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Chronic Cyclosporine Nephrotoxicity: New Insights and Preventive Strategies

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Cyclosporine (CsA) has improved patient and graft survival rates following solid-organ transplantation and has been increasingly applied with significant clinical benefits in the management of autoimmune diseases. However, the clinical use of CsA is often limited by acute and chronic nephrotoxicity, which remains a major problem. Acute nephrotoxicity depends on the dosage of CsA and seems to be caused by a reduction in renal blood flow related to afferent arteriolar vasoconstriction. However, the mechanisms underlying chronic CsA nephrotoxicity are not fully understood. Activation of the intrarenal renin-angiotensin system, increased release of endothelin-1, dysregulation of nitric oxide (NO) and NO synthase, upregulation of transforming growth factor-beta1, inappropriate apoptosis, stimulation of inflammatory mediators, and enhanced immunogenecity have all been implicated in the pathogenesis of chronic CsA nephrotoxicity. Reducing the CsA dose or withdrawing it and using combined nephroprotective drugs (mycophenolate mofetil, losartan, and pravastatin) may ameliorate chronic CsA-induced renal injury. This review discusses new insights and preventive strategies for this clinical dilemma.

Key Words: Cyclosporine, nephrotoxicity, transforming growth factor-β, renin-angiotensin system, nitric oxide, osteopontin, C-reactive protein, apoptosis; NF-kB, AP-1, aquaporin, urea transporter, immunogenecity

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INTRODUCTION

Cyclosporine (CsA) was first approved by the US Food and Drug Administration in the early 1980s for use as prophylactic antirejection therapy in patients receiving allogeneic transplants (kidney, liver, and heart). It has improved the 1- year graft survival rate with conventional therapy (prednisone and azathioprine) in renal allografts from -50% to -85%.[1]

CsA is a highly insoluble cyclic polypeptide consisting of 11 amino acids. Although its precise mechanism of action is incompletely understood, the major pathway involves the intracellular interaction of CsA and calcineurin phosphatase, thus reducing the production of interleukin-2 (IL-2). CsA initially binds to a specific family of receptors known as cyclophillins.[2] This drug-receptor complex inhibits the activation of calcineurin phosphatase, a secondary messenger in the dephosphorylation and activation of the nuclear factor of activation of T cells (NF-AT). NF-AT is a regulatory protein that promotes transcription of IL-2. Inhibition of IL-2 production by CsA stops proliferation and activation of helper and cytotoxic T cells.[3]

CsA is metabolized in the liver by the cytochrome P450 (CYP) 3A4 enzyme system. Because many drugs are metabolized via this system, they may directly or indirectly influence the rate of metabolism of CsA. These potential drug-drug interactions may enhance the risks of under-immunosuppression (rejection), over-immunosuppression (infection), and toxicity.[4] The major route

of excretion of CsA metabolites is via the biliary system, but renal elimination plays a minor role.

For over two decades, CsA has improved allograft survival and quality of life for solid-organ transplant recipients. However, several adverse effects have been reported in both transplant and nontransplant settings (autoimmune disorders), including toxicities (nephrotoxicity, hepatotoxicity, and neurotoxicity), hypertension, dyslipidemia, gingival hyperplasia, hypertrichosis, malignancies, and increased risk of cardiovascular events.[5,6] The most clinically important are chronic CsA nephrotoxicity, which is one of the known nonimmunological factors causing 6% of chronic allograft nephropathy in the total renal transplant population.[7] Chronic CsA nephrotoxicity is characterized by progressive renal dysfunction, afferent arteriolopathy, inflammatory cell influx, striped tubulointerstitial fibrosis, and increased intrarenal immunogenecity.[8-10] The exact mechanism of this complication is not well understood, and multiple factors are implicated.

Using a well-established animal model, others and we have recently demonstrated that CsA-induced renal injury includes immunological and nonimmunological pathways, as outlined in Fig. 1. This review article updates the current understanding of the pathogenesis of chronic CsA nephrotoxicity, and discusses recent literature on the prevention and delay of this complication.

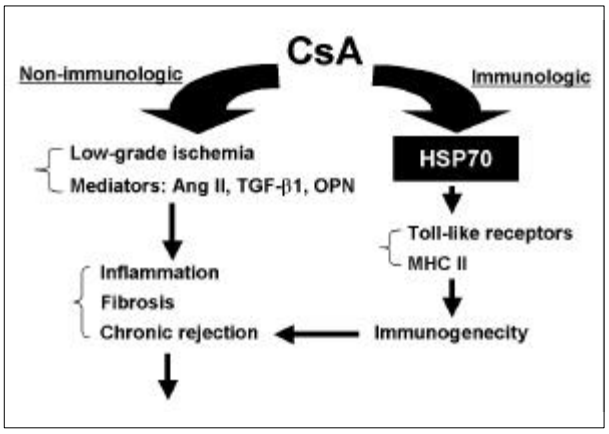

Fig. 1. Diagrammatic pathways of CsA-induced renal injury. Chronic treatment with CsA evokes renal injury by immunologic and non-immunologic pathways. Non-immunologic pathway includes hemodynamics (low-grade ischemia) and non-hemodynamics (mediators), and both ultimately results in tubulointerstitial inflammation, striped fibrosis, and increase in the rate of chronic rejection. Immunologic pathway may be triggered by the upregulated HSP-70 known as an activator of TLRs and the innate immune system, and that this correlates with enhanced intrarenal immunogenecity.

OLD AND NEW MECHANISMS

Low-grade ischemia

Long-term administration of CsA can rapidly decrease glomerular filtration rate (GFR) and renal blood flow induced by vasoconstriction or increased intrarenal vascular resistance, which ultimately results in low-grade ischemic injury.[5,6] Chronic ischemia caused by CsA is believed to be associated with reactive oxygen species (ROS) and lipid peroxidation.[11,12] This hypothesis is supported by the observation that antioxidant therapies significantly attenuate chronic CsA nephrotoxicity.[13,14]

The mechanism responsible for vasoconstriction is related in part to activation of the intrarenal renin-angiotensin system (RAS-see below) and imbalances of prostaglandins and thromboxane.[12,15,16] In addition, hypersecretion of endothelin-1 plays an important role in CsA-induced vasoconstriction.[17]

Endothelin-1 is a potent vasoconstrictor, widely released in the kidney and vascular beds, and act locally to increase vascular tone, regulate blood flow, GFR, and sodium reabsorption. Furthermore, endothelin-1 can also disrupt renal architecture because of its effect on extracellular matrix (ECM) accumulation and tubulointerstitial fibrosis. After long-term withdrawal of CsA, vasoconstriction as well as afferent arteriolopathy can be normalized. CsA may also increase systemic vascular resistance associated with activation of the sympathetic nervous system.[18]

Renin-angiotensin system

Activation of the RAS, especially the intrarenal RAS, plays an essential role in the pathogenesis of chronic CsA nephrotoxicity.[19,20] However, the mechanism of activation of RAS in this complication is still unknown. One accepted hypothesis is that CsA can increase renin release from

juxtaglomerular cells.

Rosen et al.[21] and Elzinga et al.[22] developed a reproducible animal model of chronic CsA nephrotoxicity. In this model, CsA administration to rats on a low-salt diet (LSD) induced a significant decrease in GFR and histological changes similar to those described in patients undergoing long-term CsA therapy. Salt depletion activates the RAS, which is implicated in the changes in renal hemodynamics and function that follow CsA administration. Using this model, others and we clearly demonstrated that CsA significantly increases renin and angiotensin II (Ang II) immunoreactivity in the kidney[20,23,24] as shown in Fig. 2. This supports a role for the intrarenal RAS in the pathogenesis of chronic CsA nephrotoxicity.

Activation of the intrarenal RAS induces renal injury by hemodynamic and nonhemodynamic pathways. The RAS is a potent vasoactive factor that mediates vasoconstriction hemodynamically and thus leads to low grade ischemia, as reviewed above. On the other hand, the RAS may also induce renal injury nonhemodynamically via stimulation of tubulointerstitial inflammation, TGF-β1, vascular endothelial growth factor, and increases in renal cell apoptosis.[25-28] Blocking this system with either angiotensin-converting enzyme (ACE) inhibitors or angiotensin II (Ang II) receptor type I antagonists mitigates all of the above parameters and confers a renoprotection during chronic CsA nephrotoxicity.[20,24,26-28]

Nitric oxide

In the kidney, nitric oxide (NO) is vasodilating factor that plays a key role in maintaining vascular tone. In addition, NO decreases glomerular thrombosis and ischemia, mesangial cell proliferation, ECM protein synthesis, and interstitial inflammatory cell infiltration.

NO is produced from *L*-arginine by the action of NO synthase (NOS) isoforms, of which at least three have been identified: neuronal NOS (nNOS), inducible NOS (iNOS), and endothelial NOS (eNOS). These three NOS isoforms are all found in the kidney. nNOS is specifically expressed in macula densa cells; iNOS has been observed in mesangial and proximal tubular cells; and eNOS is expressed mainly in endothelial cells of the afferent and efferent arterioles and glomerular capillaries.[29] Chronic CsA treatment has been shown to differentially influence NOS isoform production and interferes with the production of NO; however, the results obtained are conflicting.[30-33]

The role of NO in the pathogenesis of chronic CsA nephrotoxicity has been studied recently. We have demonstrated that exogenous supplementation with L-arginine effectively prevents CsA-induced renal dysfunction, arteriolopathy, and interstitial fibrosis in rats.[34] Shihab et al.[35,36] also reported that L-arginine treatment can attenuate transforming growth factor-beta1 (TGF-β1) overexpression and ECM deposition induced by CsA, and this benefit is reversed following N-nitro-L-arginine treatment, a potent competitive inhibitor of NO biosynthesis.

Osteopontin

The molecular mechanism underlying chronic CsA nephrotoxicity is multifactorial. Upregulation of chemoattractants and the resultant inflammatory cell infiltration have been proposed as important players, because interstitial inflammatory events precede ongoing fibrosis.[10]

Osteopontin (OPN) is a highly acidic phosphoprotein containing an arginine-glycine-aspartic acid (RGD) motif. It is involved in cell adhesion and migration,[37] and is expressed by several cell types in a constitutive or inducible fashion. These include osteoclasts, some epithelial cells, macrophages, T cells, smooth muscle cells, and tumor cells.[38-43] OPN acts as a chemotactic factor for macrophages and monocytes by binding to ligands including $\alpha v \beta 3$ integrin, CD44, collagen type I, and fibronectin.[44,45]

A functional role for OPN, with respect to its attracting macrophages, has been recently described *in vivo* and *in vitro*.[37,46] Moreover, the subcutaneous injection of OPN into rats produces massive macrophage accumulation, which is inhibited by the administration of an anti-OPN antibody.[47]

In the kidney, OPN is expressed constitutively in the renal medulla in the loop of Henle and the distal convoluted tubules, although it is absent from the normal renal cortex, with the exception

of the parietal epithelium of Bowman's capsule. Upregulation of OPN expression is strongly correlated with macrophage infiltration in several models of kidney diseases.[26,48-51] Young et al.[10] and Pichler et al.[26] reported that CsA treatment upregulates OPN gene expression, and that this correlates with interstitial macrophage infiltration and fibrosis.

Using immunohistochemistry, northern blotting, and in situ hybridization techniques, we found that OPN mRNA and protein were constitutively present in the tubular epithelium, collecting ducts, and uroepithelial lining cells in control rat kidneys, and most cortical structures were negative for OPN. By contrast, the levels of OPN mRNA and protein were dramatically increased in tubular epithelium and Bowman's capsule cells in CsA-treated rat kidneys. The most striking change was seen in the renal cortex, which normally expresses very little constitutive OPN. Of note, the sites of strong OPN expression were in areas of macrophage influx and severe tubulointerstitial fibrosis (Fig. 3).[24,52]

These findings imply that OPN may play a pathogenic role in CsA-induced renal injury, and this is supported by a study of OPN null mice displaying less chronic CsA nephrotoxicity.[53]

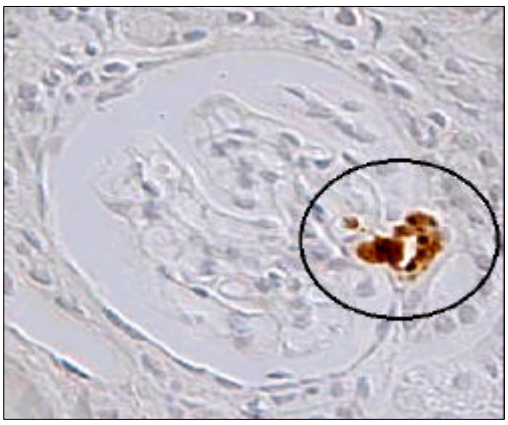

Fig. 2. Photomicrograph of immunohistochemistry for Ang II in CsA-treated rat kidney. CsA increases intrarenal Ang II immunoreactivity in the afferent arterioles of glomeruli.

Transforming growth factor beta-1

TGF-β1 is a key cytokine implicated in the pathogenesis of a wide range of kidney diseases characterized by glomerulosclerosis and tubulointerstitial fibrosis, including chronic CsA nephrotoxicity. Both *in vivo* and *in vitro* studies have proven that administration of CsA is associated with an increase in TGF-β1 expression in a dose-dependent manner.

Shihab et al. demonstrated that the upregulation of TGF-β1 by CsA causes tubulointerstitial fibrosis, probably via its actions on ECM synthesis and degradation, and plasminogen activator inhibitor-1 (PAI-1) plays a role in this process.[54,55] Administration of specific TGF-β neutralizing antibody ameliorated morphological alterations and preserved renal function in a mouse model of chronic CsA nephrotoxicity.[56]

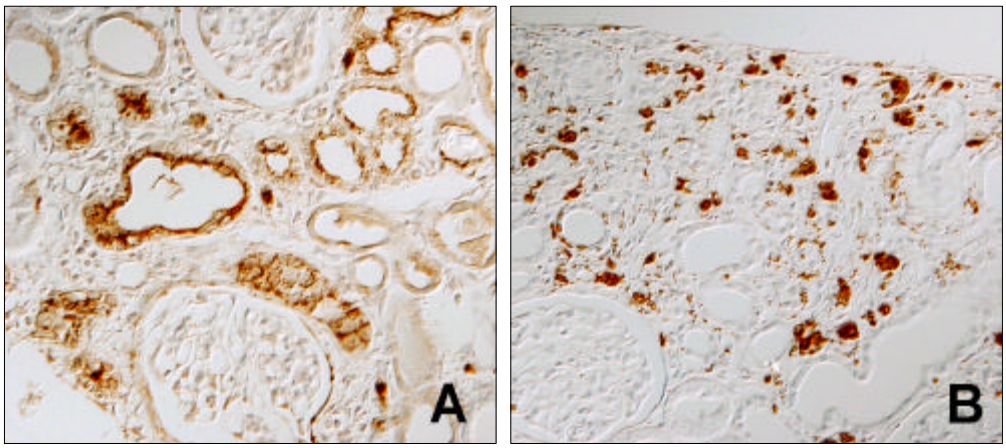

Fig. 3. Photomicrograph of immunohistochemistry for OPN and macrophages in CsA-treated rat kidney. The site of OPN overexpression and the resultant inflammatory cell infiltration (ED-1-positive cells) is localized to areas of injured tubules and fibrotic interstitium. A: OPN, 200 ×; B: ED-1, 100 ×.

TGF-β1 is secreted as a biologically inactive complex requiring *in vivo* activation. This latent TGF-β1 complex is activated by cleavage of its N-terminal latency-associated peptide to yield mature dimeric TGF-β1 through enzymatic and nonenzymatic mechanisms or by the presence of the proteoglycan decorin and the scavenging protein α_2-macroglobulin.[57-61] Therefore, increased amounts of TGF-β1 mRNA or protein may not actually represent parallel changes in its biologic activity.

Keratoepithelin (βig-h3) is a secreted matrix protein originally identified from a TGF-β1-stimulated human lung adenocarcinoma cell line (A549).[62] βig-h3 has been proposed as one of the ECM components[63]; although the precise physiologic function of βig-h3 is unclear, it may connect different matrix components and resident cells, thereby serving as a bifunctional linker protein.[64,65] Thus, βig-h3 expression has been used to assess the biological activity of TGF-β1.[66] Langham et al. reported that βig-h3 production was significantly increased in non-renal transplant recipients with chronic CsA nephropathy.[67] More recently, we found that βig-h3 mRNA and protein were normally expressed in the cortex and outer medulla, and localized in the terminal portion of afferent arterioles (vascular pole of glomerulus), S3 segment (parser recta) of the proximal tubules, and distal convoluted tubules. However, in the CsA-treated rat kidney, βig-h3 gene expression was significantly upregulated in the interstitium but not in afferent arterioles or tubules, where interstitial expansion and fibrosis developed.[68] Thus, βig-h3 may be a useful index of TGF-β1 bioactivity and may reflect the degree of tubulointerstitial injury in chronic CsA nephrotoxicity.

Apoptosis

Tubulointerstitial injury is the prominent feature of chronic CsA nephrotoxicity, and excessive loss of cellularity by apoptosis has been observed in the areas of fibrosis in renal biopsy specimens obtained from patients receiving long-term CsA therapy. Apoptosis is an active mechanism of cell clearance and plays a key role in the regulation of cell number during development, tissue homeostasis, and following insults. In the kidney, apoptosis may be beneficial,[69] but is deleterious if enough resident cells are lost.[70]

CsA has been shown to induce apoptotic cell death not only in T-lymphocytes (interfere with T cell function),[71] but also in some renal cells (deterioration of kidney structure).[28,72,73] Thomas et al.[28] were the first to characterize a close link between apoptosis and interstitial fibrosis in a rat model of chronic CsA nephrotoxicity. Others and we expanded the knowledge that CsA-induced renal cell apoptosis is associated with multigene families such as Bcl-2 proteins, Fas and Fas-ligand, p53, and caspases,[74,75] and that Ang II, NO, and intrarenal growth factors (TGF-β1) and epidermal growth factor (EGF) are also involved.[20,28,76,77]

Nuclear factor kappa B and activating protein-1

Transcription factors, such as nuclear factor kappa B (NF-κB) and activating protein-1 (AP-1), regulate the gene expression of several cytokines, chemotactic proteins, adhesion molecules, and matrix proteins involved in inflammation, immunologic responses, cell differentiation, and the control of growth.[78] Thus, there is a link between the activation of transcription factors and disease conditions.

Recent studies suggest that activation of NF-κB and AP-1 is involved in the transcription of monocyte chemoattractant protein-1 and TGF-β1 in the kidney, and that is regulated by Ang II or proteinuria.[78-81] Asai et al.[82] described that administration of CsA stimulates NF-κB and AP-1 DNA binding activity and this is blocked by the ACE inhibitor benazepril and by magnesium supplementation in rats.

Our preliminary data (unpublished data) support the involvement of activation of NF-κB and AP-1 in the pathogenesis of chronic CsA nephrotoxicity.

Intrarenal immunogenecity

CsA is a potent immunosuppressant, but may also trigger immunological injury in the kidney. Using a salt-depletion rat model, we updated the findings of immunogenecity on chronic CsA-induced renal injury.

In this model, using immunohistochemistry and

immunoblotting, we found that CsA enhanced heat shock protein (HSP)-70 production in both the cortex and the medulla compared with the vehicle-treated control rat kidney by (unpublished data). In addition, using reverse transcription polymerase chain reaction (RT-PCR) and in situ hybridization, we found that CsA increased the major histocompatibility complex class II (MHC II) immunoreactivity and upregulated both Toll-like receptor-2 (TLR-2) and TLR-4 mRNA expression in distal tubules and mononuclear cells within the interstitium (unpublished data).

HSP-70 is an activator of TLRs and the innate immune system, and TLRs are recognized as sensors for pathogen-associated molecular patterns crucial for the initiation of an innate immune response.[83,84] We assume that enhanced intrarenal immunogenecity may lead to the development of chronic allograft nephropathy. Further studies are required to clarify the relationship between TLRs and MHC II and HSP-70 in CsA-induced immunogenecity.

Tubular dysfunction

In addition to tubulointerstitial injury, CsA causes tubular dysfunction characterized by polyuria, calcium wasting, distal tubular acidosis, and hyperkalemia. Of these, impaired urine concentration is a predominant feature of chronic CsA nephrotoxicity,[10] but the molecular mechanisms are unknown.

In general, the urine-concentrating process is controlled by the aquaporin (AQP) system and the urea transporters (UTs), as previously reviewed.[85,86] AQPs are a family of membrane proteins that play an important role in the reabsorption of water in the kidney. To date, at least 11 deferent types of AQPs have been cloned.[86,87] AQP1 aids the rapid reabsorption of large quantities (70-80%) of filtered water, and is constitutively present on both apical and basolateral membranes of epithelial cells in the proximal tubule, the descending thin limbs of Henle's loop, and in endothelial cells of the descending vasa recta.[88] AQPs (2-4) are involved in the movement of water across the apical membrane in the collecting duct principal cells, and they are localized to the basolateral membrane of the collecting duct principal cells. Reabsorbed free water determines the osmolality of the final voided urine, and this AQP-related process is controlled by vasopressin.[86]

The UT family includes renal UT (UT-A) and the erythrocyte urea transporter (UT-B).[89] In the kidney, UT-A1 and UT-A3 are responsible for accumulation of urea in the inner medulla, and they are present in the inner medullary collecting duct. UT-A2 is present in the descending thin limbs of Henle's loop and UT-B in the descending vasa recta.[90-93]

We recently demonstrated that chronic CsA treatment decreases AQP (1-4) and UT (UT-A2, UT-A3, and UT-B) production in rat kidneys, and that this may account for the impairment of urinary concentrating ability (polyuria, increased fractional excretion of sodium, and decreased free-water reabsorption).[94]

Another manifestation of renal tubular dysfunction caused by CsA is calcium wasting associated with dysregulation of parathyroid hormone, which ultimately results in high bone turnover (osteopenia). Most of the filtered calcium (approximately 60%) is reabsorbed in the proximal tubule and the remainder is reabsorbed in the medullary thick ascending limb of Henle's loop, the distal convoluted tubule, and the connecting segment.[95] The calcium binding protein so-called calbindin plays a significant role in the process of calcium transport.[96] Two distinct subclasses of this protein with relative molecular masses of 9,000 and 28,000 have been recognized. Calbindin D_{9k} is present in high concentrations in the proximal small intestine and facilitates intestinal calcium absorption.[97] $Calbindin_{28k}$ is expressed in the distal nephron segments of the rat kidney and is assumed to take part in the process of calcium reabsorption.[98]

Recent experimental studies by our laboratory[99] and Steiner et al.[100] have demonstrated that chronic CsA treatment inhibits $calbindin_{28k}$ immunoreactivity in the distal nephron, and this is accompanied by a significant decrease in serum calcium concentration and an increase in urinary calcium excretion. This suggests that CsA-mediated suppression of $calbindin_{28k}$ production might be a critical factor in renal calcium wasting.

PREVENTION OF CHRONIC CSA-INDUCED NEPHROTOXICITY

Reduction or withdrawal of CsA

Is chronic CsA nephrotoxicity reversible? The answer is controversial. Traditionally, acute CsA nephrotoxicity has been regarded as reversible after CsA dose reduction or complete withdrawal of CsA, but chronic tubulointerstitial injury is considered as persistent and, in some cases, even progressive.

Weir et al.[101] investigated the long-term effect of reduction or withdrawal of calcineurin inhibitors in a cohort of renal transplant recipients (N=118). After reduction (CsA: n=67) or complete cessation of calcineurin inhibitors (CsA: n=18), there was a significant improvement in renal function. Although an improvement in renal function was not recorded in all patients, this maneuver was beneficial in most and at least reduced the rate of allograft loss in patients with nephrotoxicity.

In LSD-treated rats, Elzinga et al.[22] reported that there was progressive improvement in the GFR following 4 weeks of CsA discontinuation, but cortical fibrosis and tubular atrophy were unchanged. There is thus dissociation of GFR from tubulointerstitial fibrosis in this rat model of chronic CsA nephrotoxicity, and LSD may be an important factor.

Using the same model, Franceschini et al.[102] carried out a long-term study in which Sprague-Dawley rats were administered CsA for five weeks followed by discontinuation of CsA for two or eight weeks. They showed that the renal function and arteriolopathy returned to levels similar to placebo-treated controls after eight weeks of CsA withdrawal, and the tubulointerstitial fibrosis did not regress.

To clarify the molecular mechanism of irreversibility of chronic CsA nephrotoxicity, we carried out two prolonged studies using two dosages of CsA (7.5 mg/kg and 15 mg/kg) and multiple periods of drug washout.[103] Surprisingly, both renal function and morphological alterations (tubulointerstitial fibrosis and arteriolopathy) were reversed in rats with both dosages of CsA following five weeks of withdrawal, and a further alleviation in the tubulointerstitial fibrosis was observed when CsA was withdrawn for ten weeks. At a molecular basis, reversibility of chronic CsA nephrotoxicity is closely associated with downregulation of OPN and TGF-β1 gene expression, upregulation of EGF production, and a decrease in renal cell apoptosis.[76]

These conflicting results between our laboratory and others lead us to speculate that the effectiveness of CsA withdrawal on chronic CsA nephrotoxicity may depend on CsA dose used, or on the timing of drug elimination. Clinical trials are needed to clarify this issue.

Pharmacologic intervention

Information on chronic CsA nephrotoxicity in transplant recipients and patients with autoimmune disorders suggests that renal insufficiency is a major problem that may lead to end-stage renal disease requiring dialysis. Consequently, renal function should always be carefully monitored, even in patients whose renal function appears to be stable. In addition, early recognition and pharmacological intervention may be necessary to minimize the rate of allograft loss or chronic renal failure.

Overproduction of ROS and lipid peroxidation are pathogenic factors involved in chronic CsA-induced injury, and antioxidant agents may be therapeutic. Several lines of evidence have shown that vitamin E treatment preserves renal function and reduces free radicals, vasoconstrictive thromboxanes, and tubulointerstitial fibrosis, probably by suppressing the production of superoxide anion, H_2O_2, malonyldialdehyde, hemeoxygenase I, and some mediators (TGF-β1 and OPN).[13]

It is generally accepted that modulation of vasoactive factors (Ang II and NO) effectively prevents CsA-induced tubulointerstitial injury. Studies clearly demonstrated that concomitant administration of ACE inhibitors and Ang II type I receptors antagonists significantly abrogated arteriolopathy, interstitial fibrosis, and tubular atrophy independent of the hemodynamic effects. However, renal dysfunction did not improve. The reason for this discrepancy has been discussed above. The molecular mechanisms underlying renoprotection of these drugs in chronic CsA

nephrotoxicity may be related to its actions on inflammatory mediators, profibrotic cytokines and matrix proteins, and apoptotic cell death.[10,15,20,26-28] Similarly, exogenous supplementation of L-arginine confers a renoprotection.[34,36]

In addition to immunosuppression, mycophenolate mofetil (MMF) has anti-inflammatory and anti-fibrotic effects in subtotal nephrectomy, unilateral ureteral obstruction (UUO),[104] autoimmune glomerulonephritis,[105] and anti-glomerular basement membrane glomerulonephritis.[106] We have reported that the coadministration of MMF is negligibly effective in preventing the development of chronic CsA nephrotoxicity, but combining losartan or after CsA withdrawal affords superior protection compared with losartan monotherapy 24 and the effect of CsA withdrawal alone.[107]

Statins are competitive inhibitors of 3-hydroxy-3methylglutaryl-coenzyme A (HMG-CoA) reductase, the key enzyme which regulates the synthesis of cholesterol from mevalonic acid by suppressing the conversion of HMG-CoA.[108] Thus, statins has been widely used to govern hypercholesterolemia as well as cardiovascular disease. However, mevalonate is the precursor, not only of cholesterol, but also of many non-steroidal compounds; inhibition of HMG-CoA reductase by statins may thereby result in pleiotropic effects independent of their lipid lowering effects, such as anti-inflammation and anti-arteriosclerosis.[109,110] These benefits are mirrored in many renal disease models of ischemia-reperfusion injury,[111] subtotal renal ablation,[112] streptozotocin-induced diabetic nephropathy,[113] puromycin-induced nephrosis,[114] and UUO.[115] In a rat model of chronic CsA nephrotoxicity, we have shown that pravastatin treatment attenuates interstitial inflammation and fibrosis, and suppression of OPN, TGF-β1, and intrarenal C-reactive protein expression along with higher eNOS expression may be one of the mechanisms responsible for the renoprotective properties of pravastatin.[116]

We have recently demonstrated that preconditioning with recombinant human erythropoietin (rHuEPO), a hematopoietic growth factor, protects against subsequent ischemia-reperfusion injury in rat kidney.[117] More recently, a preliminary study (unpublished data) expands the role of rHuEPO as a useful agent to treat chronic CsA nephrotoxicity. This preventive effect of rHuEPO on chronic CsA-induced renal injury is associated with its anti-apoptotic and anti-inflammatory properties.

Future approaches to the avoidance of chronic CsA-induced nephrotoxicity

Because the pharmacokinetic pattern of CsA's action is closely associated with CsA-induced toxicity as well as CsA exposure, control of blood CsA concentration is important. In general, CsA peaks 2 hr after oral administration, and reaches a trough at 12 hr. Therefore, controlling CsA release is one possible approach to avoid CsA-induced toxicity. Recently, we reported that varying the size of polymer particles loaded with CsA can control CsA release *in vitro*.[118] By this maneuver, CsA delivery using polymer particles may produce safe and more stable graft function and optimal immunosuppression than conventional CsA formulations. An *in vivo* study is in progress in our laboratory.

CsA suppresses immune reaction by inhibiting calcineurin activity after forming complex with cyclophilins. Cyclophilin A is the most abundantly and ubiquitously expressed cyclophilin, and has been recognized for the protective role, especially as an antioxidant.[119] We have demonstrated that transgenic mice overexpressing cyclophilin A are resistant to CsA-induced nephrotoxicity via peptidyl-prolyl cis-trans isomerase activity.[120] Thus, induction of cyclophilin A production is a promising approach to prevent CsA-induced nephrotoxicity.

CONCLUSION

Current immunosuppressive drug regimens depend on CsA, even though chronic nephrotoxicity is a major limiting factor. This has resulted in an increasing cause of late allograft loss or chronic renal failure in both transplant and nontransplant patients. Fully understanding the molecular mechanisms of chronic CsA-induced renal injury is essential for nephrologists, as pharmacological intervention may delay the progres-

sion of chronic CsA nephrotoxicity.

With the introduction of newer immunosuppressive agents, strategies for calcineurin inhibitor minimization, avoidance, and withdrawal have been emerging in the literature. Reducing the dose of CsA or using protocols without CsA may ultimately minimize the incidence of nephrotoxicity and improve allograft and patient survival. A cohort of clinical trials is warranted to investigate the appropriate dose of CsA, or even the elimination of CsA with the addition of nonnephrotoxic immunosuppressants such as MMF and sirolimus, which may achieve optimal immunosuppression while avoiding the risk of acute rejection.

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