

Effect of Reduced Form of Coenzyme Q10 on Cyclosporine Nephrotoxicity

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Abstract

Objectives: Cyclosporine, a potent immunosuppressant, has nephrotoxic adverse effects that may be mediated by oxidative stress. The reduced form of coenzyme Q10 has antioxidant effects. The aim of the present study was to evaluate the effect of the reduced form of coenzyme Q10 on cyclosporine nephrotoxicity.

Materials and Methods: Six-week-old male Wistar rats were divided into 3 groups (10 animals each). Group 1 (control) received olive oil only. Group 2 received cyclosporine (30 mg/kg/d, which is an experimentally nephrotoxic dose). Group 3 received cyclosporine (30 mg/kg/d) and the reduced form of coenzyme Q10 (600 mg/kg/d). The cyclosporine and the reduced form of coenzyme Q10 were given orally for 4 weeks. Daily urinary albumin excretion, serum creatinine level, and urinary 8-hydroxydeoxyguanosine level were measured, and renal tissue was evaluated by immunohistochemistry.

Results: In rats treated with cyclosporine and the reduced form of coenzyme Q10 (group 3), there were significantly less abnormalities in mean urinary albumin excretion (group 1: 2.8 ± 0.5 ; group 2: 41 ± 7 ; group 3: 21 ± 4 $\mu\text{g/d}$), serum creatinine (group 1: 1.0 ± 0.2 ; group 2: 1.8 ± 0.4 ; group 3: 1.4 ± 0.3 mg/dL), and urine 8-hydroxydeoxyguanosine levels (group 1: 7 ± 3 ; group 2: 10 ± 3 ; group 3: 7 ± 1 mg/mL creatinine)

than rats treated with cyclosporine alone (group 2). There were 8-hydroxydeoxyguanosine deposits seen in the proximal tubular cells of group 2 that were not present in rats treated with the reduced form of coenzyme Q10 (group 3).

Conclusions: The reduced form of coenzyme Q10 may prevent or minimize cyclosporine nephrotoxicity by an antioxidant effect.

Key words: Oxidative stress, Immunosuppression, Adverse reactions, Kidney

Introduction

Cyclosporine, a potent immunosuppressive drug, has nephrotoxic adverse effects in a dose-dependent manner. The mechanism of nephrotoxicity may involve oxidative stress.^{1,2} Therefore, some antioxidants may prevent cyclosporine toxicity.

Coenzyme Q10 (CoQ10) is an oil-soluble vitaminlike substance that is a component of the electron transport chain. It is found in the membranes of many organelles, especially the inner membrane of mitochondria, and participates in aerobic cellular respiration as an electron carrier for generating energy in the form of adenosine triphosphate in vivo. There are 2 molecular forms of CoQ10: oxidized and reduced. The reduced form of CoQ10 (rCoQ10) is a common dietary supplement. It has gained attention as an antioxidant because it holds electrons loosely in the reduced form, easily giving up 1 or both electrons.³

Both oxidized and reduced forms of CoQ10 may be taken orally with few adverse effects.³ The oxidized form is changed to the reduced form when it reaches the lymphatics through the small intestine.⁴ As a result, most total CoQ10 in the human body is the reduced form.⁵ Orally administered rCoQ10 significantly increases the amount of rCoQ10 in the body.⁶

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The aim of the present animal study was to evaluate the effect of rCoQ10 on cyclosporine nephrotoxicity.

Materials and Methods

Six-week-old male Wistar rats were divided into 3 groups (10 rats each). Group 1 received olive oil only (control). Group 2 received cyclosporine (30 mg/kg/d, which is an experimentally nephrotoxic dose).⁷ Group 3 received cyclosporine (30 mg/kg/d) and rCoQ10 (600 mg/kg/d) (Kaneka, Osaka, Japan). Cyclosporine was dissolved in olive oil at a concentration of 20 mg/mL and given orally once daily using a gastric tube. The rCoQ10 was mixed at 0.6% with standard chow and fed to each rat. The cyclosporine and rCoQ10 were given for 4 weeks. Animals were handled in an accredited facility according to the institutional guidelines for animal care, and the research protocols conformed to the principles outlined by the ethical committee for animal study in the University of Tokyo.

Systolic blood pressure of each rat was monitored every week by a tail cuff method (BP-98A, Softron, Tokyo, Japan). The 24-hour urine of each rat was collected using metabolic cages on the last day of the experiment, and urinary albumin excretion was measured by an enzyme-linked immunosorbent assay (AKRAL-120, Shibayagi, Gunma, Japan). Urinary 8-hydroxydeoxyguanosine levels were measured by an enzyme immunoassay method. On the last day of the experiment, a blood sample was obtained from each rat, and serum creatinine level was measured by a traditional Jaffe method using alkaline picrate.⁸ Whole blood cyclosporine level was determined by a fluorescence polarization immunoassay method. Immunohistochemistry of the kidney using anti-8-

hydroxydeoxyguanosine monoclonal antibody (clone N45.1 / Nikken Seil Co., Ltd. Shizuoka, Japan) also was performed.

Statistical comparison of data between the 3 groups was done using analysis of variance and Fisher partial least-squares difference test. The data are reported as means \pm standard error. Statistical significance was defined by $P < .05$.

Results

Cyclosporine alone (group 2) caused significantly increased systolic blood pressure, urinary albumin, serum creatinine, and urinary 8-hydroxydeoxyguanosine, compared with olive oil control (group 1) (Table 1). The group treated with the combination of cyclosporine and rCoQ10 (group 3) had similar systolic blood pressure, but had significantly lower urinary albumin, serum creatinine, and urinary 8-hydroxydeoxyguanosine than did the group treated with cyclosporine alone (group 2) (Table 1).

No significant difference was observed between mean whole blood cyclosporine levels of groups 2 and 3 (group 2: 611 ± 102 $\mu\text{g/L}$; group 3: 588 ± 115 $\mu\text{g/L}$; not significant).

Microvacuolar changes and 8-hydroxydeoxyguanosine positive deposits were observed in the proximal tubular cells of the cyclosporine group (group 2), and these findings were not present in rats treated with cyclosporine and rCoQ10 (group 3) (Figure 1).

Discussion

In the present study, we demonstrated that rCoQ10 provided protection against the adverse effects of cyclosporine. Administration of rCoQ10 with

Table 1. Effect of Cyclosporine and Reduced Coenzyme Q10 on Systolic Blood Pressure, Urinary Albumin Excretion, Serum Creatinine Level, and Urinary 8-Hydroxydeoxyguanosine in Rats*

| Group | Systolic Blood Pressure (mm Hg) | Urinary Albumin ($\mu\text{g/d}$) | Serum Creatinine ($\mu\text{mol/L}$) | Urinary 8-Hydroxydeoxyguanosine (mg/L Creatinine) |
|----------------------------------------------|---------------------------------|-------------------------------------|----------------------------------------|---------------------------------------------------|
| Group 1: olive oil control | 113 ± 3 | $24.8 \pm 4.4^\dagger$ | 88.4 ± 17.7 | 71 ± 32 |
| Group 2: cyclosporine | $134 \pm 3^\ddagger$ | $362.4 \pm 61.9^\ddagger$ | $159.1 \pm 35.4^\ddagger$ | $102 \pm 31^\S$ |
| Group: cyclosporine and reduced coenzyme Q10 | $128 \pm 4^\ddagger$ | $185.6 \pm 35.4^\ddagger$ | $123.8 \pm 26.5^\ddagger$ | $72 \pm 13^\square$ |

*Data are presented as mean \pm SEM. For each group, n=10 rats.

$^\dagger P < .0001$ (comparison between any 2 groups in the column denoted by †)

$^\ddagger P < .0001$ vs group 1

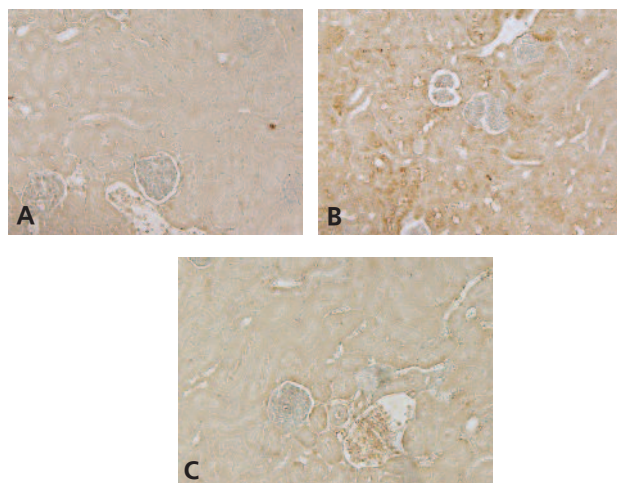
$^\S P < .0009$ vs group 1 and $.02$ vs group 2

$^\square P < .04$ vs group 1

$^\square P < .03$ vs group 2

cyclosporine caused less abnormality in markers of renal failure (albuminuria and serum creatinine elevation) compared with cyclosporine alone (Table 1). Furthermore, immunohistochemistry showed less 8-hydroxydeoxyguanosine positive deposits in the proximal tubular cells when rCoQ10 was given with cyclosporine, compared with cyclosporine alone (Figure 1).

Figure 1. Effect of Cyclosporine and Reduced Coenzyme Q10 on Renal Tissue in Rats¹



Immunohistochemistry of renal tissue stained with anti-8-hydroxydeoxyguanosine monoclonal antibody (original magnification $\times 200$); (A) Rat treated with olive oil control (group 1). (B) Rat treated with cyclosporine alone (group 2): 8-hydroxydeoxyguanosine positive deposits are noted in the proximal tubular cells. (C) Rat treated with cyclosporine and reduced coenzyme Q10 (group 3): 8-hydroxydeoxyguanosine positive deposits are not present.

Urinary 8-hydroxydeoxyguanosine, an oxidized derivative of deoxyguanosine, is a marker for oxidative stress. It has a stable molecular structure and is excreted in the urine without modification. Cyclosporine nephrotoxicity is, in part, caused by oxidative stress, and urinary 8-hydroxydeoxyguanosine levels are increased by cyclosporine.⁹⁻¹¹ This is consistent with the present finding that rCoQ10, a strong antioxidant, may be protective against the nephrotoxic adverse effects of cyclosporine (Table 1). Several previous studies had suggested that cyclosporine nephrotoxicity may be improved or prevented by antioxidants.¹²⁻¹⁷

A meta-analysis of clinical trials of CoQ10 for hypertension concluded that CoQ10 had potential for lowering blood pressure in hypertensive patients¹⁸; however, in this study, no significant reduction of systolic blood pressure was observed when rCoQ10 was administered with cyclosporine. A

possible explanation for this finding is that cyclosporine-induced hypertension may be caused by activation of the intrarenal renin-angiotensin axis,⁷ but the renal protective effect of rCoQ10 may be accomplished by another mechanism. In clinical practice, antihypertensive treatment is very important for the complete control of cyclosporine nephrotoxicity. The effect of rCoQ10 probably was not caused by a change in cyclosporine levels because there was no significant difference between whole blood cyclosporine levels of the rats treated with or without rCoQ10.

In conclusion, we demonstrated that rCoQ10 may prevent cyclosporine nephrotoxicity in rats. Clinical trials in humans are warranted to evaluate the potential benefit of supplementation with rCoQ10 for patients on cyclosporine.

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