Renoprotective effects of green tea extract on renin-angiotensin-aldosterone system in chronic cyclosporine-treated rats

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Abstract

Background. Renin-angiotensin-aldosterone system (RAAS) activation plays an important role in cyclosporine (CsA)-induced nephropathy. The main aim of this study was to test whether the administration of green tea extract (GTE) prevents the development of CsA-induced nephrotoxicity.

Methods. The rats were treated for 21 days and divided into four groups (n = 6/group): control group (0.9% saline injection), CsA group (30 mg/kg/day by intraperitoneal injection), CsA–GTE group (CsA plus GTE 100 mg/kg/day subcutaneous injection) and GTE group (GTE alone).

Results. There were significant increased levels of serum blood urea nitrogen and creatinine in the CsA group compared with that of the control group and significantly improved in the CsA–GTE group. Biochemical analysis showed that the plasma renin activity (PRA) and serum concentration of aldosterone were significantly increased in the CsA group compared with the control group and sig-

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nificantly decreased in the CsA–GTE group compared with the CsA group. The total level of renin protein expression was significantly higher in the CsA group than in the control group, and it was lower in the CsA–GTE group than in the CsA group.

**Conclusions.** CsA treatment increases the PRA and intrarenal renin levels and induces nephrotoxicity. The protective effects of GTE on CsA-induced structural and functional alternations of the kidney may be the blockage of RAAS.

**Keywords:** cyclosporine nephrotoxicity; green tea extract; renin-angiotensin-aldosterone system

**Introduction**

Cyclosporine (CsA) has been used to prevent rejection in kidney transplantation and is effective in improving 1-year renal allograft survival [1,2]. Nephrotoxicity of CsA has been found to be associated with life-long treatment of transplanted patients in experimental studies [3,4]. Chronic administration of CsA produces renal injury and is one of the known non-immunological factors causing 30–50% of chronic allograft nephropathy [5].

Activation of the renin-angiotensin-aldosterone system (RAAS), especially intrarenal RAAS, plays an important role in the pathogenesis of chronic CsA nephropathy [6,7]. Chronic CsA nephrotoxicity is characterized by tubulointerstitial fibrosis and glomerular vasoconstriction [8,9]. Administration of CsA to rats on a low-salt diet induced histological changes similar to those observed in the long-term CsA therapy patient [10,11]. Infusion of angiotensin II provoked histological changes in rat kidneys similar to those described in chronic CsA nephrotoxicity [12]. Blocking of the RAAS with either angiotensin-converting enzyme (ACE) inhibitors or angiotensin II receptor type I antagonists ameliorates these structural changes and improves the rate of decline of chronic renal allograft dysfunction with chronic CsA nephropathy patients [13,14].

A catechin of green tea, (−)-epigallocatechin 3-O-gallate (EGCG), is known to improve toxicity in renal cells and in rat kidneys. Recently, we have reported that green tea extracts (GTE) protected the mesangial cells from NO-mediated cytotoxicity by scavenging the NO against L-arginine toxicity to cultured human mesangial cells [15]. Tea polyphenols significantly inhibit apoptosis of the tubular and interstitial cells in rats with cyclosporine-induced chronic nephropathy [16]. In addition, EGCG exerts protective activity in rats with adenoine-induced renal failure as a chronic kidney disease model [17].

With this background, the aim of the present study was to investigate the level of renin in both renal tissue and plasma from rats with established chronic CsA nephropathy. We also tested whether the administration of GTE prevents the development of CsA-induced nephrotoxicity and what mechanisms are involved.

**Materials and methods**

**Chemicals**

GTE was prepared from a hot-water extract of green tea (Boseong, Chonnam, Korea) as reported by Maity et al. [18]. CsA was purchased from Chong Kun Dang (CypolTM, Seoul, Korea). All other chemicals were the highest grades of commercially available materials.

**Experimental rat protocol**

Normal male rats of the Sprague–Dawley strain, weighing between 200 and 250 g, were used in this study. The experiments were performed on four groups. Group 1 (normal control group; n = 6) rats were injected intraperitoneally (i.p.) with 0.9% saline solution; Group 2 (CsA group; n = 6) received CsA, 30 mg/kg body weight, daily for 21 days by i.p. injection; Group 3 rats (CsA + GTE group; n = 6) received CsA, 30 mg/kg body weight, daily for 21 days by i.p. injection in addition to GTE 100 mg/kg by subcutaneous injection. Group 4 (GTE group; n = 6) received GTE alone by subcutaneous injection. The reason for the different injection sites for the intraperitoneal and subcutaneous methods was to inhibit the direct pharmacologic reaction between CsA and GTE. Rats were sacrificed under light ether anaesthesia on the 22nd day and blood samples were obtained. Both kidneys from each rat were immediately extracted.

**Biochemical analysis**

Blood urea nitrogen (BUN), serum and urine creatinine levels were measured using a biochemical autoanalyser (AVIDA 1650TM, Bayer, Tarrytown, NY, USA). Trough levels of cyclosporine were measured with fluorescence polarization immunoassay technology (Abbott AxSYM™, Abbott Laboratories, IL, USA) in whole blood. Plasma renin activity (PRA), serum aldosterone and ACE levels were measured using the Quantum™ kit (Packard, Meriden, CT, USA).

**Western blot assay**

Renal tissues of rats were homogenized with ice-cold lysis buffer, pH 7.5, containing 137 mM NaCl, 20 mM Tris–HCl, 1% Tween 20, 10% glycerol, 1 mM phenylmethylsulfonyl fluoride and a protease inhibitor cocktail. For detection of renin protein expression, each sample was centrifuged at 14 000 rpm for 10 min at 4°C. Separated proteins were subjected to the 10% polyacrylamide gels and electrophoretically transferred to a nitrocellulose membrane and incubated with the primary anti-renin (Santa Cruz Biotechnology, Santa Cruz, CA, USA) and β-actin antibody overnight at 4°C. After the blots were washed, they were incubated with a conjugated horseradish peroxidase anti-mouse secondary antibody (R&D system, Minneapolis, MN, USA) for 2 h at room temperature. Each antigen–antibody complex was visualized using LumiGLO chemiluminescent substrate and detected by chemiluminescence with LabWorks software 4.0 (UVP, Upland, CA, USA). Band densities were determined by Scion Image software (Scion Corporation, Frederick, MD, USA) and quantified as a ration of the density of the β-actin band.

**Rat kidney histology**

Tissue samples of rat kidneys were fixed in 10% buffered formalin, embedded in paraffin, cut into 4 μm sections and used for histopathologic examination. The sections were stained with periodic acid-Schiff.

**Statistical analysis**

Results were expressed as mean ± SD. The statistical differences between the control and the experimental groups were analysed using Mann–Whitney test and Kruskal–Wallis test (SPSS, Statistical Package for Social Science version 11.0, Chicago, IL, USA). A P-value of <0.05 was considered significant.

**Results**

**Physiologic and laboratory studies**

**Serum CsA levels.** The rats treated with CsA only failed to gain body weight when compared with those receiving ve-
There was no statistical difference in water intake and urine output between the CsA-treated group and the CsA–GTE group. The CsA–GTE group showed no significant difference in the level of cyclosporine concentration when compared with the CsA group (5621 ± 1289 ng/mL vs 5765 ± 1320 ng/mL, P > 0.05) (Table 1).

Table 1. Effects of GTE on serum CsA level, body weight change, water intake and urine output in the experimental groups

<table>
<thead>
<tr>
<th>Group</th>
<th>CsA level (ng/mL)</th>
<th>Body weight change (g)</th>
<th>Water intake (mL/24h)</th>
<th>Urine output (mL/24h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>–</td>
<td>9.11 ± 2.12</td>
<td>51.4 ± 7.2</td>
<td>15.4 ± 3.2</td>
</tr>
<tr>
<td>GTE</td>
<td>–</td>
<td>10.63 ± 3.29</td>
<td>55.2 ± 8.7</td>
<td>17.1 ± 4.7</td>
</tr>
<tr>
<td>CsA + GTE</td>
<td>5621 ± 1289</td>
<td>8.38 ± 1.97**</td>
<td>49.1 ± 7.8</td>
<td>16.4 ± 3.5</td>
</tr>
<tr>
<td>CsA</td>
<td>5765 ± 1320</td>
<td>2.11 ± 0.52*</td>
<td>45.4 ± 5.2</td>
<td>13.7 ± 2.2</td>
</tr>
</tbody>
</table>

Abbreviations: CsA, cyclosporine-A; Cr, creatinine; GTE, green tea extract. The results are expressed as mean and standard deviation. *P < 0.05 as compared with the control group. **P < 0.05 as compared with the CsA group. (None of the comparisons showed significant differences.)

Effects of GTE on cyclosporine-induced renal dysfunction. Figure 1 shows the effects of GTE on serum parameters of renal function. The BUN level significantly increased in the CsA group (55.8 ± 4.95 mg/dL) compared with the control group (20.1 ± 1.29 mg/dL, P < 0.01) and significantly decreased in the CsA–GTE group (23.1 ± 5.27 mg/dL, P < 0.01) compared with the CsA group. The creatinine level significantly increased in the CsA group (1.10 ± 0.082 mg/dL) compared with the control group (0.32 ± 0.055 mg/dL, P < 0.01) and significantly decreased in the CsA–GTE group (0.48 ± 0.084 mg/dL, P < 0.01) compared with the CsA group. The creatinine clearance was lower in the CsA-treated rats compared with the CsA + GTE-treated group (P < 0.01, Figure 1).

Effects of GTE on PRA and serum aldosterone level. The PRA was significantly higher in the CsA group (18.5 ± 4.88 ng/mL/h) than in the control group (13.3 ± 2.96 ng/mL/h, P < 0.01). The increases in PRA were significantly suppressed by GTE treatment (5.1 ± 4.08 ng/mL/h, P < 0.01). The serum aldosterone level was significantly increased in the CsA group (65.2 ± 36.44 ng/dL) compared with the control group (36.3 ± 19.96 ng/dL, P < 0.01) and significantly decreased in the CsA–GTE group (14.3 ± 4.09 ng/dL, P < 0.01) compared with the CsA group. The animals receiving CsA–GTE showed no significant difference in ACE levels when compared with the control group (P > 0.05). [The serum level of ACE was increased in the CsA group (153.5 ± 33.4 IU/L) compared with the control group (131.2 ± 32.1 IU/L) and decreased in the CsA–GTE group (137.7 ± 9.8 IU/L) but was not statistically different in each group (P > 0.05).] The serum potassium level was significantly higher in the CsA group (33.2 ± 16.39 mmol/L, P < 0.01) than in the control group (12.3 ± 0.98 mmol/L) and significantly lower in the CsA–GTE group (13.7 ± 3.99 mmol/L, P < 0.01) than in the CsA group (Figure 2).

Expression of renin protein by western blot assay. We examined the expression of renin protein in the kidneys of rats. As shown in Figure 3, a low level of renin was observed in the control group without CsA stimulation. Total level of renin protein expression was significantly higher in the CsA group than in the control group and was lower in the CsA–GTE group than in the CsA group (P < 0.05, Figure 3).

Renal pathologic examination. Periodic acid-Schiff-stained specimens from the experimental rats revealed proximal tubular necrosis and mild interstitial inflammation in the
CsA group. The CsA–GTE group showed no changes compared with the control group (Figure 4).

**Discussion**

In this study, we showed that PRA, serum aldosterone and intrarenal renin levels are increased in rats with CsA-induced chronic nephropathy. The present study also demonstrated that treatment with GTE prevents chronic CsA nephropathy as evidenced by restoration of serum BUN and creatinine. In addition, we also found that GTE decreases the PRA, serum aldosterone and intrarenal renin levels in these animals. These data suggest that GTE has a renoprotective effect on chronic CsA nephropathy via suppression of the RAAS, such as the renin inhibitor rather than ACE inhibitor.

Green tea contains various types of catechins such as (−)-epigallocatechin 3-O-gallate (EGCG), (−)-gallocatechin 3-O-gallate, (−)-epicatechin 3-O-gallate, (−)-epigallocatechin, (+)-gallocatechin, (−)-epicatechin and (+)-catechin [19]. Catechins are known to have many physiological functions, such as anti-tumour activity, anti-mutagenic activity, hypocholesterolaemic effects, protective effects on liver injury, suppressive effects on renal failure and have antioxidant and anti-diabetic effects [20–23]. In addition, EGCG inhibits the proliferation of mesangial cells and induces apoptosis in renal interstitial fibroblast cells in *in vitro* studies [24,25]. Recently, we have reported that green tea polyphenol treatment has antiproteinuric effects in CsA-induced acute renal injury [26]. However, it is unknown what effects GTE has on chronic cyclosporine nephropathy.

Long-term treatment of CsA decreases glomerular filtration rate and renal blood flow by intrarenal vasoconstriction which results in low-grade ischaemic injury. The mechanism for intrarenal vasoconstriction is related to the activation of the intrarenal RAAS and hypersecretion of endothelin-1 [6]. The exact mechanisms of activa-
tion of the intrarenal RAAS are multifactorial, and one hypothesis is that the CsA increases renin release directly from juxtaglomerular cells [7]. Activation of intrarenal RAAS also induces nephrotoxicity by stimulation of tubulointerstitial injury, transforming growth factor-beta 1, osteopontin as well as increasing renal cell apoptosis [27–29]. Klar et al. [30] have shown that aldosterone enhances renin gene expression by stabilizing renin mRNA in primary cultures of mouse juxtaglomerular cells. In the present study, we found that chronic CsA administration increased serum creatinine and BUN and induced nephrotoxicity in rats. These data probably result from increased renal vasoconstriction induced by stimulation of PRA and serum aldosterone level and an increased expression of renin protein in the rat kidney. The beneficial effects of GTE were associated with prevention of the increase in PRA and serum aldosterone levels and decreased expression of intrarenal renin, and also pathological states.

In this study, we have shown that the serum potassium level was significantly higher in the CsA group than in the control group and significantly lower in the CsA–GTE group than in the CsA group. As to the potassium channel, it has been reported that CsA induces the opening of a potassium-selective channel in higher plant mitochondria [31]. The decrease in Na⁺-K⁺ ATPase activity caused by CsA is thought to be one of the mechanisms for the observed potassium ion secretion defects [32].

Efforts to define optimal drugs for the prevention of chronic CsA nephropathy and studies of other materials are potentially valuable. This study was limited by the GTE components. In the present study, we did not use a single component of green tea catechins. In general, EGCG is the most potent catechin for the inhibition of growth of cancer cells. However, the other catechins in green tea act synergistically with EGCG in their anticancer properties [33]. Therefore, further research using EGCG is required to determine the efficacy of GTE in chronic CsA nephropathy.

In summary, GTE has renoprotective effects against chronic CsA nephropathy in rats, because it is highly possible that GTE can inhibit the activation of the RAAS via suppression of PRA, serum aldosterone levels and expression of intrarenal renin. This study may provide strong supporting evidence for the renoprotective effects in chronic CsA nephropathy, suggesting that it would be a potential aid for the management of transplant patients with CsA administration.

Conflict of interest statement. None declared.

References


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