Effects of coenzyme Q10 supplementation on activities of selected antioxidative enzymes and lipid peroxidation in hypertensive patients treated with indapamide. A pilot study

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Abstract

Introduction: An increase in oxidative stress is strongly documented in hypertensive patients. In blood vessels, oxidative stress increases the production of superoxide anion (O$_2$•⁻) that reacts with nitric oxide (NO) and impairs the ability of endothelium to relax. Many reports indicate a beneficial effect of coenzyme Q10 (CoQ) in hypertension. Coenzyme Q10 therapy may lower O$_2$•⁻ and thus decrease the complications associated with hypertension. The aim of our study was to evaluate the effects of CoQ supplementation on antioxidative enzyme activities and lipid peroxidation in elderly hypertensive patients.

Material and methods: We determined the activities of superoxide dismutase (SOD-1) and glutathione peroxidase (GSH-Px) and the concentration of malondialdehyde (MDA) in erythrocytes of 27 elderly (mean age 72.5 ± 6.1 year) hypertensive patients treated with indapamide at baseline and after 12 weeks of CoQ supplementation (60 mg twice a day) in comparison with 30 healthy elderly volunteers (mean age 76.8 ± 8.5 year).

Results: Decrease of SOD-1 (p < 0.001) and insignificant reduction of GSH-Px activities and increase of MDA (p < 0.001) level were observed in hypertensive patients in comparison to healthy volunteers before supplementation. Coenzyme Q10 administration resulted in a significant increase only in SOD-1 activity (p < 0.001).

Conclusions: The present study indicates that CoQ improves the most important component of the antioxidant defence system – SOD-1, which is responsible for O$_2$•⁻ scavenging. Coenzyme Q10 may be used as an additional therapeutic agent for prophylaxis and treatment of hypertension in elderly patients.

Key words: aging, glutathione peroxidase, hypertension, oxidative stress, superoxide dismutase.
Introduction

In 1972 Igarashi et al. [1] reported that coenzyme Q10 (CoQ) reduced elevated blood pressure in rats treated with desoxycorticosterone and saline. Subsequently, CoQ supplementation has been demonstrated to have a hypotensive effect [2-4]. A possible explanation of these findings may be the antioxidative properties of CoQ. The pathogenesis of primary hypertension is multifactorial. It has been accepted that oxidative stress and augmented production of reactive oxygen species (ROS), mainly superoxide anion (O2•−), may have a key role [5-7]. Overproduction of O2•− results from increased tissue renin-angiotensin system (RAS) activation [6-8]. The RAS is responsible for angiotensin II (AngII) synthesis, and additionally aldosterone potentiates the actions of this peptide [9]. Angiotensin II enhances the generation of O2•− via an increase of nicotinamide adenine dinucleotide (phosphate) reduced oxidase (NAD(P)H-oxidase) activity in endothelium, vascular smooth muscle and leukocytes [5, 8, 10]. In a reaction with O2•−, vasodilatory nitric oxide (NO) is inactivated by conversion to peroxynitrite [11, 12]. This powerful oxidant reacts with polyunsaturated fatty acids and in this way initiates lipid peroxidation [11]. As a result, vessel wall degradation and endothelium damage may occur [12]. Moreover, ROS increase the synthesis of several vasoconstrictors, such as F2α-isoprostanes, endothelin and thromboxane A2, and decrease synthesis of vasodilatory prostacyclin [5]. Experimental evidence indicates that reduction in NO availability, endothelium cell damage and disturbances in synthesis of several tissue hormones via impaired endothelium-dependent vasodilation may lead to primary hypertension development [5, 8, 13].

Coenzyme Q10 exerts some impact on the generation and half-life of O2•− [15]. One of the intriguing ideas is that CoQ may exert an influence on activities of antioxidative enzymes and in this way reduce the level of ROS. Therefore the aim of the study was to investigate the effects of CoQ supplementation on SOD-1 and GSH-Px activities in elderly hypertensive patients. In addition a lipid peroxidation marker, malondialdehyde (MDA) concentration, was determined.

Material and methods

Participants

Twenty-seven elderly (mean age 72.5 ±6.1 year) hypertensive patients treated with indapamide at baseline and after 12 weeks of CoQ supplementation (60 mg twice a day) in comparison with 30 healthy elderly volunteers (mean age 76.8 ±8.5 year) were included in the study. The patients were evaluated by a standard physical examination and routine clinical laboratory tests. Persons addicted to alcohol and/or tobacco, and patients with diabetes mellitus, ischaemic heart disease, a history of stroke, renal failure or other conditions of known free radical aetiology were excluded from the study.

All participants provided written consent to participate in the experiment, which was approved by the local ethics committee of Collegium Medicum in Bydgoszcz, Poland.

Experimental protocols

Each patient was treated with a pharmaceutical preparation of 60 mg of CoQ twice a day for 12 weeks. Blood samples were collected after overnight fasting from the cubital vein into polypropylene heparinized tubes (6 ml) a day before supplementation, and after 12 weeks of CoQ treatment.

All samples were centrifuged (2500 g for 10 min). After plasma removal, the haemolysate was assayed according to the methods of Placer et al. [16], Misra and Fridovich [17], and Paglia and Valentine [18], respectively. The MDA level was expressed as the concentration of thiobarbituric acid reactive substances, read at 532 nm. The SOD-1 activity was determined.

Biochemical analysis

Erythrocytic MDA, SOD-1, and GSH-Px were assayed according to the methods of Placer et al. [16], Misra and Fridovich [17], and Paglia and Valentine [18], respectively. The MDA level was expressed as the concentration of thiobarbituric acid reactive substances, read at 532 nm. The SOD-1 activity was determined at 37°C by recording the increase in absorbance at 480 nm following the auto-oxidation of adrenaline, inhibited by SOD-1. One unit (U) of this activity is defined as the amount of enzyme inhibiting the adrenaline auto-oxidation by 50%. The GSH-Px activity was
determined at 25°C by recording the decrease in absorbance at 340 nm following the oxidation of NADPH in the presence of tert-butyl hydroperoxide as a substrate. One unit (U) of this activity is defined as the amount of the enzyme oxidizing 1 µmol NADPH/min. The haemoglobin concentration in the haemolysate was estimated after conversion into cyanmethaemoglobin form using a commercial reagent (Biomed, Poland) read at 540 nm.

**Statistical analysis**

All the results were expressed as mean ± SEM. One-way analysis of variance followed by the Tukey post hoc test was performed to determine the statistical significance of differences. The level of significance was set at \( p < 0.05 \).

**Results**

The clinical characteristics of the examined groups are presented in Table I. The parameters of oxidative stress in erythrocytes of elderly hypertensive subjects differed from the results obtained for healthy elderly volunteers: MDA level (0.288 ±0.039 µmol/g Hb and 0.223 ±0.038 µmol/g Hb, respectively) (Figure 1) was significantly higher (\( p < 0.001 \)), activity of SOD-1 (2431 ±266 U/g Hb and 2916 ±352 U/g Hb, respectively) (Figure 2) was significantly lower (\( p < 0.001 \)) and activity of GSH-Px (14.7 ±3.2 U/g Hb and 16.3 ±1.6 U/g Hb, respectively) (Figure 3) was insignificantly lower.

Twelve weeks of CoQ supplementation caused a significant decrease (\( p < 0.001 \)) in the erythrocytic MDA level (0.255 ±0.026 µmol/g Hb) (Figure 1) and significant increase (\( p < 0.001 \)) in erythrocytic SOD-1 activity (2677 ±321 U/g Hb) (Figure 2) in elderly hypertensive patients. Coenzyme Q supplementation did not influence the erythrocytic activity of GSH-Px (16.1 ±2.3 U/g Hb) (Figure 3) in elderly hypertensive patients.

**Discussion**

Many authors have observed that augmented ROS production exceeds the capacity of the antioxidative system in the elderly [19, 20].

| Parameters | Healthy elderly volunteers | Elderly hypertensive group |
|------------|---------------------------|---------------------------|
| Number of subjects | 30 | 27 |
| Age [years] | 76.8 ±8.5 | 72.5 ±6.1 |
| Sex (number of subjects) | M/F | 7/23 | 7/20 |
| BMI [kg/m²] | 24.8 ±4.6 | 30.9 ±3.4 |
| Systolic blood pressure [mmHg] | 120.6 ±4.8 | 152.8 ±9.3 |
| Diastolic blood pressure [mmHg] | 80.0 ±5.0 | 82.5 ±6.9 |
| Plasma total cholesterol [mg/dl] | 178.9 ±15.8 | 195 ±28 |
| Plasma triglyceride [mg/dl] | 135.4 ±18.4 | 154.5 ±11.2 |
| Plasma glucose [mg/dl] | 93.28 ±16.26 | 82.9 ±8.7 |
| Serum creatinine [mg/dl] | 1.0 ±0.3 | 0.9 ±0.13 |

*BMI – body mass index, M – male, F – female

* \( p < 0.001 \) hypertensive patients before supplementation vs. healthy elderly subjects, *\#p < 0.001 hypertensive patients before supplementation vs. after supplementation

* Figure 1. Effect of coenzyme Q10 (CoQ) supplementation on the concentration of malondialdehyde (MDA) in erythrocytes of elderly hypertensive patients in comparison with healthy elderly volunteers. All parameters were measured at baseline in both groups and after 12 weeks of CoQ administration in the hypertensive group. Each value is mean ± SEM

* Figure 2. Effect of coenzyme Q10 (CoQ) supplementation on the activity of superoxide dismutase (SOD-1) in erythrocytes of elderly hypertensive patients in comparison with healthy elderly volunteers. All parameters were measured at baseline in both groups and after 12 weeks of CoQ administration in the hypertensive group. Each value is mean ± SEM

* \( p < 0.001 \) hypertensive patients before supplementation vs. healthy elderly subjects, *\#p < 0.001 hypertensive patients before supplementation vs. after supplementation
levels of ROS by-products, such as MDA, carbonyl groups, advanced end products of glycation and damaged 8-oxo-2′-deoxyguanosine, were found in elderly people [21]. Hypertension is an age-dependent disorder and oxidative stress may be involved in the development of this disease. In our study erythrocyte MDA level was significantly higher in the essential hypertensive patients as compared to the control group before treatment with CoQ. Significantly higher MDA concentrations in erythrocytes and plasma of hypertensive patients have been observed in many studies [22-26]. Uddin et al. [27] noticed a 2.6-fold higher level of MDA in the aorta of hypertensive mice than that of normotensive ones. These findings suggest that increased lipid peroxidation may be associated with elevated blood pressure. Additionally, increased concentration of MDA may contribute to the development of hypertension complications [22, 28].

Decreased activities of the antioxidative enzymes SOD-1 and GSH-Px in hypertensive patients before treatment were found in the present study but only SOD-1 activity was diminished significantly. In our previous study a significant age effect on decreasing activity of GSH-Px was found [29]. As opposed to the present results, Se-dependent GSH-Px activity was reported to be unchanged or increased in several publications [27, 30, 31]. Uddin et al. [27] observed unchanged GSH-Px activity in hypertensive mouse aorta. Patients with essential hypertension exhibited increased GSH-Px activity compared to normotensive persons in the study of Simic et al. [31].

Lowered SOD-1 activity in erythrocytes of patients with essential hypertension was reported by Baykal et al. [23], Kumar and Das [24] and Donmez et al. [32]. A possible explanation of decreased SOD-1 activity in elderly people could be a Zn²⁺ ion deficiency, which may be connected with poor diet of elderly people [30]. According to some authors the reason for diminished activity of SOD-1 may be the oxidative modifications of enzymatic proteins. Researchers emphasize that protein damage induced by ROS may be related to increasing age [33, 34].

Kontush et al. [35] reported a significantly lower level of CoQ in blood of hypertensive patients. The studies by Sohal et al. [36] indicate that supplementation of CoQ elevates the endogenous content of this compound. Generally, the administration of CoQ directly improved the efficiency of antioxidative defence in the present study. A particularly noteworthy result is a significant rise of SOD-1 activity after CoQ supplementation. Three isoforms of SODs exist, but SOD-1 is the major vascular isofrom and is important in scavenging of O₂⁻ and enhancing availability of endothelial NO [37]. The reaction between O₂⁻ and NO occurs 6 times faster than the removal of O₂⁻ by SOD-1. So, increased SOD-1 activity via CoQ supplementation may be very important in the improvement of endothelial function. Tiano et al. [38] reported an improvement in endothelium-dependent vasodilation and an increase in extracellular SOD-1 activity in the CoQ-treated group of patients. Sena et al. [39] reported that CoQ therapy increases Mn-SOD activity in the pancreas of type 2 diabetic rats. The present study showed that also the activity of GSH-Px increased after CoQ supplementation compared to the activity observed in the control group, but this rise was insignificant. Contrary to these results, Sohal et al. [36] found that intake of CoQ had no effect on SOD-1 and GSH-Px activities.

Moreover, we observed that CoQ treatment decreased erythrocytic MDA concentration, suggesting that this compound may diminish ROS concentration. Decreased MDA levels in the plasma after administration of CoQ were also noted by Ankola et al. [40]. In the study by Sena et al. [39] CoQ treatment decreased pancreatic MDA level in diabetic rats.

In conclusion, the results from the present study provide evidence for an increase in MDA level and a decrease in activities of antioxidative enzymes in hypertensive patients before Q10 supplementation and suggest that these changes may be associated with the process of hypertension. The improvement of oxidative stress parameters after CoQ supplementation confirms the antioxidative properties of this compound. As was mentioned above, clinical studies indicate a deficiency of CoQ concentration in hypertensive patients. Therefore, taking into account both hypotensive and antioxidative properties of CoQ, we suggest the supplementation of elderly hypertensive patients with this compound as an alternative complementary treatment. However, due to the small number of patients,
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