Mechanisms of Cyclosporine-Induced Renal Cell Apoptosis: A Systematic Review

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**Key Words**
Cyclosporine A • Apoptotic mechanisms • Renal cells • Chronic cyclosporine A nephrotoxicity • Systematic review

**Abstract**

**Background/Aims:** Chronic cyclosporine A (CsA) nephrotoxicity (CCN) is an important cause of chronic renal dysfunction with no effective clinical intervention. To further elucidate the mechanisms of renal cell apoptosis in CCN, all relevant in vivo studies on this subject were analyzed. **Methods:** We searched for in vivo studies on the mechanisms of CsA-induced renal cell apoptosis in Medline (1966–July 2010), Embase (1980–July 2010) and ISI (1986–July 2010). The studies were evaluated for their quality according to a set of in vivo standards, data extracted according to PICOS, and then synthesized. **Results:** Renal cell apoptosis was an important feature of CCN and an important factor of renal dysfunction. First, CsA could upregulate Fas/Fas ligand, downregulate Bcl-2/Bcl-XL, and increase caspase-1 and caspase-3. Second, it could induce oxidative stress and damage the antioxidant defense system. Third, it could increase endoplasmic reticulum stress protein in a dose- and time-dependent manner. Fourth, CsA could impair the urine concentration and decrease the expression of hypertonicity-induced genes. Fifth, CsA-induced renal cell apoptosis was significantly decreased by blocking the angiotensin II type 1 receptor using losartan. **Conclusions:** The in vivo mechanisms for CCN are more complex than those found in vitro. CsA can induce renal cell apoptosis using five pathways in vivo and activated caspses might be the ultimate intersection of these pathways and the common intracellular pathway mediating apoptosis. These data provide new potential points for intervention and need to be confirmed by further studies.

**Introduction**

Cyclosporine A (CsA) is widely used for organ transplantation and autoimmune disorders [1]. Chronic CsA nephrotoxicity (CCN) is an important cause of chronic renal dysfunction, the primary cause of graft loss [2–7]. Our previous systematic review [8] showed that coculturing CsA with renal tubular epithelial cells, vascular en-
dothelial cells and mesangial cells induced their apoptosis in a time- and dose-dependent manner. At least four pathways were found to be involved in CsA-induced renal cell apoptosis in cultured cells. These findings indicated that apoptosis in renal cells was an important cause of CCN. However, in vitro studies do not always reflect the in vivo context. To further elucidate the in vivo apoptotic mechanisms and provide new ideas for prevention, intervention for future studies of chronic renal dysfunction, we systematically evaluated all in vivo studies on the mechanisms of CsA-induced renal cell apoptosis.

Methods

Search Strategy

Two reviewers (Z.X. and J.S.) independently searched articles in electronic databases using the search strategy (Nephrotoxicity AND (Cyclosporine A OR CsA OR CyA OR Cyclosporine) AND Apoptosis) or the search strategy (Cyclosporine A OR CsA OR CyA OR Cyclosporine) AND (Renal tubular epithelial cell OR Renal cells) AND Apoptosis). The databases used were Medline (1966–July 2010), Embase (1974–July 2010) and ISI (1986–July 2010). No language restrictions were placed on the search.

Included/Excluded Criteria

All in vivo studies on the mechanisms of CsA-induced renal cell apoptosis (no limitation of research design) were eligible for inclusion. We excluded duplicated articles, meeting abstracts and reviews without specific data.

Evaluation Standards

There is no generally accepted evaluation standard for in vivo research. We therefore defined a grading system based on the ‘Evidence Pyramid’ [9]. The quality of all articles was evaluated according to the following in vivo standards: A = systematic reviews (i.e. meta-analyses) of studies in animals; B = randomized controlled study, or inbred animal study; C = controlled study; D = noncontrolled study.

Selection and Evaluation of Articles

Two reviewers (Z.X. and J.S.) independently selected and evaluated articles according to the above standards. Any disagreements were resolved by discussion with each other or with Youping Li.

Data Extraction and Synthesis

All studies were classified into two groups: characteristics of animal models and apoptotic mechanisms. We used descriptive methods to synthesize the studies because of the heterogeneity of the data. After reviewing all the articles, we designed data extraction tables according to research themes, extracted the experimental data following PICOS (Animal (P), Interventions (I), Control study (C), Outcomes (O) and Study Design (S)) and then synthesized the studies.

Results

Search Results

The initial database search identified 454 articles using our search strategies (fig. 1). After successively applying the study exclusion criteria, 25 articles were selected.

Study Quality

Of the 25 articles, 10 were randomized controlled studies (grade B), and 15 were control studies (grade C). The grades for individual articles are shown in the data extraction tables.

Main Characteristics of Animal Models

After reviewing all articles, 19 studies were included in this group. There were 10 studies that scored B and 9
studies that scored C (table 1). Sprague-Dawley (SD) rats (14 studies), Wistar rats (4 studies) or imprinting control region (ICR) mice (one study) were used to establish CCN animal models. Most animals (80%) were pretreated by feeding full low-salt diet and treated for about 4 weeks with CsA (15 mg/kg/day s.c.). The dose of CsA in mice was higher than that in rats [10]. Wistar rats and ICR mice were treated about 4 weeks with CsA. CsA treatment dramatically induced CCN as shown by interstitial inflammatory infiltration, renal tubular injury and fibrosis in renal tissue. CsA treatment decreased kidney function and increased both tubular and interstitial cell apoptosis in different animals [11–15]. Renal cell apoptosis was identified by the terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assay in all studies and various in situ apoptosis detection kits were used. All results showed that CsA treatment could cause CCN with interstitial inflammatory infiltration, renal tubular cells apoptosis and fibrosis in the animals studied.

### Apoptotic Mechanisms of CsA Nephrotoxicity

Twenty-seven studies from 25 articles were analyzed (tables 2–6). Six apoptotic pathways were found to be involved in nephrotoxicity; namely Fas/Fas ligand, mitochondrion, endoplasmic reticulum (ER), angiotensin II (AngII), hypertonicity pathway and the nitric oxide (NO)-related pathway. These pathways could synergistically mediate renal cell apoptosis.

#### Fas/Fas Ligand Pathway

Eight studies were included, three of which scored B and five scored C (table 2). In seven studies, SD rats were...
### Table 2. Fas/Fas ligand pathway

<table>
<thead>
<tr>
<th>Reference</th>
<th>Animals (n)</th>
<th>Interventions</th>
<th>Capsule</th>
<th>Outcome</th>
<th>Study in vivo</th>
<th>Quality grade</th>
</tr>
</thead>
<tbody>
<tr>
<td>Li et al. [14]</td>
<td>SD (39)</td>
<td>15 mg/kg/day s.c.</td>
<td>5</td>
<td>yes</td>
<td>Fas</td>
<td>Bcl-2 no</td>
</tr>
<tr>
<td>Shihab et al. [15]</td>
<td>SD (32)</td>
<td>15 mg/kg/day s.c.</td>
<td>4</td>
<td>yes</td>
<td>Fas ligand</td>
<td>Bax Bcl-2 caspase-1,-3</td>
</tr>
<tr>
<td>Lee et al. [17]</td>
<td>SD (26)</td>
<td>15 mg/kg/day s.c.</td>
<td>4</td>
<td>yes</td>
<td>no</td>
<td>Bax Bcl-2 caspase-3</td>
</tr>
<tr>
<td>Lee et al. [19]</td>
<td>SD (30)</td>
<td>15 mg/kg/day s.c.</td>
<td>6</td>
<td>yes</td>
<td>Fas</td>
<td>Bax Bcl-2 caspase-3</td>
</tr>
<tr>
<td>Li et al. [21]</td>
<td>SD (24)</td>
<td>15 mg/kg/day s.c.</td>
<td>4</td>
<td>yes</td>
<td>no</td>
<td>Bax Bcl-2 caspase-3</td>
</tr>
<tr>
<td>Shihab et al. [24]</td>
<td>SD (32)</td>
<td>7.5 mg/kg/day s.c.</td>
<td>4</td>
<td>yes</td>
<td>Fas ligand</td>
<td>no Bcl-X1 caspase-3</td>
</tr>
<tr>
<td>Rawat et al. [23]</td>
<td>SD (10)</td>
<td>25 mg/kg/day s.c.</td>
<td>4</td>
<td>yes</td>
<td>no</td>
<td>Bax Bcl-2 caspase-3</td>
</tr>
<tr>
<td>Yang et al. [10]</td>
<td>Imprinting control region mice (24)</td>
<td>30 mg/kg/day s.c.</td>
<td>4</td>
<td>yes</td>
<td>Fas ligand</td>
<td>Bax Bcl-2 caspase-1,-3</td>
</tr>
</tbody>
</table>

O1 = Increased Fas/Fas-l; O2 = translocation of Bax; O3 = decreased antiapoptotic factors; O4 = increased caspases; O5 = increased apoptosis of tubular and interstitial cells.

### Table 3. Mitochondrial pathway

<table>
<thead>
<tr>
<th>Reference</th>
<th>Animal strain (n)</th>
<th>Interventions</th>
<th>Control, olive or saline</th>
<th>Outcome</th>
<th>Study in vivo</th>
<th>Quality grade</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ghee et al. [12]</td>
<td>SD (24)</td>
<td>15 mg/kg/day 4</td>
<td>yes no no no no no MΦ</td>
<td>OPN no yes</td>
<td>B</td>
<td></td>
</tr>
<tr>
<td>Lee et al. [17]</td>
<td>SD (26)</td>
<td>15 mg/kg/day 4</td>
<td>yes no no no no no no</td>
<td>Bax Bcl-2 caspase-3 MΦ</td>
<td>OPN, CRP no yes</td>
<td>B</td>
</tr>
<tr>
<td>Chung et al. [18]</td>
<td>SD (26)</td>
<td>15 mg/kg/day 4</td>
<td>yes no no no no no no</td>
<td>Bax Bcl-2 caspase-3 MΦ</td>
<td>AngII, OPN no yes</td>
<td>B</td>
</tr>
<tr>
<td>Jin et al. [29]</td>
<td>SD (19)</td>
<td>15 mg/kg/day 2</td>
<td>yes no no no no no no</td>
<td>Bax Bcl-2 caspase-3 MΦ</td>
<td>LIX, MCP-1 no yes</td>
<td>C</td>
</tr>
<tr>
<td>Lee et al. [19]</td>
<td>SD (30)</td>
<td>15 mg/kg/day 6</td>
<td>yes no no no no no no</td>
<td>Bax Bcl-2 caspase-3 MΦ</td>
<td>no no yes</td>
<td>B</td>
</tr>
<tr>
<td>Mazzali et al. [30]</td>
<td>Imprinting control region mice (17)</td>
<td>30 mg/kg/day 2</td>
<td>no no no no no no no</td>
<td>MΦ no no no no no no yes</td>
<td>C</td>
<td></td>
</tr>
<tr>
<td>Khan et al. [31]</td>
<td>Wistar (40)</td>
<td>15 mg/kg/day 2</td>
<td>yes MDA GPx, SOD no no no no no no no no</td>
<td>MΦ no no yes</td>
<td>C</td>
<td></td>
</tr>
<tr>
<td>Disel et al. [27]</td>
<td>Wistar (24)</td>
<td>15 mg/kg/day 4</td>
<td>yes MDA no no no no no no no no</td>
<td>no yes yes</td>
<td>B</td>
<td></td>
</tr>
<tr>
<td>Kasap et al. [25]</td>
<td>Wistar (26)</td>
<td>25 mg/kg/day 4</td>
<td>yes TRARS SOD no no no no no no no no</td>
<td>caspase-3 no yes</td>
<td>C</td>
<td></td>
</tr>
<tr>
<td>Josephine et al. [32]</td>
<td>Wistar (24)</td>
<td>50 mg/kg BW 3</td>
<td>yes ROS SOD, GPx, GSH no no no no no no no no</td>
<td>no no no no yes</td>
<td>C</td>
<td></td>
</tr>
<tr>
<td>Rawat et al. [23]</td>
<td>SD (10)</td>
<td>25 mg/kg/day 4</td>
<td>yes no no no no no no</td>
<td>Bax Bcl-2 caspase-3 no no no no no no no no yes</td>
<td>C</td>
<td></td>
</tr>
<tr>
<td>Li et al. [21]</td>
<td>SD (24)</td>
<td>15 mg/kg/day 4</td>
<td>yes no no no no no no</td>
<td>Bax Bcl-2 caspase-3 no no no no no no no no yes</td>
<td>C</td>
<td></td>
</tr>
</tbody>
</table>

O1 = Increased reactive oxygen species; O2 = decreased antioxidant factors; O3 = increased proapoptotic factors; O4 = decreased antiapoptotic factors; O5 = increased caspases; O6 = increased inflammatory cell infiltration; O7 = increased proinflammatory cytokines: LIX, MCP-1, OPN, CRP, AngII; O8 = increased apoptosis of tubular and interstitial cells; SOD = superoxide dismutase; GSH = glutathione; LPO = lipid peroxidation; GPx = glutathione peroxidase.
Table 4. Endoplasmic reticulum pathway

<table>
<thead>
<tr>
<th>Reference</th>
<th>Animal strain (n)</th>
<th>Interventions</th>
<th>Control vehicle</th>
<th>Outcome</th>
<th>Study in vivo</th>
<th>Quality grade</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pallet et al. [33]</td>
<td>SD (18)</td>
<td>15 mg/kg/day 4</td>
<td>yes</td>
<td>GRP78, PDI, CHOP, HERP</td>
<td>no</td>
<td>yes</td>
</tr>
<tr>
<td>Han et al. [11]</td>
<td>SD (24)</td>
<td>15 mg/kg/day 4</td>
<td>yes</td>
<td>BiP (4), CHOP</td>
<td>yes</td>
<td>no</td>
</tr>
</tbody>
</table>

O1 = UPR-related genes unfolded protein response, glucose-regulated protein 78 (GRP78, also known as BiP), increased CHOP; O2 = increased endoplasmic reticulum cisternae dilatations; O3 = increased caspases; O4 = increased apoptosis of tubular and interstitial cells.

Table 5. Angiotensin II pathway

<table>
<thead>
<tr>
<th>Reference</th>
<th>Animal strain (n)</th>
<th>Interventions</th>
<th>Control vehicle</th>
<th>Outcome</th>
<th>Study in vivo</th>
<th>Quality grade</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chung et al. [18]</td>
<td>SD (26)</td>
<td>15 mg/kg/day s.c.</td>
<td>vehicle</td>
<td>AngII, OPN</td>
<td>increased</td>
<td>yes</td>
</tr>
<tr>
<td>Thomas et al. [16]</td>
<td>SD (31)</td>
<td>CsA + losartan¹</td>
<td>yes</td>
<td>no</td>
<td>no</td>
<td>no</td>
</tr>
</tbody>
</table>

O1 = Increased proinflammatory cytokine (OPN, AngII); O2 = increased inflammatory cell infiltration; O3 = increased proapoptotic factors; O4 = increased or decreased apoptosis of tubular and interstitial cells.

¹CsA + losartan rats received daily CsA 15 mg/kg s.c. and losartan 10 mg/kg by gavage for 35 days.

Table 6. Hypertonicity pathway

<table>
<thead>
<tr>
<th>Reference</th>
<th>Animal strain (n)</th>
<th>Interventions</th>
<th>Control vehicle</th>
<th>Outcome</th>
<th>Study in vivo</th>
<th>Quality grade</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lim et al. [13]</td>
<td>SD (36)</td>
<td>15 mg/kg/day</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
</tr>
<tr>
<td>Sheikh-Hamad et al. [34]</td>
<td>SD (10)</td>
<td>25 mg/kg/day 4</td>
<td>yes</td>
<td>yes</td>
<td>no</td>
<td>no</td>
</tr>
</tbody>
</table>

O1 = Decreased urine-concentrating ability: increased urine volume and fractional excretion of sodium, urine osmolality and free-water reabsorption; O2 = decreased vital enzymes (Na-K-ATPase, aldose reductase, betaine/γ-amino-n-butyric acid transporter-1, myo-inositol transporter, HSP70); O3 = decreased urea transporter family (UT-A2, UT-A3, UT-B); O4 = increased aquaporins (AQP1, AQP3, AQP4); O5 = increased tubular damage, interstitial infiltrates and fibrosis; O6 = increased apoptosis of tubular and interstitial cells.
treated for 4 or 6 weeks with CsA (7.5–25 mg/kg/day). CsA treatment dramatically induced renal cells apoptosis and upregulated the expression of pro-apoptotic factors (Fas and Fas ligand) in SD rats. CsA downregulated Bcl-2 and Bcl-XL and resulted in the translocation of Bax to the mitochondria and perturbed the balance of Bcl-2 and Bax in renal tubular cells. The expression and activity of caspase-1 and caspase-3 were significantly increased in CsA-treated rats. In the murine study, ICR mice were treated with CsA (30 mg/kg/day). CsA also induced strong expression of Fas, Fas ligand, caspase-1 and caspase-3 in renal tubular cells.

**Mitochondrial Pathway**

Twelve studies were included; these were split equally between groups B and C (table 3). In seven studies, SD rats were treated for 2–4 weeks with CsA (15–25 mg/kg/day). CsA treatment dramatically increased proinflammatory cytokines such as lipopolysaccharide-induced CXC chemokine, monocyte chemoattractant protein 1, osteopontin (OPN), C-reactive protein and AngII in SD rats. In four studies, Wistar rats were treated for 2 or 4 weeks with CsA (15–25 mg/kg/day). CsA treatment significantly increased malondialdehyde in plasma and kidney tissue. It significantly induced oxidative stress, increased thiobarbituric acid-reacting substance and damaged the antioxidant defense system. This resulted in decreased levels of superoxide dismutase, catalase and glutathione peroxidase, and increased lipid peroxidation. Furthermore, the activities of tricarboxylic acid cycle and electron transport chain enzymes were decreased in Wistar rats. In one study, ICR mice were treated for 2 weeks with CsA (30 mg/kg/day). CsA treatment induced early features of CsA nephropathy with arteriolar hyalinosis and cortical and tubulointerstitial fibrosis in wild-type mice and fewer features of nephropathy in OPN−/− mice.

**Endoplasmic Reticulum Pathway**

Two studies were included, one of which scored B and the other C (table 4). In both studies, SD rats were treated for 4 weeks with CsA (15 mg/kg/day). CsA significantly induced the mRNA expression of 78 kDa glucose-regulated protein, protein disulfide isomerase, C/EBP homologous protein (CHOP) and homocysteine-induced ER protein in a dose- and time-dependent manner. Short-term treatment of CsA for 1 week activated both the ER stress response (induction of binding immunoglobulin protein mRNA and protein) and the apoptotic response (upregulation of both caspase-12 and CHOP mRNA and protein). However, long-term treatment with CsA for 4 weeks decreased binding immunoglobulin protein and further increased CHOP. The imbalance between the two responses coincided with the kinetics of apoptotic cell death and disruption of the ER structure.

**Angiotensin II Pathway**

Two studies were included, one of which scored B and the other C (table 5). In one study, SD rats were treated for 4 weeks with CsA (15 mg/kg/day) with or without losartan (to block AngII type 1 receptor). Tubular and interstitial apoptotic cells were decreased in the group treated with CsA and losartan, compared with the group treated with CsA alone. In another study, SD rats were treated for 4 weeks with CsA (15 mg/kg/day). CsA treatment induced renal cells apoptosis and increased AngII, OPN and transforming growth factor-β1 (TGF-β1). The number of macrophages and caspase-3 levels were also increased significantly in CsA-treated rat kidneys.

**Hypertonicity Pathway**

Two studies were included, one of which scored B and the other C (table 6). SD rats were treated for 4 weeks with CsA (15 mg/kg/day). CsA treatment decreased the expression of AR and Na-K-ATPase-(α)1 and increased the number of TUNEL-positive renal tubular cells in both the cortex and medulla. Long-term CsA treatment increased urine volume and fractional excretion of sodium and decreased urine osmolality and free-water reabsorption compared with VH-treated rats. These functional changes were accompanied by decreased expression of aquaporins (1–4) and urea transporters (UT) (UT-A2, UT-A3 and UT-B). However, there was no change in aquaporin-2 in the cortex and outer medulla and UT-A1 in the inner medulla. SD rats were treated daily for 4 weeks with CsA (25 mg/kg/day s.c.). CsA prevented the nuclear translocation of the transcription nuclear factor of activated T lymphocyte 5 and inhibited osmotic response element-mediated reporter gene expression. It also decreased mRNA expression of hypertonicity-induced genes (aldose reductase, betaine/gamma-amino-n-butyric acid transporter 1 and heat-shock protein 70) in the medulla. Histologic examination showed a significant increase in apoptosis in the renal medulla where hypertonicity normally prevails.

**Nitric Oxide-Related Pathway**

Only one study was included which scored C for data quality (table 7). SD rats were treated daily for 4 weeks...
with CsA with or without L-NAME (N\textsubscript{o}-nitro-L-arginine methyl ester), to block NO. Animals treated with CsA + L-NAME had a statistically significant increase in apoptosis of tubular and interstitial cells compared with the CsA-treated animals. This indicates that CsA-induced apoptosis is partially mediated by NO inhibition.

**Discussion**

The molecular mechanisms for CsA-induced chronic nephrotoxicity remain poorly understood. To further verify elucidate the in vivo mechanisms, we systematically analyzed all studies on this subject and report our results here. There is no generally accepted evaluation standard for in vivo research. We therefore defined a grading system based on the ‘Evidence Pyramid’ [9]. According to this standard, all studies used in this evaluation were of high quality. Our results indicated that this standard was more suited to the needs of our study, but further studies using this standard should clarify its efficacy.

CCN is an important cause of chronic renal dysfunction. In the clinic, many studies have confirmed that long-term use of CsA could induce CCN after renal transplantation [2–7]. In this systematic review, our results showed that CsA treatment caused CCN in renal tissue. CsA treatment (20 mg/kg/day) for approximately 3 weeks caused nonspecific tubular lesions and glomerular modifications in dogs [35]. The same dose over 30 days caused leukocyte infiltration, tubular atrophy, interstitial fibrosis and arteriopathy in rabbits [36, 37]. Other reports [38, 39] showed that this dose for 4 weeks caused renal functional and structural changes in rats similar to those reported in humans. These findings suggest that these animal models are appropriate tools for studying CCN in humans. CsA treatment (15–20 mg/kg/day) for 4 weeks induced nephrotoxicity in rats, dogs and rabbits. Cell apoptosis is the process of programmed cell death involved in both beneficial and harmful processes within the mammalian body. Our results showed that CsA caused renal cell apoptosis in vivo. In vitro results demonstrated that CsA (0.1–1.0 g/ml) induced apoptosis in human cells (HK-2s, HECs and human mesangial cells) after about 24 h [8]. Taken together, this evidence suggests that CsA-induced renal cell apoptosis is one of the primary causes in CCN and understanding the complexities of its mechanism is critical for preventing CCN.

In this systematic review, most animals (80%) were pretreated by feeding a full low-salt diet. Salt sensitivity (changes in blood pressure in response to alterations in salt intake) is a risk factor for hypertension [40]. A high-sodium diet has been shown to significantly upregulate mRNA expression of the angiotensin 1 receptor in the renal artery and aggravate nephropathy [41]. High sodium (8% salt diet) increased blood pressure, plasma renin concentration, urinary protein excretion and renal nitroxidative stress while decreasing renal blood flow and angiotensin 1–7 receptor (mas) protein expression in spontaneously hypertensive rats [42]. To exclude the effects on angiotensin receptor expression in kidney induced by a high-salt diet, most animals (80%) were pretreated by feeding full low-salt diet in all studies. However, other studies have demonstrated that sodium depletion could promote CsA-induced tubulointerstitial injury [43]. Therefore, the effect of a low-salt diet in a CCN animal model needs to be confirmed by further studies.

To reveal the apoptotic mechanisms of CCN in vivo, we systematically analyzed all relevant articles and collected 27 experiments on this subject. The in vivo mechanisms are more complex than those in vitro. There are at least five pathways which mediate renal cell apoptosis.
Renal Cell Apoptosis Induced by Cyclosporine A

Our results showed that CsA treatment dramatically induced renal cell apoptosis through Fas/Fas ligand and mitochondrial pathways in vivo. In our previous systematic review of in vitro studies, we showed that CsA could induce renal cell apoptosis through the Fas/Fas ligand and mitochondrial pathways [8]. We believe that there is now sufficient evidence that the Fas/Fas ligand and mitochondrial pathways are involved in CCN.

ER stress is an important mediator of kidney injury in diabetes mellitus, ischemic injury, neurodegenerative disorders, cancer, atherosclerosis and chemical toxicity [44–48]. Pallet et al. [49] found that CsA exposure was associated with the upregulation of the ER stress marker binding immunoglobulin protein in kidney transplant biopsies. In our results, CsA treatment significantly increased two ER stress responses, caused the imbalance between these responses and induced renal cell apoptosis. ER stress also mediated renal cells apoptosis in amyloid-β neurotoxicity and caspase-12 was an important factor in the ER-specific apoptosis pathway [50]. In vitro, evidence has shown that CsA might induce renal cell apoptosis through ER stress [8]. Therefore, we believe that CsA can induce nephrotoxicity through ER-specific apoptosis pathways and that ER stress is a new prognostic factor for CCN. This finding suggests that managing ER stress could provide a target for novel renoprotective drugs.

AngII plays an important role in renal injury through the type 1 (fig. 1) receptor and the type 2 (fig. 2) receptor, which invoke different molecular mechanisms [51–52]. AngII mediated renal cell apoptosis in spontaneously hypertensive rats [53] and unilateral ureteral obstruction in rats [54]. Our results showed that CsA increased AngII and caspase-3 in the rat kidney [18] and CsA-induced renal cell apoptosis was significantly decreased through losartan inhibition of the AT1 receptor [16]. In kidney transplantation patients, renin and AngII expression were significantly higher in kidneys from CCN patients compared with those from patients who suffered from chronic rejection [55]. When AngII was infused into SD rats on day 28, it induced podocyte apoptosis [56]. AngII stimulated intracellular formation of reactive oxygen species such as superoxide anion and hydrogen peroxide [57] and upregulated pro-apoptotic factors such as Fas, Fas ligand, Bax, Bak and Bcl-2 [58, 59]. In vitro, AngII induced renal cell apoptosis in a dose- and time-dependent manner. This occurred in mouse mesangial cells [60], rat renal proximal tubular cells [58] and rat glomerular visceral epithelial cells [61]. Together, this evidence...

Fig. 2. Renal cell apoptotic mechanisms of CsA-induced nephrotoxicity.
suggests that AngII can induce renal cell apoptosis in different diseases through activating the Fas/Fas ligand pathway and the mitochondrial pathway. Therefore, we believe that CsA may induce CCN through an AngII pathway. Direct evidence for this is currently insufficient and needs to be confirmed by further studies.

Renal medullary cells are normally exposed to high NaCl concentrations as part of the urinary concentrating mechanism, yet they survive and function [62]. Once the microenvironement is damaged, hypertonicity will induce renal cell apoptosis. Our results showed that CsA damaged the urinary concentrating mechanism and induced renal cell apoptosis. This reveals that renal medullary cell survival is dependent on protective genes and normal urinary concentration in the hypertonic environment. Horii et al. [63] reported that when MDCK were exposed to 700 mosm medium for 24 h, 40% of cells underwent apoptosis with increased activity of caspase-3, caspase-8, and caspase-9. Other studies [64, 65] showed that hypertonicity-induced renal medullary cell apoptosis was an important cause of radiocontrast-induced nephropathy. Extreme acute hypertonicity could induce renal medullary cell apoptosis via mitochondrial dysfunction [66] and oxidative stress [65]. Osmolytes are compounds affecting osmotic and play a role in maintaining cell volume and fluid balance. Zhang et al. [67] reported that organic osmolytes prevented hypertonicity-induced apoptosis by preventing dissipation of mitochondrial membrane potential through the stabilization of the permeability transition pore. These findings suggest that hypertonicity can induce renal cell apoptosis through mitochondrial pathways. In all studies, most animals (80%) were pretreated by feeding a full low-salt diet. We did not find evidence that a high-sodium diet has an impact on hypertonicity. Therefore, our data suggest that CsA induces nephrotoxicity through a hypertonicity pathway and provides a new mechanism for CCN.

NO is an endothelial-derived factor which is involved in both beneficial and harmful processes. Endogenous and exogenous NO contributed to renal cell apoptosis via caspase-8 [68]. Kipari et al. [69] reported that inducible NO synthase (iNOS) blockade reduced interstitial cell apoptosis. However, Martinez-Mier et al. [70] reported that NO demonstrated a protective effect in ischemic kidney injury and exerted an antiapoptotic action by down-regulating the expression of p53 [71]. Our results suggested that NO prevented renal cells from CsA-induced apoptosis in vivo. Other data suggested that docosahexaenoic acid had a protective effect on CsA nephrotoxicity through increasing total NO bioavailability in rat renal tissues [72]. NO could modulate vascular endothelial growth factor and its receptors. Vascular endothelial growth factor expression was increased by NO blockade and decreased by NO enhancement in CCN [73]. These data indicate that NO has a protective effect in CCN. CsA treatment has been shown to increase NO in HK-2 cells [8]. When cocultured with human mesangial cells, HK-2, HUVECs or ECs for 12–24 h, CsA significantly increased the protein expression of iNOS, p53 and the iNOS activity in all cell lines [8]. These data show that NO plays an important role in both anti- and proapoptotic mechanisms. Whether NO promotes CsA-induced renal cell apoptosis needs to be confirmed by further studies.

In all studies, CsA induced renal cell apoptosis through a number of different pathways. These provide potential points for intervention. In vitro, caspase activation is a common feature of all these pathways which ultimately leads to cellular apoptosis. Therefore, we think that the activated caspases might be the ultimate intersection of these pathways and the common intracellular pathway mediating apoptosis in vivo.

In summary, the evidence indicates that the in vivo mechanisms for CCN are more complex than those in vitro. There are at least five apoptotic pathways including Fas/FasL, mitochondrial, ER, AngII and hypertonicity pathways. These pathways synergistically mediate renal cell apoptosis and lead to CCN. Whether NO promotes CsA-induced renal cell apoptosis needs to be confirmed by further studies. Of note, CsA induced renal cell apoptosis through the AngII and hypertonicity pathways. Activated caspases might be the ultimate intersection of these pathways and the common intracellular pathway. These might provide new potential points for interventions.

Acknowledgment

This work was funded by grants from the National 973 Program of China, No. 2009CB522401.

Disclosure Statement

The authors have no conflicts of interest to declare.
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