Reduction of Kidney Damage by Supplementation of Vitamins C and E in Rats With Deoxycorticosterone-Salt-Induced Hypertension

Behjat Seifi,[1] Mehri Kadkhodaee,[1] Seyed Morteza Karimian,[1] Maryam Zahmatkesh,[1] Sedighe Shams,[2] Enayatolla Bakhshi[3]

[1]Department of Physiology, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran
[2]Children Medical Center, Tehran University of Medical Sciences, Tehran, Iran
[3]Department of Epidemiology and Biostatistics, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran

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Introduction. We assessed whether cosupplementation of vitamins C and E has additive beneficial effects on reducing the kidney damage and attenuation of the arterial pressure elevation compared to administration of either vitamin C or vitamin E alone in deoxycorticosterone acetate-salt-induced hypertension.

Materials and Methods. Forty rats were divided into 4 study groups and 1 sham-operated group. Unilateral nephrectomy was carried out in the study groups and hypertension was induced by deoxycorticosterone injection and 1% sodium chloride and 0.2% potassium chloride added to the drinking water. Vitamins C and E (200 mg/kg/day) or combination of them were administered with DOCA-salt for 4 weeks in 3 study groups. The effects of DOCA and salt and treatment with vitamins were compared in terms of blood pressure, urinary protein excretion, antioxidant activity of the kidneys, and renal histological changes.

Results. Four weeks of supplementations of vitamins C, vitamin E, and both in the DOCA-salt-treated rats had comparable significant effects in decreasing systolic blood pressure. Urinary protein excretion and histological damage did not significantly change with the combination therapy of vitamins C and E compared to the vitamin C or E alone. The renal levels of glutathione and ferric reducing/antioxidant power in combination therapy group were similar to the two other treatment groups and were significantly higher than non-treated group.

Conclusions. Co-administration of vitamin C and E does not have an additive beneficial effect on reducing the kidney damage and hypertension compared to either vitamin C or E alone in DOCA-salt-induced hypertension.

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INTRODUCTION

Excessive production of reactive oxygen species (ROS) in the kidney has been reported in different models of experimental induction of hypertension along with progressive kidney injury.[1,2] Reactive oxygen species can react with proteins, lipids, nucleic acids, and carbohydrates, therefore causing inflammation, apoptosis, fibrosis, and cell proliferation.[3,4] Some studies have indicated that humans with essential hypertension have oxidative stress.[5,6] More than half of these hypertensive patients have a salt-sensitive type of hypertension.[7] In one study on salt-sensitive hypertensive patients, 1-day sodium loading and depletion using an established protocol produced corresponding increases and decreases, respectively, in plasma

F2-isoprostanes.[8] Salt-sensitive hypertensive patients experience end-stage renal damage much more frequently than salt-resistance hypertensive patients.[9] Salt-sensitive hypertensive animal models have been used to investigate the relationship between hypertension, ROS, and end-stage renal damage.[4] In these studies, administration of deoxycorticosterone acetate (DOCA) and salt are used to induce a low-renin hypertension that is generated in a rapid-time course.

Cellular defense against ROS injury is provided by enzymatic and nonenzymatic scavenging systems present in the cell. Vitamins C and E are the main dietary antioxidants. Studies on experimental hypertension have reported that vitamin C, a water-soluble antioxidant, has a beneficial effect on blood pressure.[5,6] Vitamin E is a lipid-soluble antioxidant demonstrated to prevent development of increased blood pressure and lipid peroxidation in spontaneously hypertensive rats.[7] In addition, Vitamin E supplementation can has protective effects against deterioration of kidney function in rats with streptozotocin-induced Type 1 diabetes mellitus.[10]

The ability of vitamin C in reducing tocopheroxyl radical to generate tocopherol has been demonstrated in many in-vitro and in-vivo studies.[8,9] Since there is a synergistic action between vitamins E and C, an important question is whether a combination of antioxidants provides better protection. Vitamins C and E supplements to the high-sodium diet of Dahl S rats decreased arterial pressure, renal oxidative stress, and kidney damage, and increased renal hemodynamics.[11] In addition, vitamins C and E reduce oxidative stress, improve vascular function and structure, and prevent progression of hypertension in stroke-prone spontaneously hypertensive rats.[12] A large clinical trial demonstrated that 6-year supplementation of daily vitamin E and vitamin C reduced progression of carotid atherosclerosis.[13,14] Thus, the purpose of this study was to determine the effects of supplementation with a combination of vitamins E and C on kidney damage and blood pressure in DOCA-salt-induced hypertension in rats.

MATERIALS AND METHODS

Experimental Protocol

The study was performed on 40 male Sprague-Dawley rats weighing between 200 g and 250 g. The rats were maintained in the animal quarters under standardized conditions of a 12-h light/day cycle, an environment temperature of 20 ºC to 22ºC, and a 40% to 50% humidity. All of the rats received standard laboratory rat chow and water ad libitum. Animal care was in compliance with the guidelines of the Animal and Human Ethical Committee of Tehran University of Medical Sciences.

Unilateral Nephrectomy

The rats were anesthetized with a combination of ketamine (50 mg/kg; Rotexmedica, Trittau, Germany) and xylazine (5 mg/kg; Alfasan, Woerden, The Netherlands). Right unilateral nephrectomy was performed with the use of a retroperitoneal approach. After a right flank incision, the right kidney was visualized. The right ureter and renal vessels were isolated and then sectioned between two ligatures. Fat and connective tissues surrounding the right kidney were removed, while care was taken to avoid damaging the adjacent adrenal gland. The flank incision was closed with 3-0 silk suture materials.

One week after the unilateral nephrectomy, the following 5 groups of rats (8 in each group) were studied: sham-operated rats received vehicle injections and normal tap drinking water (sham group); DOCA-salt-treated rats received subcutaneous injection of DOCA (Iran Hormone Co, Tehran, Iran) at a dose of 20 mg/w and were provided with drinking water supplemented with 1% sodium chloride and 0.2% potassium chloride for 4 weeks (DOCA-salt group); and the three groups with vitamin supplementation received vitamin C or vitamin E, 200 mg/kg/d, or both of them along with DOCA and salt, 20 mg/w, for 4 weeks.

At the end of the experiment, the rats were placed in metabolic cages and 24-hour urine was collected for the measurement of urinary protein excretion. The rats were then anesthetized with ketamine and xylazine, and the left kidney was harvested; one-half was frozen in liquid nitrogen and homogenized for measurements of oxidative indexes, and the other half was fixed in 10% formalin for histological analysis. Renal tissues and urine samples were stored at -80°C until analysis.

Blood Pressure Measurement

Systolic blood pressure was measured in conscious rats by the tail-cuff method connected

to a pneumatic transducer using a PowerLab/4sp data acquisition system (software Chart, version 5, ADInstruments, Castle Hill, Australia). Systolic blood pressure was determined once a week in the morning. At least 3 determinations were made in every session and the mean of the lowest of the three values within 5 mm Hg was taken as the systolic blood pressure. The rats who received DOCA and salt were considered hypertensive if the systolic blood pressure was higher than 140 mm Hg.

Renal Antioxidant Status

Glutathione Measurement. Renal glutathione concentration was assessed as a measure of nonenzymatic antioxidant. Total glutathione concentration of kidney tissues was measured according to the modified method of Tietze by Griffith,[15,16] which was based on conversion of 5,5´-dithiobis-2-nitrobenzoic acid to 5-thio-2-nitrobenzoate by nicotinamide adenine dinucleotide phosphate in the presence of glutathione redoctase. Formation of 5-thio-2-nitrobenzoate was measured by spectrophotometry at 412 nm and comparing that to a glutathione standard curve.

Ferric Reducing/Antioxidant Power Assay. The ferric reducing/antioxidant power (FRAP) assay measures the alteration in absorbance at 593 nm owing to the formation of a blue-colored ferrous-tripyridyltriazine compound from the colorless oxidized ferric form by the action of electron-donating antioxidants.[17] The FRAP reagent consists of 300-mM acetate buffer (3.1 g of sodium acetate plus 16 mL of glacial acetic acid, made up to 1 L with distilled water; pH, 3.6), 10-mM 2,4,6-tris(2-pyridyl)-1,3,5-triazine in 40 mM of hydrochloric acid and 20 mM of $FeCl_3.6H_2O$ in the ratio of 10:1:1. Briefly, 50 µL of kidney homogenate was added to 1.5 mL of freshly prepared and prewarmed (37 °C) FRAP reagent in a test tube and incubated at 37 °C for 10 minutes. The absorbance of the blue-colored complex was read against reagent blank (1.5-mL FRAP reagent plus 50-µL distilled water) at 593 nm. Standard solutions of ferrous in the range of 100 mM to 1000 mM were prepared from ferrous sulphate ($FeSO_4.7H_2O$) in distilled water. The data was expressed as mmol ferric ions reduced to ferrous form per gram of tissue (FRAP value).

Urinary Protein Excretion

Urinary excretion of proteins was assayed by the colorimetric method, while the rats were kept in metabolic cages.

Histological Analysis of Kidneys

After fixation in formalin (10% phosphate-buffered) and dehydration process, the paraffin-embedded renal section (4 µm) was stained by hematoxylin-eosin. Tubular dilations, cellular vacuolation, cell destruction, loss of brush borders of the proximal tubules, and presence of luminal cast and proteinaceous materials were used as evidence of tubular damage. Increase in glomerular size and the Bowman space were used as evidence of glomerular damage.

Statistical Analyses

Data are expressed as the mean ± standard deviation. Comparisons between groups were made by the 1-way analysis of variance followed by the Duncan multiple range test. The SPSS software (Statistical Package for the Social Sciences, version 13.0, SPSS Inc, Chicago, Ill, USA) was used for data analyses. *P* values less than .05 were considered significant.

RESULTS

Blood Pressure

Systolic blood pressure increased significantly after treatment with DOCA and salt compared to that in the sham group throughout the 4 weeks of experimental periods ($P < .05$; Figure 1). Treatment

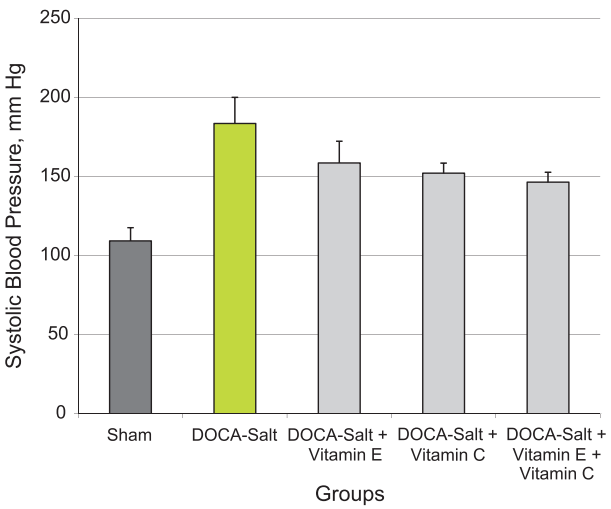

Figure 1. The mean systolic blood pressure in rats with unilateral nephrectomy that received deoxycorticosterone (DOCA) and salt with or without vitamins.

with vitamin C and/or vitamin E significantly decreased systolic blood pressure in the DOCA-salt hypertensive rats compared with no vitamin supplementation ($P < .05$; Figure 1).

Urinary Protein Excretion

Urinary protein excretion was significantly greater in the rats of the DOCA-salt group compared to those of the sham group ($P < .05$). As shown in Figures 2, treatment with vitamin C and/or vitamin E significantly decreased protein excretion induced by the DOCA and salt compared with no vitamin supplementation ($P < .05$).

Renal Antioxidant Status

Deoxycorticosterone and salt induced significant decreases in renal glutathione and FRAP levels compared to the sham group ($P < .05$). Figures 3 and 4 show that administering vitamin C and/or vitamin E significantly maintained the glutathione and FRAP levels despite of DOCA-salt-induced decrease in these factors ($P < .05$).

Renal Histological Changes

Rats treated with DOCA and salt for 4 weeks exhibited a marked increase in glomerular size and the Bowman space compared to the sham

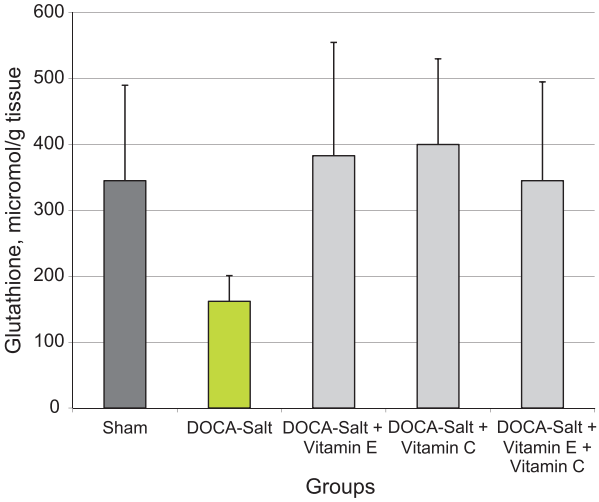

Figure 3. The mean renal glutathione concentration in rats with unilateral nephrectomy that received deoxycorticosterone (DOCA) and salt with or without vitamins.

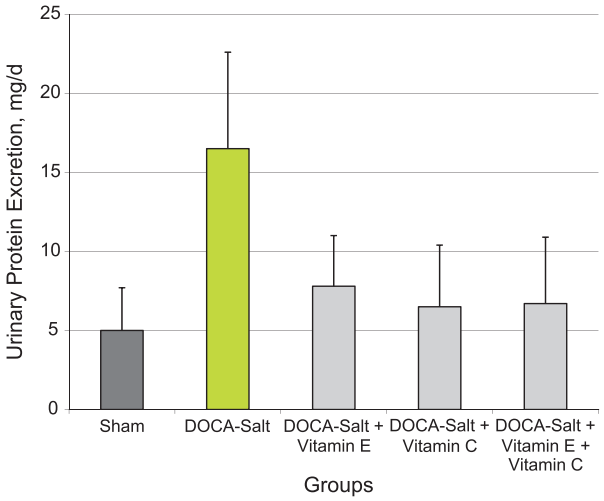

Figure 2. The mean urinary protein excretion in rats with unilateral nephrectomy that received deoxycorticosterone (DOCA) and salt with or without vitamins.

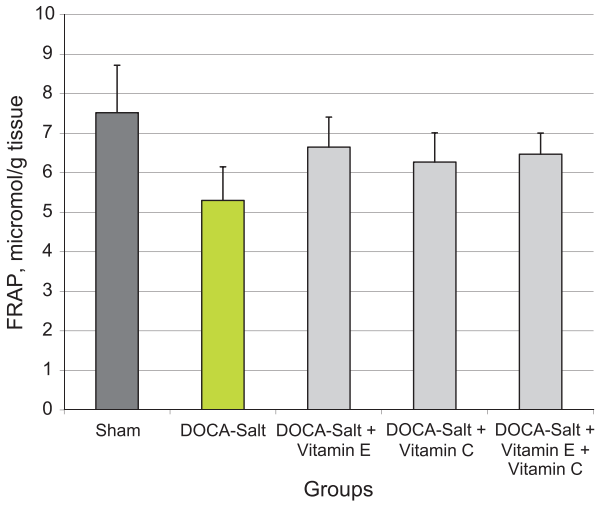

Figure 4. The mean renal ferric reducing/antioxidant power (FRAP) levels in rats with unilateral nephrectomy that received deoxycorticosterone (DOCA) and salt with or without vitamins.

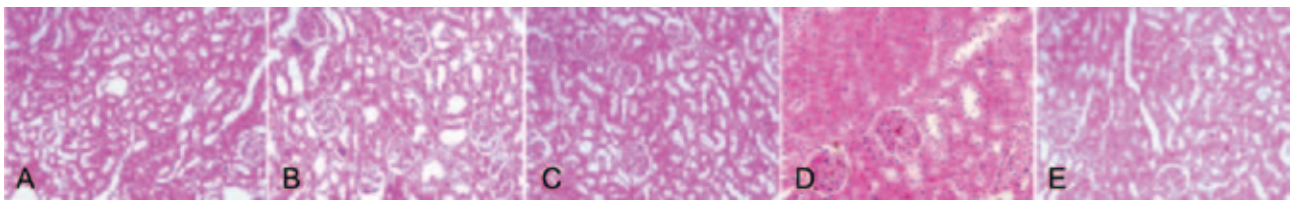

Figure 5. Histopathological features in kidney tissue of different groups of rats (hematoxylin-eosin, × 400). **A,** a rat with sham operation without nephrectomy. **B,** a rat that received deoxycorticosterone (DOCA) and salt. **C,** a rat that received DOCA-salt and vitamins C and E. **D,** a rat that received DOCA-salt and vitamin **E,** E a rat that received DOCA-salt and vitamin C.

group (Figures 5A and 5B). Also, tubular dilation and cellular vacuolization and damage were profoundly abundant in the DOCA-salt group of rats compared to the corresponding sham group. In the group of rats treated with vitamin E plus vitamin C, histological evaluation revealed a profound improvement compared to the DOCA-salt group (Figure 5C). Similar features were observed with solitary administration of vitamin E or vitamin C (Figures 5D and 5E).

DISCUSSION

In the present study, DOCA-salt-treated rats developed severe hypertension and excessive urinary protein excretion in 4 weeks. The increase in urinary protein excretion suggested a significant kidney injury induced by DOCA. The presence of tissue damage was further substantiated by structural abnormalities in the kidney (Figure 4B). In addition, the antioxidants levels (glutathione and FRAP) in the kidney decreased after 4 weeks of treatment with the DOCA and salt, while supplementation with vitamin C, vitamin E, or a combination of them significantly preserved renal antioxidant levels and prevented renal damage and elevation of systolic blood pressure induced by the DOCA and salt.

There are many studies suggesting that hypertension is associated with increased oxidative stress. In a recent study, Meng and coworkers showed that urinary isoprostane excretion, an index of oxidative stress, reached maximum levels after only 1 week of increased dietary sodium intake, whereas arterial pressure and urinary protein excretion continued to increase during the 3rd week of the experiment.[18] The authors suggested that oxidative stress might have contributed to the increases in arterial blood pressure and kidney damage. In another study, Chabrashvili and colleagues[2] showed that the expression of nicotinamide adenine dinucleotide phosphate oxidase, an enzymatic source of superoxide anion production, was enhanced in the kidneys of young spontaneously hypertensive rats prior to the development of high blood pressure. Our study provides support for the effect of antioxidant therapy in improving kidney damage and elevation of blood pressure in the DOCA-salt-induced hypertension.

In the present study, the doses of the vitamins were determined according to the previous report of beneficial effect of these doses. Vitamin C, 200 mg/kg/d, reduces the elevated arterial pressure in spontaneously hypertensive rats on a high-sodium diet.[19] Previous studies have also shown a beneficial effect of 200 mg/kg of vitamin E supplementation to correct the overproduction of vascular superoxide anion in rats who received DOCA.[20]

Combined treatment with vitamins C and E has beneficial effects on endothelium-dependent vasodilation, arterial stiffness, and plasma markers of oxidative stress in untreated patients with essential hypertension.[21] The ability of vitamin C to reduce tocopheroxyl radical has been demonstrated in many in-vitro and in-vivo studies.[22,23] Because of the known synergistic action between vitamins E and C, we assessed in this study weather a combination of these antioxidants provided a better protection. Vitamin C or vitamin E alone and a combination of them preserved renal antioxidant levels and prevented kidney damage and elevation of blood pressure in the rats receiving DOCA. In a recent study, cosupplementation of single and multiple doses of vitamins C and E ameliorated cisplatin-induced acute kidney failure in mice.[24] In our study, although the combination of vitamins C and E was effective, the effect was not significantly different from that seen with either vitamin C or vitamin E alone in DOCA-salt-induced hypertensive rats.

Kidney damage in this study was evidenced by proteinuria and renal morphological changes. The decreases in urinary protein excretion and the improvement in renal histopathology in antioxidant groups suggest a major role of oxidative stress in the developing of kidney damage in DOCA-salt-induced hypertension. Co-administration of vitamins C and E did not show an additive protection against kidney damage. The protection might be partially due to the elevated level of glutathione and FRAP and may also be attributed to the direct antioxidant effect of these vitamins. In a study on Dahl S rats with salt-sensitive hypertension, antioxidant treatment with vitamins C and E improved renal dysfunction and decreased arterial pressure.[11] A large clinical trial demonstrated that 6-year supplementation of daily vitamin E and vitamin C reduced progression of carotid atherosclerosis.[13,14]

CONCLUSIONS

We concluded that co-administration of vitamins

C and E may not have an additive beneficial effect on reducing the kidney damage and hypertension in DOCA-salt-induced hypertensive rats compared to the administration of either vitamin C or vitamin E alone.

CONFLICT OF INTEREST

None declared.

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Correspondence to:
Mehri Kadkhodaee, PhD
Department of Physiology, School of Medicine, Tehran Medical Sciences University, Enghelab St, Tehran 141556447, Iran
Tel: +98 21 6641 9484
Fax: +98 21 6641 9484
E-mail: kadkhodm@tums.ac.ir

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