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Original Article

Protective Effects of Vitamin E and/or Quercetin Co-Supplementation on the Morphology of Kidney in Cyclosporine A-Treated Rats

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Abstract

Background: Cyclosporine A (CsA) is a nephrotoxic immunosuppressive drug. Antioxidants might attenuate its toxicity. In the present study, the effects of vitamin E and quercetin on the morphology of kidney in CsA-treated rats were investigated.

Methods: Six groups of rats were used in this gavage feeding study either for 4 or 8 weeks. Groups 1 and 2 received either olive oil or 25% ethanol in olive oil per day. Group 3 received CsA (25 mg/kg/day) in olive oil. All other groups received CsA plus the following: group 4, vitamin E (100 mg/kg/day) in olive oil; group 5, quercetin (15 mg/kg/day) in 25% ethanol in olive oil; and group 6, vitamin E plus quercetin. In the final day of the study, the animals were sacrificed and kidney sections were prepared for morphologic studies using light microscopy.

Results: Acute morphologic alterations induced by CsA in the kidney tubules included isometric vacuolization, brush border loss, microcalcification, and presence of inclusion bodies. Smooth muscle degeneration and necrosis were developed in arterioles.

Treatment with vitamin E plus quercetin prevented severe, moderate, and mild abnormalities of the tubules. However fibrosis was the only microscopic change of the interstitium that was not present in animals treated with vitamin E plus quercetin after both periods.

Some mild morphological changes of the blood vessels such as arteriolar medial smooth muscle degeneration and necrosis, arteriolar myocyte dropout and arteriolar wall hyalinization caused by CsA disappeared with administration of vitamin E, quercetin or vitamin E plus quercetin in both periods.

Conclusion: Co-administration of vitamin E plus quercetin with CsA in renal transplant patients may be beneficial in reducing the nephrotoxic effects of CsA.
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Keywords ● Cyclosporine A ● kidney ● morphology ● vitamin E ● quercetin ● rats

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Introduction

Cyclosporine A (CsA), an immunosuppressive drug of choice in kidney transplantation, is nephrotoxic and produces a series of biochemical changes in serum and the kidney tissues in addition to the acute and chronic

renal morphological alterations. In rats, the biochemical changes include gradual increase in blood urea nitrogen (BUN) and serum level of creatinine together with diminished kidney tissue antioxidant enzymes and elevated thiobarbituric acid reactive substances (TBARS).[1]

Combination of quercetin and vitamin E attenuates adverse biochemical effects of CsA on both the kidney antioxidant enzymes and serum levels of BUN and creatinine.[1] Morphological changes of the kidney tissue as a result of CsA nephrotoxicity include both acute and chronic changes. Acute changes affect tubules, glomeruli, interstitium, and vessels.[2] Acute tubular changes consist of loss of brush borders and isometric vacuolization, which are usually observed in proximal tubules as early as 4 days after treatment with CsA.

Tubular microcalcification and the presence of inclusion bodies are among other features of tubular nephrotoxicity caused by CsA.[2] In acute CsA nephrotoxicity, prominent morphological changes in the glomeruli or interstitium are often not observed. However, acute changes in the kidney arterioles of the rats treated with CsA include smooth muscle degeneration and necrosis or apoptosis.[2]

Chronic CsA nephrotoxicity has been observed as early as 2 weeks after treatment with CsA. These nephrotoxic effects include interstitial fibrosis, tubular atrophy, arteriolar hyalinosis, and sometimes focal glomerular scarring.[2] Kim and Suh,[3] noted that at high doses of CsA, renal tubular changes characterized by vacuolization, presence of inclusion bodies and microcalcification, and interstitial fibrosis were commonly found both in humans and rats. Satyanarayana and co-workers,[4] demonstrated that severe striped interstitial fibrosis, arteriopathy, glomerular basement membrane thickening, tubular vacuolization, and hyaline casts produced by subcutaneous injection of 20 mg/kg CsA for 21 days could be attenuated by co-administration of 2mg/kg quercetin combined with CsA. In another study, Satyanarayana and Chopra reported the beneficial effects of an anti-ischemic agent, trimetozine, in attenuating renal dysfunction and morphological changes induced by CsA treatment in rats.[5]

Effects of flavonoids and polyphenols in attenuating the adverse morphological changes induced by CsA in kidney tissues are reviewed by Rezzani.[6] Sabry and colleagues showed focal tubular atrophy and interstitial fibrosis in the inner medulla and the inner strip of outer medulla in CsA treated rats.[7] They also compared the effects of colchicine and omega-3 fatty acids in reversing the morphological abnormalities in the animals' kidneys.

In the present investigation, morphological changes induced in rat kidney by high doses of CsA for either 4 or 8 weeks were studied using light microscopy.

The primary objective of the present study was to evaluate the possible beneficial effects of vitamin E and/or quercetin in attenuating the abnormal morphological changes induced by CsA in rat kidney.

Materials and Methods

The study protocol and the experiments on animals were approved by the Ethics Committee of Shiraz University of Medical Sciences. Six groups of male Sprague Dawley rats each consisting of 7-10 animals weighing 270 ± 15 g were used in this study for either 4 or 8 weeks as reported previously.[1,8] Rats in all groups received drug, supplements, or vehicles by gavage in the following manner: group 1, olive oil (0.5 ml) as vehicle; group 2, ethanol, 25% in olive oil (0.5 ml) plus olive oil (0.5 ml) as vehicle; group 3, CsA (Neoral, Novartis Pharma AG, Basel, Switzerland) 25mg/kg in 0.5 ml olive oil; group 4, CsA (25mg/kg) plus vitamin E (Fluka Chemical Company, Buchs, Switzerland) 100 mg/kg in olive oil (0.5 ml); group 5, CsA (25mg/kg) plus quercetin (Sigma Chemical Company, St. Louis, MO, USA) 15 mg/kg in 25% ethanol in olive oil (0.5 ml); and group 6, CsA (25mg/kg), plus vitamin E and quercetin.

The animals were weighed at the beginning of the experiments and every week thereafter for the rest of the study. On the final day of the treatments, blood sample was collected by cardiac puncture under mild ether anesthesia to measure the whole blood CsA concentration and serum levels of BUN and creatinine. The procedures and the results of the measurement of biochemical parameters and the weight gain of the animals in each group have been reported previously.[1] The animals were then sacrificed by decapitation on the 4th or the 8th week of the study and the kidneys were removed and washed with phosphate buffered saline (PBS). Each kidney was cut longitudinally into two lobes and each lobe was further cut longitudinally into two halves. Three quarters of each kidney was used for biochemical studies. One quarter of each kidney was fixed in 10% neutral buffered formalin for 24 hr, embedded in paraffin and 5 µm thick sections were prepared. Staining of kidney sections was performed by hematoxylin and eosin (H & E), periodic acid Schiff (PAS), Masson's trichrome (Masson), and silver stain (Jone's).

Two slides of H&E, one PAS, one Masson, and one Jone's were studied from each animal's

kidney under light microscope by a nephropathologist who was not aware of the study protocol. Morphological changes studied in the tubules, interstitium, vessels and glomeruli were graded as – (normal), + (mild), ++ (moderate), and +++ (severe). The mean percentage grade of all replicates for a given morphological abnormality in each group was calculated and if 75% or more of the replicates demonstrated a given grade, that grade was assigned to the morphological abnormality in that group. The morphological changes in the kidney tubules included epithelial cell vacuolization, presence of inclusion bodies, microcalcification, epithelial cell regeneration, tubular atrophy, brush border loss, presence of red blood cell casts, and tubular cell debris.

In the interstitium, the pathologist searched for inflammation, edema, and striped, regular and patchy fibrosis. Morphological features studied in the vessels consisted of arteriolar medial smooth muscle degeneration and necrosis, arteriolar myocyte drop out, arteriolar wall hyalinization, mucoid intimal thickening, extravasated red blood cells, vascular necrosis, and thrombosis. In glomeruli, glomerulitis, intraglomerular thrombosis or necrosis, focal or diffuse glomerular basal membrane duplication, mesangial expansion, focal global and focal segmental glomerulosclerosis were among the morphological changes studied.

Results

Biochemical Findings

The effects of vitamin E and/or quercetin in attenuating renal dysfunction in rats after 4 or 8 weeks of treatment with CsA were previously reported.[1] Whole blood concentrations of CsA in animals receiving CsA alone and those receiving CsA and vitamin E plus quercetin were not significantly different (602 ± 3 ng/ml *v* 606 ± 4 ng/ml respectively).

Histopathological Findings

Typical morphological changes induced by CsA in kidney tubules, interstitium, and arterioles after 4 or 8 weeks are shown in figure 1. The effects of vitamin E and/or quercetin cosupplementation in preventing the morphological changes are demonstrated in figures 2-4 and summarized in tables 1 and 2. Morphological changes observed in the tubules after 4 weeks (figure 1A, 1B) or 8 weeks (figure 1C, 1D, 1E, 1F) of CsA administration included moderate tubular epithelial cell cytoplasmic isometric vacuolization (figure 1A), mild tubular inclusion bodies in all segments of proximal tubules, focal and mild loss of brush borders (figure 1B), mild microcalcification, mild tubular epithelial regenerative changes and moderate tubular atrophy (table 1).

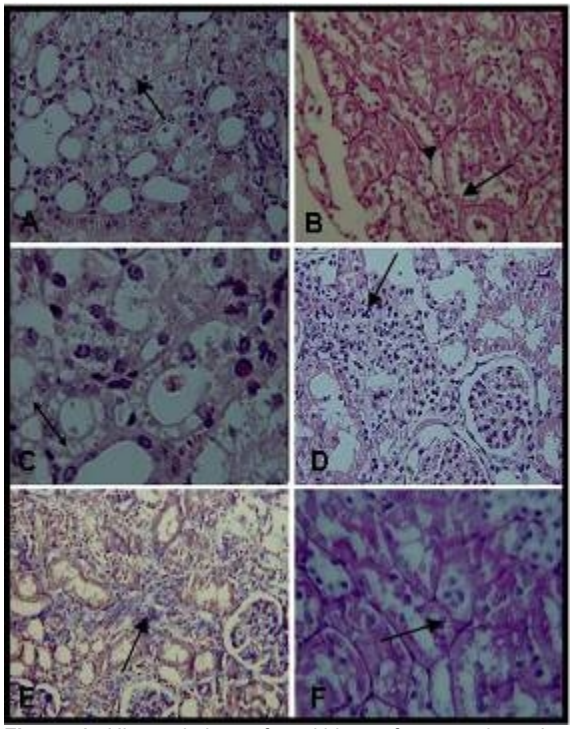

Figure 1: Histopathology of rat kidney after 4 or 8 weeks of CsA administration. A) Isometric vacuolization of proximal tubular epithelial cells (arrow), H&E x 400. B) Loss of proximal tubular brush borders (arrow) and presence of tubular epithelial cell inclusion bodies (arrow head), PAS x 400. C) Tubular epithelial cell vacuolization (double head arrow) H&E x 400. D) Degenerative changes of tubular epithelial cells, H&E x 250. E) Interstitial fibrosis, Masson's x 250. F) Presence of tubular epithelial inclusion bodies (arrow), PAS x 400.

After 8 weeks of CsA administration, tubular epithelial cell regenerative changes were moderate (figure 1D), in all segments of the proximal tubules inclusion bodies were seen (figure 1F), and all of the other tubular changes were the same as the 4-week experimental groups (table 2, and figure 1C-F).

Treatment with vitamin E for 4 weeks only abolished the presence of inclusion bodies and tubular atrophy (table 1), however, the kidney tubules demonstrated some mild degenerative changes (figure 2A). Treatment with quercetin for the same period of time abolished epithelial cell vacuolization in addition to the morphological improvements observered for vitamin E (table 1) while the loss of brush borders still persisted (figure 2B). After 8 weeks of treatment, vitamin E or quercetin had similar effects as 4 weeks (figure 3A-D), except for the presence of moderate or mild inclusion bodies in vitamin E or quercetin groups, respectively (table 2). Combination therapy with vitamin E

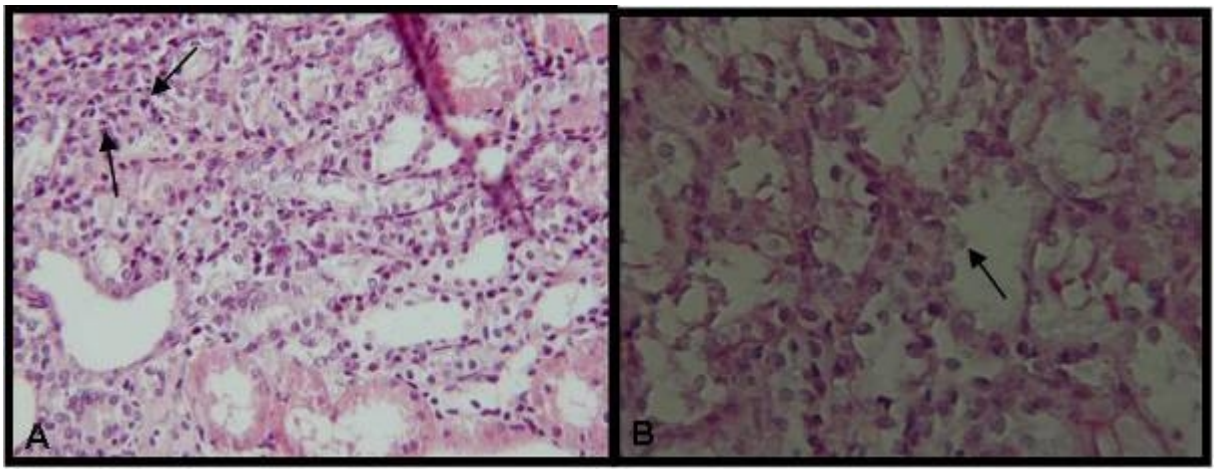

Figure 2: Effects of co-supplementation of vitamin E or quercetin on the morphology of kidney in rats receiving CsA for 4 weeks. A) Tubular epithelial cell degenerative changes of the kidney (arrows) in vitamin E plus CsA group, H&E x 400. B) Loss of tubular brush borders (arrow) in quercetin plus CsA group, PAS x 400.

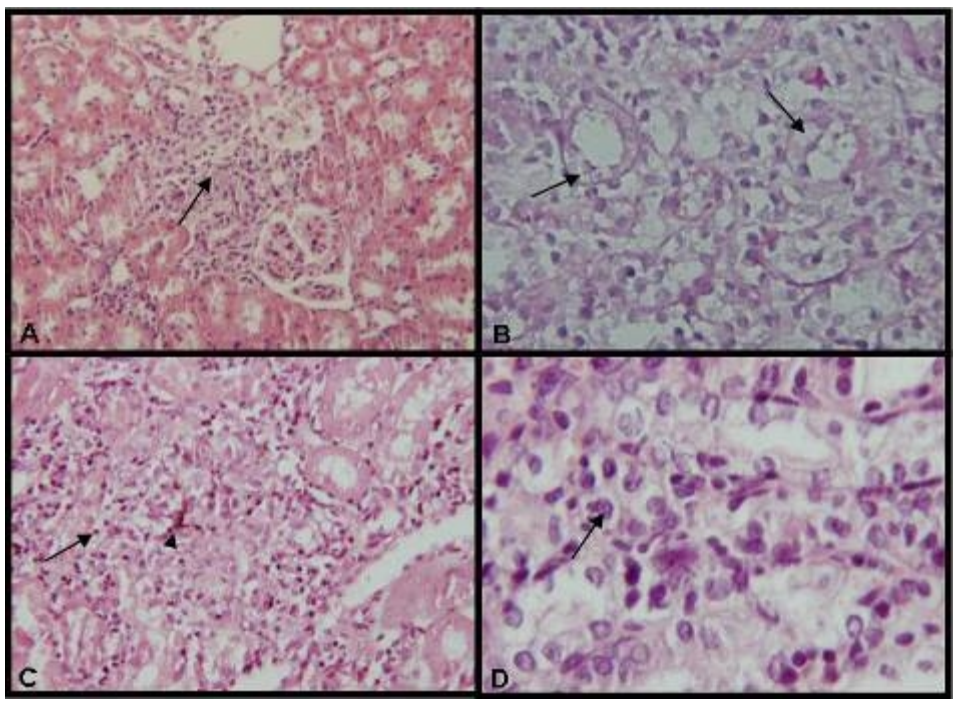

Figure 3: Effects of co-supplementation of vitamin E or quercetin on the morphology of kidney in rats receiving CsA for 8 weeks. A) Tubular epithelial cell degenerative changes of kidney in vitamin E plus CsA group, H&E x 250. B) Isometric vacuolization (arrows) in vitamin E plus CsA group, H&E x 400. C) Inflammatory cell infiltration (arrow) and epithelial cell degenerative changes (arrow head) in quercetin plus CsA group, H&E x 250. D) Tubular epithelial cell regenerative changes (arrow) in quercetin plus CsA group, H&E x 400.

plus quercetin prevented all abnormal tubular morphological changes after 4 or 8 weeks of treatment (tables 1 and 2, and figure 4A-C).

Pathological changes in the interstitium after 4 or 8 weeks of CsA administration included moderate inflammation, mild edema, and moderate (tables 1 and 2) or severe fibrosis (8 weeks, table 2 and figure 1E) that were not completely prevented by vitamin E, however, some of the changes were attenuated or abolished by quercetin or a combination of vitamin E plus quercetin (tables 1 and 2).

Vascular changes in the kidney as a result of 4 or 8 weeks of CsA administration included mild arteriolar medial smooth muscle degeneration and necrosis, mild arteriolar myocyte drop out and mild arteriolar wall hyalinization (tables 1 and 2) that were completely prevented by either vitamin E, quercetin or both (tables 1 and 2). No morphologic abnormality was seen in the glomeruli by CsA treatment or combination of CsA with vitamin E and/or quercetin (data not shown).

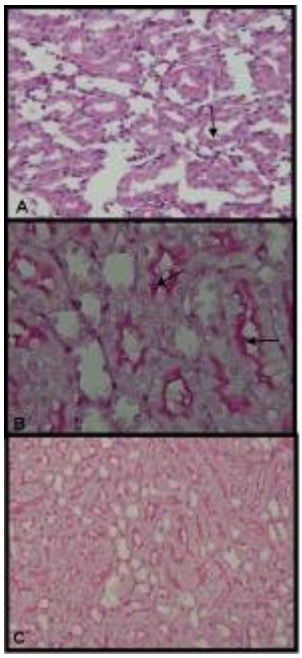

Figure 4: Effects of co-supplementation of vitamin E plus quercetin on the morphology of kidney in rats receiving CsA. A) Preservation of brush borders (arrow) in vitamin E plus quercetin group (4 weeks), PAS x 250. B) Preservation of brush borders (arrows) in vitamin E plus quercetin group (8 weeks), PAS x 400. C) Presence of normal epithelial cells (8 weeks), PAS x 250.

Discussion

The effects of combination therapy with vitamin E and quercetin on kidney dysfunction, the diminution of TBARS and the increase in antioxidant enzymes by vitamin E and/or quercetin in CsA treated rats have been previously reported.[1] We observed a series of acute and chronic morphologic changes in the kidneys of CsA treated rats and demonstrated protective effects of combination therapy with vitamin E and quercetin. The acute morphologic changes of the kidney tubules including isometric vacuolization, loss of brush borders, tubular microcalcification, and presence of inclusion bodies have been reported by Rezzani and Kim and Suh.[2,3] Similarly, CsA –induced acute morphologic changes in the kidney arterioles including smooth muscle degeneration and necrosis were also reported by Rezzani.[2]

Among the chronic features of CsA nephrotoxicity in our study, only interstitial fibrosis was reported by Kim and Suh,[3] and interstitial fibrosis, tubular atrophy, and arteriolar hyalinosis were reported by Rezzani.[2] Sabry and coworkers also reported some chronic morphologic changes such as interstitial fibrosis and focal tubular atrophy in CsA treated animals and demonstrated the protective effects of colchicine and omega-3 fatty acids.[7]

In agreement with our results, Satyanarayana and colleagues,[4] reported that CsA –induced renal morphological changes can be partially reversed by intraperitoneal injection of quercetin in rats.

In the present study, although all tubular and arteriolar changes of rat kidneys were completely prevented by the combination of vitamin E and quercetin, the interstitial changes including inflammation, edema, and fibrosis were only partially attenuated. Vitamin E and quercetin can only partially reverse the morphological changes observed in CsA nephrotoxicity.

Co-administration of vitamin E plus

Table 1: Effects of vitamin E and/or quercetin co-supplementation on the kidney morphology of CsA-treated rats (4- week treatment groups).

Kidney morphology	Control	CsA	CsA+ vitamin E	CsA+ quercetin	CsA+ vitamin E plus quercetin
Tubules:					
Epithelial cell vacuolization	—[1]	++	+	—	—
Inclusion bodies	—	+	—	—	—
Microcalcification	+	+	+	+	—
Epithelial degeneration	—	+	+	+	—
Tubular atrophy	—	++	—	—	—
Loss of brush borders	—	+	+	+	—
Red blood cell casts & tubular cell debris	—	—	—	—	—
Interstitium:					
Inflammation	—	++	+	+	+
Edema	—	+	+	—	+
Fibrosis (striped, regular, patchy)	—	++	+	—	—
Vessels:					
Arteriolar medial smooth muscle degeneration & necrosis	—	+	—	—	—
Arteriolar myocyte dropout	—	+	—	—	—
Arteriolar wall hyalinization	—	+	—	—	—
Mucoid intimal thickening	—	—	—	—	—
Vascular thrombosis	—	—	—	—	—
Intimal thickening	—	—	—	—	—

—[1]: absent, +: mild, + +: moderate, + + +: severe

Table 2: Effects of vitamin E and/or quercetin co-supplementation on the kidney morphology of CsA-treated rats (8- week treatment groups).

Kidney morphology	Control	CsA	CsA+ vitamin E	CsA+ quercetin	CsA+ vitamin E plus quercetin
Tubules:					
Epithelial cell vacuolization	—[1]	++	+	+	—
Inclusion bodies	—	+++	++	+	—
Microcalcification	—	+	+	+	—
Epithelial degeneration	—	++	+	+	—
Tubular atrophy	—	++	—	—	—
Loss of brush borders	—	+	+	+	—
Red blood cell casts & tubular cell debris	—	—	—	—	—
Interstitium:					
Inflammation	+	++	+	+	+
Edema	—	+	+	—	+
Fibrosis(striped, regular, patchy)	—	+++	++	+	—
Vessels:					
Arteriolar medial smooth muscle degeneration & necrosis	—	+	—	—	—
Arteriolar myocyte dropout	—	+	—	—	—
Arteriolar wall hyalinization	—	+	—	—	—
Mucoid intimal thickening	—	—	—	—	—
Vascular thrombosis	—	—	—	—	—
Intimal thickening	—	—	—	—	—

—[1], absent; +, mild; + +, moderate; + + +, severe

quercetin with CsA in patients who need renal transplantation may be beneficial in reducing the nephrotoxic effects of CsA.

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Conflict of Interest: None declared

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