New insights into the pathophysiology of cyclosporine nephrotoxicity: a role of aldosterone

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Bobadilla NA, Gamba G. New insights into the pathophysiology of cyclosporine nephrotoxicity: a role of aldosterone. Am J Physiol Renal Physiol 293: F2–F9, 2007. First published April 11, 2007; doi:10.1152/ajprenal.00072.2007.—Cyclosporine A (CsA), a calcineurin inhibitor, has improved allograft survival in solid organ transplantation and has been increasingly applied in the management of autoimmune diseases. While marked progress has been made in patient and allograft survival rates, clinical use of CsA is often limited by its nephrotoxic effect, which can be presented as two distinct and well-characterized forms: acute and chronic nephrotoxicity. The acute form is characterized by renal vasoconstriction, induced by an imbalance of vasoactive substances release, which leads to renal dysfunction. This form is reversible. The chronic toxicity, in contrast, is characterized by the vasoconstriction plus the development of structural damage that includes arteriolopathy and tubulointerstitial fibrosis that are often not reversible. The exact mechanisms of these deleterious effects are not fully understood, but major advances have occurred over the last few years. Here we review the current literature regarding the pathogenesis and strategies that have been used to ameliorate renal injury in chronic CsA nephrotoxicity. Recent observations suggest that aldosterone plays a central role in the pathogenesis of CsA nephrotoxicity and that spironolactone could be a useful agent to prevent it. These studies and the use of mineralocorticoid receptor blockade are discussed.

cyclosporine A

Two forms of CsA renal toxicity have been described: acute and chronic nephropathy. The acute form is characterized by renal vasoconstriction induced by an imbalance of vasoactive substance release, which in turn produces a fall in renal function. This form of toxicity is presented as a reversible acute renal failure when CsA administration is diminished or withdrawn. In contrast, the chronic form of nephrotoxicity is characterized not only by renal vasoconstriction but also by the development of structural damage, including arteriolopathy and tubulointerstitial fibrosis, which is irreversible and may lead to end-stage renal disease (3, 29, 39). This review is focused on studies using CsA, because most of the studies addressing the issue of nephrotoxicity by calcineurin inhibitors have been performed with this agent.

Mechanisms of CsA Nephrotoxicity

Experimental models of CsA nephrotoxicity in rats have been helpful in the study of pathophysiological mechanisms involved in the development and maintenance of renal toxicity. Acute and chronic forms of CsA nephrotoxicity can be reproduced in rats. The acute form is induced by administration of repeated doses of CsA by subcutaneous injection, from a range that varies from 15 to 50 mg·kg⁻¹·day⁻¹ for 7–28 days. The chronic model requires that, in addition to CsA administration at similar doses for at least 15 days, the animals should be fed a low-sodium diet (3, 19, 20, 27, 44, 55), suggesting that for some reason activation of the renin-angiotensin-aldosterone
system is required in rats to induce the CsA-related structural injury in the kidney.

Using the models described above, studies in which glomerular hemodynamics were analyzed by renal microcuvure techniques revealed that CsA administration is associated with afferent and efferent arteriolar vasoconstriction, with predominating pregglomerular vasoconstriction that results in a significant reduction of renal plasma flow ($Q_\Lambda$). A reduction of the ultrafiltration coefficient ($K_f$) has also been observed. The decrease in these two hemodynamic variables lead to a significant reduction of the single-nephron glomerular filtration rate and thus renal dysfunction (7, 10, 11). The precise mechanism by which CsA induced renal vasoconstriction has not been clearly established. Results from several studies indicate that vascular dysfunction induced by CsA results from an increase in vasoconstrictor factors that include endothelin (28), thromboxane (46), and angiotensin II (45, 65) as well as a reduction of vasodilator factors such as prostacyclin (45) and nitric oxide (NO) (10, 11, 17, 34, 69). Thus an imbalance in the release of vasoactive substances seems to be responsible for renal vasoconstriction.

Several factors have been implicated in the development of structural injury during chronic CsA nephrotoxicity. These are 1) activation of the renin-angiotensin-aldosterone system, in which angiotensin II, through the activation of $\text{AT}_1$ receptors, not only participates in renal vasoconstriction but also promotes fibrotic processes and the release of aldosterone (47, 58); and 2) renal hypoxia that results from renal vasoconstriction induced by CsA, leading to the formation of reactive oxygen species (11, 34) that cause cellular injury and promote cellular death by apoptosis (77, 78); and 3) upregulation of transforming growth factor-$\beta$ (TGF-$\beta$), which promotes renal fibrosis by increasing the production and decreasing the degradation of extracellular matrix proteins (20, 33, 58, 72).

**Strategies to Prevent or Reduce Chronic CsA Nephrotoxicity**

Because the use of CsA as an immunosuppressive agent to prevent allograft rejection has been limited by its toxic effects, several groups have studied potential strategies to prevent or reduce CsA nephrotoxicity. Acute toxicity is reversible, while chronic toxicity in which histological changes occur is nonreversible and increases the risk of end-stage renal disease. Thus most of the studies have been performed using the chronic model of toxicity, in which rats are fed a low-salt diet during the period of CsA administration. Results of these strategies on renal function and tubulointerstitial fibrosis are shown in Table 1.

The vasoconstrictor effect of CsA on renal vasculature has been shown to be involved in the pathogenesis of CsA toxicity. As we mentioned before, a group of strategies have been developed toward blocking the effect of vasoconstrictor factors such as losartan as an antagonist of angiotensin II receptor 1 (64), BQ123 for its properties as endothelin receptors blocker (26), and l-arginine as substrate for NO synthase, producing NO to counteract renal vasoconstriction (5, 57). Although losartan was able to reduce tubulointerstitial fibrosis by $\sim$50%, no improvement was observed in renal function. In fact, a further decrease in creatinine clearance was observed in rats treated with CsA and losartan, probably secondary to the losartan-induced fall in efferent resistance. Antagonism of both receptors, endothelin A and B, with BQ123 improved renal function by $\sim$30% but was unable to reduce renal structural injury. These observations agree with Kon et al. (28), who suggested that endothelin is at least partially responsible for afferent vasoconstriction. However, the slight increase in renal function was not associated with improvement of renal structural damage. In contrast, chronic l-arginine administration increased renal function by $\sim$70% and reduced tubulointerstitial injury by $\sim$50%. This protective effect was associated with a reduction of vascular endothelial growth factor (VEGF) and suggests that the reestablishment of renal blood flow due to increased availability of NO was associated with lesser structural injury.

A second group of studies included two different treatments with anti-inflammatory drugs such as pentosan polysulfate (55) and mycophenolate mofetil (61). Schwadler et al. (55) observed that pentosan polysulfate was able to reduce arteriolar lesions and tubulointerstitial fibrosis by $\sim$45%, but this treatment did not prevent the CsA-induced decrease in creatinine clearance. Shihab et al. (61) reported that mycophenolate mofetil is capable of improving neither renal function nor structural injury produced by CsA. Thus anti-inflammatory drugs are not able to improve renal function in CsA-treated rats and had limited benefit in preventing renal structural injury.

A third group of studies included drugs with antifibrotic properties such as the TGF-$\beta$ antibody, statins, pirfenidone, and hepatocyte growth factor. Because of the central role of TGF-$\beta$ in the development of renal injury induced by calcineurin inhibitors, the effect TGF-$\beta$-neutralizing monoclonal antibody administration was studied. As Table 1 shows, this

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Improvement of Renal Function</th>
<th>Reduction of Renal Fibrosis</th>
<th>Reference(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Losartan</td>
<td>No</td>
<td>50%</td>
<td>64</td>
</tr>
<tr>
<td>BQ123</td>
<td>30%</td>
<td>No</td>
<td>26</td>
</tr>
<tr>
<td>l-Arginine</td>
<td>70%</td>
<td>50%</td>
<td>5, 57</td>
</tr>
<tr>
<td>Pentosan polysulfate</td>
<td>No</td>
<td>45%</td>
<td>55</td>
</tr>
<tr>
<td>Mycophenolate mofetil</td>
<td>No</td>
<td>40%</td>
<td>33</td>
</tr>
<tr>
<td>Anti-TGF-$\beta$</td>
<td>ND</td>
<td>No</td>
<td>61</td>
</tr>
<tr>
<td>Pirfenidone</td>
<td>No</td>
<td>50%</td>
<td>59, 60</td>
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<tr>
<td>Pravastatin</td>
<td>75%</td>
<td>55%</td>
<td>32</td>
</tr>
<tr>
<td>Hepatocyte growth factor</td>
<td>No</td>
<td>25%</td>
<td>38</td>
</tr>
<tr>
<td>Magnesium supplementation</td>
<td>82% (No)</td>
<td>80% (ND)</td>
<td>4, 6, 12</td>
</tr>
<tr>
<td>Spironolactone</td>
<td>100%</td>
<td>50%</td>
<td>20, 44</td>
</tr>
</tbody>
</table>

CSA, cyclosporine A; TGF, transforming growth factor; ND, not determined.
strategy reduced renal structural injury by ~40%. Although renal function was not determined, serum creatinine levels were significantly lower in mice that received the TGF-β antibody than in untreated mice, suggesting that neutralization of TGF-β, in addition to expected reduction in renal fibrosis, also improved renal function by a mechanism that remains to be determined. Previous studies in cultured mesangial cells (23) and in rats with diabetic nephropathy (22) have shown that TGF-β upregulation induced glomerular injury through activation of calcineurin, which in turn induced greater NFAT translocation to the nucleus, resulting in mesangial proliferation and extracellular matrix (ECM) accumulation. In the diabetic rats, these effects were reduced by CsA administration in the glomerular but not in tubulointerstitial area (22), suggesting that calcineurin activation promotes ECM accumulation in the glomerulus. On the other hand, calcineurin inhibitors are associated with TGF-β overexpression and tubulointerstitial, but not glomerular, ECM accumulation (58, 62), suggesting that calcineurin activity results in different effects on ECM accumulation between the glomerulus and tubulointerstitium. Pravastatin, a drug with anti-inflammatory and antifibrotic properties, was evaluated in this sort of kidney injury. As shown in Table 1, pravastatin administration improved renal function by 70% and reduced tubulointerstitial fibrosis by ~55%. Mechanisms implicated were suppression of osteopontin, TGF-β, and intrarenal C-reactive protein expression together with higher eNOS expression (70). However, the beneficial effect of pravastatin in very high doses (20 mg/kg) was not observed in rats that received lower doses (5 mg/kg). Another antifibrotic agent that has been tested in chronic CsA nephrotoxicity is pirfenidone. The mechanism of action of this compound is unclear, but available data suggest that pirfenidone inhibits the actions of TGF-β. Shihab et al. (59, 60) showed that simultaneous administration of pirfenidone to CsA-treated and salt-depleted rats significantly reduced tubulointerstitial fibrosis by ~50%. This antifibrotic effect was accompanied by a reduction of TGF-β protein expression and matrix deposition, as well as by a reduction of cell death by apoptosis and expression of regulatory genes of apoptosis. Finally, hepatocyte growth factor has also been reported to protect against renal injury as an antifibrotic factor. Mizui et al. (38) showed a slight reduction of renal fibrosis by hepatocyte growth factor gene transfer in chronic CsA nephrotoxicity (~25%), an effect that was accompanied by a reduction of TGF-β mRNA levels and apoptosis. Thus antifibrotic approaches such as TGF-β antibody, pravastatin, pirfenidone, and hepatocyte growth factor can be of help in reducing renal fibrosis.

Supplementation of magnesium is another strategy that has been shown to be helpful in correcting hypomagnesemia in Sprague-Dawley rats with chronic CsA nephrotoxicity (37). Asai et al. (6) observed that magnesium supplementation prevented renal dysfunction induced by CsA. In this study, vehicle-treated rats had a creatinine clearance of 2.1 ± 0.04 ml/min. Renal function was decreased by CsA to 0.8 ± 0.1 ml/min, and this effect was partially reversed by magnesium supplementation to a value of 1.7 ± 0.1 ml/min. Simultaneously, renal structural damage was also prevented. Authors observed that rats treated with CsA in which magnesium was supplemented exhibited a reduction of osteopontin, monocyte chemoattractant protein-1, TGF-β, and endothelin mRNA levels, associated with a decrease in monocyte/macrophage influx into renal tissue. Because the beneficial effects of magnesium supplementation were not observed when the renin-angiotensin system was inhibited with the angiotensin-converting enzyme inhibitor benazepril, the authors concluded that the protective effect of magnesium supplementation was independent of angiotensin II. The mechanism by which magnesium supplementation was able to prevent CsA toxicity was not elucidated. Interestingly, however, the protective effect of magnesium supplementation was not observed in two other studies. Burdmann et al. (12) and Andoh et al. (4) also analyzed the effect of magnesium supplementation in Sprague-Dawley rats with chronic CsA nephrotoxicity. Although in both studies hypomagnesemia was successfully corrected by exposing rats to drinking water containing 2% MgCl₂, the reduction in glomerular filtration rate was not prevented by this maneuver. In addition, we have observed that hypomagnesemia does not occur in Wistar rats with acute or chronic CsA nephrotoxicity. In our experience, plasma magnesium levels in vehicle- and CsA-treated animals under a low-sodium diet for 21 days were 2.1 ± 0.1 and 2.6 ± 0.1 mg/dl, respectively. Thus in Wistar rats CsA nephrotoxicity is developed in the absence of hypomagnesemia, suggesting that, at least in this model, CsA toxicity is not related to magnesium deficiency.

Finally, in clinical practice, there are also several studies that have evaluated different strategies to reduce CsA nephrotoxicity, but only a few of them focused on chronic nephropathy. Use of the calcium channel blocker nifedipine is one strategy that has shown a beneficial effect on renal dysfunction and interstitial fibrosis in patients treated with CsA for 6 and 12 mo after transplantation (35), suggesting that renal vasodilatation induced by nifedipine protects the kidney from detrimental CsA actions. Another strategy reported is dietary supplementation with fish oil. van der Heide et al. (68) observed that recipients of a primary cadaveric kidney transplant who were treated with CsA and who ingested fish oil daily during the first postoperative year had higher glomerular filtration rate and renal plasma flow than those not ingesting fish oil. However, a more recent randomized, double-blind, placebo-controlled in transplant patients taking CsA showed no beneficial effect of fish oil on glomerular filtration rate and renal plasma flow, as well as on renal histopathology. In addition, two studies tested the effect of randomized pentoxifylline administration, a hemorheological agent, to cardiac transplant patients treated with CsA. Renal dysfunction induced by CsA was similar between the pentoxifylline and placebo group (21, 74). A similar effect has been observed in CsA-treated renal transplant recipients also receiving CGS 12970, a thromboxane synthase inhibitor (63). Thus, of all potential preventive treatments tested in humans to reduce CsA nephrotoxicity, the only one that showed beneficial effects was the use of renal vasodilators like calcium channel blockers.

Role of Aldosterone in CsA Nephrotoxicity

Table 1 shows that the blockade of aldosterone receptors with spironolactone has been shown to be a successful strategy to ameliorate CsA nephropathy. In recent years, there has been a growing interest in the role of aldosterone and mineralocorticoid receptors (MR) in the pathophysiology of cardiovascular and renal diseases. The role of aldosterone in promoting...
cardiovascular injury is underlined by the Randomized Aldosterone Evaluation Study (RALES) (49) and the Eplerenone Post-Acute Myocardial Infarction Heart Failure Efficacy and Survival Study (EPHESUS) trials (48). In these studies, it was observed that addition of MR blockers spironolactone or eplerenone to standard therapy resulted in reduced cardiac mortality that could not be explained solely by blood pressure reduction. In addition, recent animal and clinical studies support a beneficial effect of MR blockade on the progression of renal injury. In rats, MR antagonists markedly ameliorated glomerular and/or tubulointerstitial injury in several models of nephropathy, including spontaneously hypertensive stroke-prone rats (50, 51), angiotensin II and NO synthase inhibitor-treated rats (52), aldosterone-treated rats (25), and in a ureteral obstruction model (66). Moreover, Aldigier et al. (2) reported that MR blockade not only reduced the development of glomerulosclerosis but also induced regression of existing glomerulosclerosis in rats after 5/6 nephrectomy. In humans, pilot studies showed that the addition of spironolactone to angiotensin-converting enzyme inhibitors had no hemodynamic effects but markedly reduced proteinuria in patients with renal failure (8) and in patients with type 2 diabetes (53). The protective effect of spironolactone in patients with mild renal insufficiency has recently been corroborated by a large double-blind, placebo-controlled randomized trial (15).

Given the evidence that MR blockade protects the kidney in several models of renal disease, Feria et al. (20) analyzed the effect of spironolactone administration in rats with chronic CsA nephrotoxicity. Administration of CsA during 21 days induced renal structural damage characterized by arteriolopathy and tubulointerstitial fibrosis. This effect was associated with an upregulation of TGF-β, fibronectin, and collagen I and IV mRNA levels. In contrast, rats that received simultaneous administration of CsA and spironolactone presented a significant reduction of arteriolopathy and tubulointerstitial fibrosis. This renoprotective effect was related to the prevention of overexpression of TGF-β and extracellular matrix proteins. Interestingly, as shown in Fig. 1A, Feria et al. (20) also observed that spironolactone administration completely prevented the reduction of creatinine clearance, suggesting that aldosterone is an important mediator of both functional and structural injury in this model of nephropathy. We later observed that protection conferred by spironolactone was associated with prevention of the CsA-induced pro-renin mRNA upregulation and ETB receptor downregulation (44).

The fact that spironolactone not only reduced structural injury in chronic CsA nephrotoxicity but also completely prevented renal dysfunction suggested that aldosterone is implicated in regulating renal vascular tone in this model. Since acute CsA nephrotoxicity results mainly from renal vasoconstriction, Perez-Rojas et al. (44) studied the effect of spironolactone on renal blood flow and glomerular filtration rate in rats treated with CsA for 7 days, in the absence of a low-salt diet, to produce the reversible CsA-acute model. As shown in Fig. 1B and C, it was observed that spironolactone administration completely prevented the fall in glomerular filtration rate as measured by inulin clearance. This effect was accompanied by the reestablishment of renal blood flow. A similar improvement of renal blood flow and function by aldosterone blockade in acute CsA nephrotoxicity was recently reproduced by

![Fig. 1. Protective effect of spironolactone on cyclosporine (CsA) toxicity. A: creatine clearance (CCR) in chronic toxicity. B and C: glomerular filtration rate (GFR) and renal blood flow (RBF) in acute toxicity, respectively. D: creatinine clearance in already preexisting chronic CsA nephrotoxicity. V, vehicle-treated rats; Sp, spironolactone-treated rats; CsA, cyclosporine-treated rats; CsA+Sp, rats that received cyclosporine and spironolactone. Data are modified from Refs. 20, 43 and 44. *P < 0.05 vs. the same group at 0 days. **P < 0.05 vs. the same group at 18 days.]
Nielsen et al. (40). The mineralocorticoid receptor is a protein complex that includes a steroid-binding protein receptor and heat shock proteins (Hsps) of 56, 70, and 90 kDa. Interestingly, Tumlin et al. (67) reported that when steroid hormone bound to its receptor, the release of the Hsps complex is able to activate calcineurin phosphatase activity. Also, it has been demonstrated that calcineurin might regulate Na⁺/K⁺-ATPase activity through dephosphorylation of its catalytic subunit (30). These studies suggest that aldosterone could mediate actions through at least two different mechanisms: by the classic pathway at the transcriptional level and by releasing the Hsps complex that could change the activity of different proteins including the phosphatase calcineurin. These findings, together with our previous observations of chronic CsA toxicity, showed that mineralocorticoid receptor blockade prevented renal vasoconstriction, suggesting that aldosterone participates in CsA renal homodynamic effect that could be mediated by both mechanisms describe before. Thus our findings in acute and chronic models of CsA nephrotoxicity indicated that spironolactone is an effective prophylactic agent to prevent the development of CsA nephrotoxicity. Finally, Perez-Rojas et al. (43) analyzed whether spironolactone blockade can contribute to prevent the progression of existing tubulointerstitial injury and renal dysfunction in the model of chronic CsA nephropathy. As shown in Fig. 1D, once chronic CsA nephrotoxicity was established after 18 days of treatment and confirmed through renal function and structural analysis, one group of rats simultaneously received CsA and spironolactone during an additional 18 days and was compared with a group that received only CsA for a similar period of time. It was observed that MR blockade increased rat survival, and although renal dysfunction already established in CsA-treated rats was not reversed by spironolactone, this treatment was able to avoid further renal function deterioration. On a structural level, spironolactone produced significant renal protection associated with a reduction of arteriolar thickening, apoptosis, and the area affected by tubulointerstitial fibrosis. The renoprotection conferred by spironolactone in rats with preexisting chronic CsA nephrotoxicity was associated with a reduction of TGF-β and procaspase-3 mRNA levels.

The potential role of aldosterone as a promoter of vasoconstriction was originally suggested by the observations that primary aldosteronism, a disease due to oversecretion of aldosterone, is associated with impaired vascular reactivity (9, 18). In addition, it was reported by Gros et al. (24) that acute aldosterone exposure induced a dose-dependent vasoconstriction through myosin light chain phosphorylation in clonal adult human vascular smooth muscle cells. This effect was prevented by spironolactone, suggesting that aldosterone-mediating vasoconstriction may represent an important pathophysiological mechanism of vascular disease. The mechanisms by which aldosterone increases vascular resistance has not been elucidated, but some studies suggest possible mechanisms, such as an increased catecholamine vasoconstrictor effect (73) and upregulation of angiotensin II receptors (54, 75, 76). A recent report indicates that aldosterone induces a downregulation of endothelial glucose-6-phosphate dehydrogenase that results in impaired oxidative stress and decreased NO availability. Interestingly, these effects of aldosterone were completely prevented by spironolactone (31). In support to this, we have recently shown that spironolactone completely prevented renal acute injury induced by ischemia-reperfusion by a mechanism that involved preservation of renal plasma flow, reestablishment of urinary NO₂/NO₃ excretion that was accompanied by increased expression of eNOS and phosphorylation at its residue S1177, as well as by a reduction of lipoperoxidation and cell apoptotic death, indicating indeed that aldosterone also participates in hypoperfusion observed in this model (36).

A New Scheme of CsA-Induced Nephrotoxicity

As shown in Table 1, there is a clear dissociation between improvement of structural changes and normalization of renal function. Some strategies, like antifibrotic agents, were able to...
improve renal structural damage but had no beneficial effect on renal function. In contrast, strategies such as L-arginine (5, 57) and spironolactone (20, 43, 44), which improved renal function probably due to an effect on vascular resistances, had an additional beneficial effect on structural injury, suggesting that the increase in vascular resistances, with consequent renal ischemia, seems to be a major pathophysiological mechanism involved in the development of structural injury. In this regard, we propose that aldosterone plays a central role in the establishment of renal dysfunction and structural injury observed in chronic CsA nephrotoxicity (Fig. 2). The cascade of events seems to begin with the well-known effect of CsA, inducing an imbalance of vasoactive substances release, such as an increase in endothelin, thromboxane, and angiotensin II and a decrease in prostacyclin and NO, resulting in renal vasocstriction (for a review, see Refs. 13 and 42). Angiotensin II stimulates aldosterone secretion from adrenal glands, although it is also possible that local synthesis of aldosterone in the kidney might be also stimulated. Despite the increase in vasoconstrictor factors induced by CsA, we propose that aldosterone itself could be the major player in producing renal vasocstriction, since aldosterone receptor blockade completely prevented the fall in renal plasma flow and glomerular filtration rate induced by CsA (20, 44). The resulting renal vasocstriction is responsible for both renal dysfunction and hypoxia observed during CsA nephrotoxicity. It is also known that renal ischemia promotes greater generation of reactive oxygen species that contribute to structural damage by increasing apoptosis and cellular tubular injury. We suggest that aldosterone also participates in the development of structural injury through increased TGF-β expression and cellular death by apoptosis. However, in contrast to renal vasocstriction, interstitial inflammation and macrophage infiltration are not only caused by aldosterone. Instead, CsA and angiotensin II themselves may contribute to the development of tubulointerstitial fibrosis. As a consequence, although aldosterone receptor blockade with spironolactone is able to fully reverse the renal dysfunction induced by CsA, it does not completely prevent structural injury.

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