6, 7]. Atherosclerosis, being an inflammatory disease, several criteria of inflammation are associated with increased risk of myocardial infarction [8]. This polyphenol compound resveratrol, having biological activities as the inhibition of cyclooxygenase, induction of apoptosis, has an effect on the cell division cycle and modulation of nuclear factor kappa B (NF-κB) activation and seems to affect immune regulatory mechanisms efficiently. Hence, many *in vitro* experiments have proven that resveratrol has immuno-modulatory activity [4, 9–11].

In their previous studies, the authors also have demonstrated that resveratrol has a significant effect on lipid metabolism; in fact, they have found significant decreases in body weights and total cholesterol levels of rats in a preliminary experimental study [12]. Therefore, regarding its wide spectrum of multipotential effects, the aim of the present study was designed to determine the effects of resveratrol on blood glucose, blood pressure, heart rate, oxidant/antioxidant systems and related trace elements copper and zinc concentrations in rats.

Material and Methods

In this study the **c**ontrol group consisted of 12 male Wistar albino rats, 10–12 weeks of age, weighing approximately 279 g. The experimental group consisted of 12 male Wistar albino rats, in the same age, weighing 285 g. The rats were housed in individual cages in a climate-controlled room with an ambient temperature of 22±1°C for six weeks and were in a light regulated space in approximately 12 hour day and night cycles and at a relative humidity of 60±10% and given a standard laboratory diet and water before experimental procedure. Also, before the experimental procedure, a training period of 10 days, in which each rat from both groups was addicted to measurement by tail-cuff pletysmography, after this period systolic arterial blood pressures and heart rates were recorded. The experimental group was administered resveratrol (20 mg/kg) in drinking water for six weeks. Body weight was evaluated. During 10 days of training, daily measurements of blood pressure by tail-cuff pletysmography were performed. In order to determine the arterial blood pressure, a minimum of four consecutive measurements were taken and recorded by a computer.

At the end of the study, blood samples of control and experimental groups were drawn, blood glucose levels were determined. Red cell copper-zinc superoxide dismutase (Cu-Zn/SOD) and catalase activities, malondialdehyde (MDA) concentrations, plasma and red cell copper, zinc concentrations were determined. Superoxide dismutase [13], catalase [14] activities and MDA [15] concentrations were determined spectrophotometrically. Plasma and red cell zinc and copper concentrations were determined by atomic absorption spectrophotometry, according to Perkin-Elmer's principles [16, 17].

All the laboratory procedures were performed at Pathophysiology Department Laboratories. Researchers adhered to the Helsinki Convention and the study was approved by Ethics Committee of the Ankara University. All the data is expressed as median (minimum-maximum). Mann-Whitney U test was used to perform comparisons between groups. The difference within groups was assessed by Wilcoxon-Signed Ranks test. P values less than 0.05 was considered significant. SPSS for Windows 11.5 was used for statistical analysis.

Results

After resveratrol administration, there were increases in SOD ($p < 0.001$) and catalase ($p < 0.001$) activities. Plasma copper ($p = 0.005$) and red cell zinc ($p < 0.001$) concentrations were increased respectively in experimental group compared to control group. Weight gain in control and experimental groups was shown to have significant difference when compared at the end of the study ($p < 0.001$), while there was an increase in weight gain of control group ($p = 0.002$), there was a decrease in experimental group ($p = 0.021$). Decreases in blood glucose ($p < 0.001$), blood pressure ($p = 0.002$), lipid peroxidation product MDA ($p < 0.001$) and plasma zinc ($p < 0.001$), red cell copper ($p = 0.008$) concentrations were determined in resveratrol administered group. Heart rates were not changed in experimental group ($p = 0.259$).

The results of this study were shown in Table 1 and 2.

Discussion

Resveratrol has many different targets and mechanisms of action including; inhibition of lipid peroxidation, chelation of copper, free-radical scavenging activity, inhibition of platelet aggregation, anti-inflammatory activity, modulation of lipid metabolism, regulation of blood glucose, anticancer activity and estrogenic activity [4, 9, 10] related with its wide range of biological activities.

In this study, the results showed that there was a significant decrease in body weight gains in resveratrol treated group ($p < 0.001$). It was reported in some studies, resveratrol administration (5 and 25 mg/kg) leads to a decrease in body weight, consistent with present results. On the other hand, there is conflicting data reporting that resveratrol treatment had no significant effect on body weight. In some assays, it was reported that resveratrol administration led to relatively low levels of glucose in mice [18, 19]. Confirming these results, blood glucose levels were also decreased in present study ($p < 0.001$).

Systolic arterial blood pressure was decreased in resveratrol treated group ($p = 0.002$), and heart rate was slightly increased correspondingly in present study ($p = 0.259$). All the parameters mentioned above were also studied in author's previous study and were found consistent with this study [12]. Recently, it has been shown that resveratrol upregulates the expression of iNOS, indicating the ability of resveratrol to induce eNO synthesis. In some tissue assays, resveratrol was found to exert its protective action through upregulation of NO synthesis proving the suggestions above [20–22]. Additionally, resveratrol shows many common physiological functions with NO, as vasodilatation and augments NO availability [20–24].

The Cu/Zn-SOD enzyme activity represents the first line of intracellular defense against cellular damage from ROS. In this study, a significant increase in Cu-Zn/SOD activity in resveratrol treated group were detected ($p = 0.001$). Thus, the significant increase of Cu/Zn-SOD activity in this study was interpreted as an enhancing effect of resveratrol on SOD activity. The same enhancement on intracellular antioxidant defense system was also determined in catalase activity ($p < 0.001$) [20, 21]. In some literature data shows the activity of catalase was found to be higher in polyphenol consuming rodents than controls [20]. In resveratrol treated group, MDA levels were decreased compared to controls ($p < 0.001$). The decrease in MDA level might come from the suppressive effect of this polyphenol on lipid peroxidation. Through its inhibitory effect on membrane lipid peroxidation, resveratrol neutralizes the effects of ROS [20, 21] with a significant antioxidant manner.

Table 1. Body weights of control and experimental groups

Tabela 1. Masa ciała grupy kontrolnej i badanej

	First day (g) (Dzień pierwszy)	Last day (g) (Dzień ostatni)	Change (Zmiana)	p^+
Control (Kontrolna)	279 (261–323)	340 (310–370)	60 (47–68)	0.002
Experimental (Badana)	285 (265–300)	281.5 (261–297)	–1.5 (–6–1)	0.021
p^*	0.514	< 0.001	< 0.001	

All data are expressed as median (minimum–maximum).
Wszystkie dane wyrażono jako średnie (minimum–maksimum).
* Difference between control and experimental groups.
* Różnica między grupą kontrolną a badaną.
+ Difference between first and last days.
+ Różnica między pierwszym a ostatnim dniem.

Table 2. The results of control and experimental groups

Tabela 2. Wyniki badań grupy kontrolnej i badanej

	Control (Kontrolna)	Experimental (Badana)	p
Blood glucose (mg/dL) (Stężenie glukozy we krwi)	209 (189–300)	155 (138–195)	< 0.001*
Systolic arterial blood pressures (mm Hg) (Skurczowe ciśnienie krwi)	165 (141–280)	139 (117–169)	0.002*
Heart rates (beat/min) (Tętno (uderzeń/min))	373 (340–389)	377.5 (350–409)	0.259
Red cell Cu-Zn/SOD activity (U/g Hb) (Aktywność Cu-Zn/SOD w czerwonych krwinkach)	4494.5 (4000–5128)	5128 (5000–5882)	< 0.001*
Red cell Catalase activity (k/g Hb) (Aktywność katalazy w czerwonych krwinkach)	158.4 (156–159.1)	169.4 (161.2–176)	< 0.001*
MDA (nmol/mL)	10.54 (8.5–11.15)	6.25 (2.9–7.6)	< 0.001*
Plasma Zn (μg/dL) (Cynk w osoczu)	129 (109.3–156)	93 (74–122)	< 0.001*
Plasma Cu (μg/dL) (Miedź w osoczu)	100 (90–120)	122 (94–146)	0.005*
Red cell Zn (μg/mL) (Cynk w czerwonych krwinkach)	6.75 (6.3–7.4)	8.21 (7.29–11.01)	< 0.001*
Red cell Cu (μg/mL) (Miedź w czerwonych krwinkach)	0.30 (0.22–0.34)	0.25 (0.16–0.31)	0.008*

*statistically significant.
*istotne statystycznie.
All data are expressed as median (minimum–maximum).
Wszystkie dane wyrażono jako średnie (minimum–maksimum).

Hence Zn, being a cofactor of Cu-Zn/SOD, was also measured in this study. It was interesting that in resveratrol treated group plasma Zn concentration was decreased and red cell zinc was increased ($p < 0.001$, $p < 0.001$). Indeed, zinc has been shown to antagonize the catalytic properties of the redox-active transition metals, such as copper [25]. Under resveratrol treatment, an increase in red cell zinc concentration was seen. On the other hand, the concentration of the transition metal copper, which has another important role in Cu/Zn-SOD activity, was expected to show a decrease in red cell in this circumstance. As expected, present results showed that the red cell copper concentration was decreased significantly in resveratrol treated group when compared with the control group ($p = 0.008$) respectively. On the other hand, it was reported that resveratrol has copper chelating properties. It has been postulated that the position of the hydroxyl groups is important for the chelation of copper [10]. The decrease in red cell copper concentration might also be due to elevated Cu utilization for increased Cu-Zn/SOD activity [26, 27].

Briefly, this study provides beneficial influences of resveratrol on anti-atherosclerotic effects through the oxidant/antioxidant system [28, 29]. In conclusion, it was suggested in this preliminary experimental study that, confirming its antioxidant and antiaging properties, resveratrol has led to an increase in antioxidant enzyme activities, a decrease in blood systolic pressure and corresponding changes in zinc and copper concentrations.

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