Cyclosporine Nephrotoxicity

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After more than 20 years of cyclosporine use its nephrotoxicity remains a significant clinical problem. Cyclosporine-induced renal injury has been described in solid organs recipients and in patients treated for autoimmune diseases. It is manifested in 2 distinct and well characterized forms, acute nephrotoxicity and chronic nephrotoxicity. This communication reviews the current literature analyzing the available data about the pathogenesis and mechanisms of acute and chronic cyclosporine-induced nephrotoxicity. A working hypothesis for the possible mechanisms of chronic cyclosporine nephrotoxicity will be provided.
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CYCLOSPORINE A (CSA), the first calcineurin inhibitor available for clinical use, was launched in the 1980s and radically changed the field of organ transplantation. The incidence of acute rejection in solid organ transplantation decreased significantly, making this event an unusual cause of graft failure nowadays. Early (1-2 y) graft and patient survival have increased to unparalleled levels. CSA also has proven to be effective in bone marrow transplant immunosuppression and in the treatment of autoimmune diseases.[1] The later development of self-emulsifying formulations of CSA has improved bioavailability and decreased inter- and intrapatient variability of the drug.

CSA inhibits interleukin-2 gene transcription and the transition of T lymphocytes from the G0 to G1 phase of the cell cycle. It binds to a cytoplasmic immunophilin called cyclophilin. The complex CSA-cyclophilin decreases calcium signaling and blocks calcineurin, a calcium-dependent enzyme responsible for the nuclear translocation and dephosphorylation of the cytosolic activating nuclear factor of T lymphocytes. The cytosolic activating nuclear factor of T lymphocytes regulates the transcription of genes responsible for several cytokines, including interleukin 2.[1]

Ironically, although extensively used in kidney transplantation, CSA causes important renal adverse effects: acute and chronic renal dysfunction, hemolytic-uremic syndrome, hypertension, electrolyte disturbances (hyperkalemia, hypomagnesemia, and hypocalcemia), tubular acidosis and defects in urinary concentrating ability. Indeed, nephrotoxicity is the most significant adverse effect caused by CSA. When calcineurin is inhibited, many genes besides interleukin 2 have their transcription impaired, including other interleukins, interleukin 2 receptor, nitric oxide synthase, transforming growth factor β (TGF-β), endothelin, collagen I and IV, and bcl-2, which seems to be implicated in cellular protection against apoptosis.[2] It is conceivable that calcineurin inhibition blocks the immune cell-mediated response against the transplanted tissue and at the same time triggers a sequence of undesirable events that will eventually lead to renal toxicity.[2] The recent development of selective calcineurin inhibitors that disrupt particular gene transcription without affecting other genes may clarify this issue.[3]

It is essential to be aware that CSA-induced nephrotoxicity is manifested in 2 particular and very distinct forms of renal injury. CSA acute nephrotoxicity is a hemodynamically mediated event, characterized by the absence of permanent structural injury and by the normalization of renal function when CSA is decreased or discontinued. Conversely, chronic CSA nephrotoxicity is an insidious lesion, characterized by an irreversible and progressive renal interstitial fibrosis, which may evolve to chronic or even end-stage renal disease.

This article discusses the available information on the pathogenesis of CSA-induced renal injury and describes the most relevant mechanisms of CSA acute and chronic nephrotoxicity.

ACUTE NEPHROTOXICITY

Acute CSA nephrotoxicity is a functional and reversible abnormality related to a renal imbalance of vasoconstrictor and vasodilators mediators. The main feature of this form of nephrotoxicity is an intense intrarenal vasoconstriction reflected by in-

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creased renal vascular resistance and reciprocal renal blood flow (RBF) decrease, followed by variable degrees of glomerular filtration rate (GFR) impairment. This vasoconstriction occurs preferentially in the afferent arterioles but also in adjacent small arteries, including the glomerular tuff.[4,5]

The experimental model for acute CSA nephrotoxicity is consistent and has been shown after different doses and routes of CSA administration in animals. Similarly, acute impairment of renal hemodynamics and function has been observed after CSA administration to patients and healthy human volunteers. Experimental or clinical histology changes are minimal and nonspecific or absent, even when renal dysfunction is striking.

There is a long list of possible mediators of acute CSA nephrotoxicity (Table 1). Attempts at individual manipulation of those mediators generally resulted only in partial improvement of renal function indicating that the pathogenesis of CSA-induced vasoconstriction is complex and multifactorial.

Table 1. Possible Mediators of Acute CSA Nephrotoxicity

Angiotensin II
Endothelin
Nitric oxide
Prostaglandins
Leukotrienes
Sympathetic system
Free radicals
Adenosine
Vasopressin
Platelet activation factor
Atrial natriuretic factor
Kallikrein-kinin system
Cholesterol
Hypomagnesemia
Extracellular volume depletion
Cremophor
Direct contraction—mesangial and smooth vascular cells
Direct tubular epithelial cell toxicity

Vascular/Hemodynamic Mechanisms of Injury

Renin-Angiotensin-Aldosterone System

The interaction of cyclosporine and the plasma and tissue renin-angiotensin-aldosterone systems (RAS) has been studied extensively with excellent reviews available. Sodium depletion, a condition that stimulates renin release, enhances acute CSA nephrotoxicity. There are abundant in vivo and in vitro experimental data indicating systemic and tissue activation of the RAS by CSA (Table 2). Increased levels of pro-renin and total renin and juxtaglomerular apparatus (JGA) hyperplasia have been found in CSA-treated heart and liver transplant recipients. Clinical evidence of renal tissue RAS activation by CSA has been offered by Gardiner et al,[6] who showed that conversion from CSA to azathioprine decreased the number of renin-containing cells in renal allograft biopsy samples.

Table 2. Experimental Evidence of RAS Activation by CSA

In vivo
Increased plasma renin activity
Juxtaglomerular hypertrophy and hyperplasia
Increased renal renin content
Increased renin-staining cells in juxtaglomerular apparatus
Increased renin-containing cells in the afferent arterioles
Increased number of renal angiotensin II AT_1 receptors.
In vitro
Up-regulation of angiotensin II receptors in cultured human smooth muscle cells
Renin release in renal cortical slices of rats
Renin release and synthesis in cultured juxtaglomerular cells

Although CSA clearly affects the RAS, its blockage in experimental models of acute CSA nephrotoxicity produced conflicting results. Attenuation of the decrease in GFR and RBF caused by CSA[7] or lack of improvement in renal function and/or hemodynamics when angiotensin-converting enzyme inhibitor was given concomitantly with CSA both were shown.[8] Attempts of prevention or attenuation of CSA-induced renal functional changes by pharmacologic blockade of RAS in humans mostly have been disappointing. One study found RBF and renal vascular resistance but not GFR improvements when angiotensin II receptor antagonists were administered to patients under CSA therapy.[9]

Endothelin

Evidence linking endothelin (ET) to acute CSA nephrotoxicity first emerged when it was shown that CSA stimulated ET release from cultured renal epithelial cells.[10] Numerous animal studies con-

firmed and extended these observations by showing that anti-endothelin antibodies partially prevented CSA-induced renal vasoconstriction and GFR decrease.[11] Subsequently, increased levels of ET have been shown after CSA administration to solid organ transplant recipients.[12]

Experimental use of ET_A or ET_A/ET_B receptor blockers has yielded conflicting results. Fogo et al[13] found that an ET_A antagonist attenuated a CSA-induced decrease in GFR and RBF only when it was infused in the renal artery before CSA administration, but not when it was systemically infused or followed CSA infusion. Davis et al[14] were unable to prevent CSA-induced renal vasoconstriction in rats using a selective ET_A antagonist or a combination of an ET_A and ET_B receptor antagonists.

Endothelial Injury and Nitric-Oxide System

CSA was found to have a direct cytotoxic effect on cultured endothelial cells and to inhibit human umbilical endothelial cell proliferation.[15] CSA increases the plasma level of many markers of endothelial damage such as Von Willebrand factor, P-selectin, and thrombin-antithrombin complexes in renal transplant patients.[16] Similarly, the release of Von Willebrand factor, endothelin tissue factor pathway inhibitor, and thrombomodulin was enhanced by CSA in the supernatant of endothelial cells in culture.[17]

The pattern of hemodynamic changes caused by CSA is consistent with disturbances in the L-arginine–nitric oxide (NO) pathway. Indeed, numerous investigators have shown that CSA impairs endothelium-dependent vasodilatation mediated by NO in human vessels and in a range of in vitro, ex vivo, and in vivo studies evaluating the aorta, arteries, and afferent and efferent arterioles of rats.[18,19] On the other hand, studies assessing CSA influence on tissue, plasma, and urinary NO levels and on tissue expression of NO synthase isoforms have provided contradictory results. Unaltered, increased, or decreased urinary NO metabolites were found after CSA administration to rats. Studies performed in healthy volunteers showed that CSA increases NO synthase activity whereas in renal transplant recipients CSA impaired basal and stimulated NO production. Clinical trials assessing the effects of L-arginine supplementation in CSA-treated renal or heart transplant patients generally were negative, without improvement in renal function and/or hemodynamics,[20] with the exception of Andres et al[21] who found increases in RPF, GFR, and natriuresis after administration of L-arginine to stable renal transplant recipients.

Eicosanoids

Eicosanoids (ie, arachidonic acid metabolites) have an important role in the local control of RBF, mainly in the setting of systemic or intrarenal hemodynamic disorders. The cyclooxygenase pathway produces the vasodilators prostaglandins PGI_2 or prostacyclin (that undergoes spontaneous hydrolysis to 6-keto-$PGF_{1\alpha}$) and PGE_2 and the vasoconstrictor thromboxane A_2 (TXA_2), whereas the lipoxygenase pathway produces the vasoconstrictor leukotrienes. CSA-induced imbalance in the vasodilator/vasoconstrictor ratio of eicosanoids favors vasoconstriction and probably is linked to the renal functional changes observed in acute CSA nephrotoxicity.[1] CSA administration to rodents consistently resulted in increased urinary excretion of TXA_2 metabolites, reflecting enhancement of renal and systemic thromboxane production.[22] Clinical studies also showed CSA-related increases in urinary TXA_2 metabolites in transplant recipients.[16] The enhancement of thromboxane production by CSA has been related to activated infiltrating platelets and macrophage in renal tissue, increased renal lipid peroxidation and reactive oxygen species production, endothelial injury, and systemic platelet activation.[23] Recently it has been shown in rats that CSA suppressed renal cyclooxygenase-2 expression whereas cyclooxygenase-1 expression did not change. This event may be connected to the CSA-induced decrease in renal excretion of vasodilatory eicosanoids.[24]

Disappointingly, the clinical use of selective thromboxane synthase inhibitors did not prevent CSA acute nephrotoxicity despite decreasing urinary and systemic levels of thromboxane metabolites.[25] In the same way, the use of fish oil in CSA-treated patients resulted in contradictory results, with some investigators finding improvement in renal function and a decrease in urinary TXB_2, and others finding no effect at all.

A role for the 5-lipoxygenase pathway in CSA acute nephrotoxicity is suggested by the demonstration that CSA increased urinary excretion of leukotriene metabolites in a rat model of renal transplantation. In this study the use of a leukotri-

ene receptor antagonist totally prevented the GFR impairment caused by CSA.[26]

Although manipulation of vasodilative prostaglandins by prostacyclin analogues or PGE precursors resulted in protection against the functional changes caused by CSA in laboratory animals, data derived from clinical studies or human tissue is conflicting. Although some benefit was shown in some studies, other studies did not show beneficial effects after the administration of PGE_2 or prostacyclin analogues to patients receiving CSA.[27]

Sympathetic Nervous System

CSA-stimulated activation of the sympathetic system has been linked to hypertension and to systemic and renal hemodynamic abnormalities caused by this drug. Some of the mechanisms implicated in adrenergic stimulation by CSA in rats include augmented norepinephrine release from terminal nerves, blockade of neuronal calcineurin, activation of excitatory neural reflexes in the subdiaphragmatic area, and elevation of plasma and platelet catecholamines.[28] Zhang et al[29] showed that knockout mice lacking synapsin (synaptic vesicle proteins that regulated neurotransmitter release at synapses) were protected against efferent sympathetic nerve activation and blood pressure increase after CSA administration.

Adrenergic pharmacologic blockade, renal denervation, chemical sympathectomy, administration of glycine (an inhibitory neurotransmitter), and catecholamine stores depletion by reserpine prevented or significantly reduced CSA-induced hemodynamics changes and hypertension in experimental studies.[30] However, other investigators did not find protection against experimental CSA-induced renal functional impairment when renal denervation was performed in CSA-treated rats.[31] Furthermore, clinical studies did not find an association between increased sympathetic activity and the renal and systemic hemodynamic changes found in patients treated with CSA.[32]

Free Radicals

There is an increasing body of data suggesting the participation of free radicals in CSA acute nephrotoxicity. Experimental in vivo and in vitro studies have found that cyclosporine-induced functional derangements were accompanied by oxidant stress.[30,33-35] Results regarding CSA effect on glutathione renal content showed decreased glutathione levels or increased tissue concentrations of oxidized and reduced glutathione.[34] If we consider the pivotal role of glutathione in cellular protection from free radical damage these results can be reconciled. It is possible that the increases in oxidized and reduced glutathione were caused by accelerated glutathione peroxidase activity and the adjustment of glutathione pathway to counterbalance excessive free radical production and that reduced glutathione levels indicated an exhaustion of the system. This excessive free radical production has been attributed to renal underperfusion and hypoxia-reoxygenation injury or to direct effects of CSA on their production. How CSA provokes oxidant stress and the interaction of these events with the cell defense systems described in other articles in this issue is a fruitful area of research.

The use of antioxidants or free radical scavengers such as lazaroid, vitamin E, melatonin, trimetazidine, carvedilol and N-acetylcysteine, xanthine oxidase inhibition by allopurinol, and viral delivery of superoxide dismutase genes consistently resulted in renal function improvement in experimental models of acute CSA nephrotoxicity.[30,34-37] The results of the few clinical studies that addressed the role of free radicals in acute CSA nephrotoxicity, however, are negative.[38]

Other Mediators

Other mediators also have been related to CSA-induced functional nephrotoxicity. Adenosine, vasopressin, platelet activating factor, atrial natriuretic factor degrading enzyme, hypomagnesemia, and the renal kallikrein system may yet prove to be important in mediating acute cyclosporine nephrotoxicity.

CSA also augments vasoconstrictor responses in animal and human mesangial cells, smooth vascular cells, and resistance vessels with obvious consequence for renal function and hemodynamics. These CSA effects are associated with augmented intracellular influx of calcium, which also impair relaxation responses to endothelium-derived relaxing factor (EDRF).[19]

The vehicle used for CSA solubilization may take part in acute nephrotoxicity caused by the intravenous formulation of the drug. Cyclosporine is a very lipophilic and hydrophobic compound, making it necessary to use lipids to obtain stable preparations. The commercial intravenous formulation of CSA uses Cremophor-EL (Novartis,

USA), a polyethylene castor oil, as a vehicle, which possess important hemodynamics effects. This intravenous formulation has been incriminated in episodes of acute renal failure in transplant recipients and patients with autoimmune diseases and has caused abrupt GFR decrease after a single dose in healthy volunteers. Substitution of Cremophor by a soybean lipid used for parenteral nutrition (Intralipid, Fresenius Kabi, Brazil) reduced the effect of CSA on GFR and maintained its immunosuppressive activity. In addition, these favorable effects were related to increased CSA clearance and lower trough levels but a similar tissue amount of CSA, all of which probably reduced exposure of the drug to the vascular compartment.[39]

Tubular Mechanisms of Injury

The seminal study by English et al[4] clearly showed that necrosis of the proximal tubule was not a feature of acute CSA nephrotoxicity. On the other hand, experimental and clinical evidence points to a more subtle CSA-induced tubular cell injury. Increased urinary excretion of proximal tubular-derived enzymes, increased fractional excretion of magnesium in the presence of hypomagnesaemia, impaired urinary concentrating ability, and hyperkalemia consequent to impaired tubular excretion of potassium all have been documented. Experiments using cultured renal tubular cell lines showed a dose-dependent CSA direct toxicity manifested in many different ways. This toxicity has been related to lipid peroxidation, increased p53b protein expression, intracellular influx of calcium, and reduced NO production.[40-42] Other indirect evidence of CSA-induced tubular cell damage is the induction of heat shock proteins in renal tubular cells after CSA administration to rats.[43] Using freshly isolated rat proximal tubules, da Costa et al[44] showed that only very high concentrations of CSA caused direct tubular injury, which was prevented by low calcium or high magnesium concentrations in the medium. These studies suggest subtle but important effects of cyclosporine that may be relevant to the survival of cells, especially after long-term exposure.

CHRONIC NEPHROTOXICITY

Chronic CSA-induced nephropathy is characterized by irreversible tubulointerstitial fibrosis in a striped pattern beginning in the medulla and progressing to the medullary rays of the cortex, generally accompanied by some degree of renal dysfunction.[45] Unfortunately, many investigators have used the term *chronic CSA nephrotoxicity* inadequately to describe the renal functional changes of CSA administration in humans and animals without histologic evaluation. The term must be reserved for the description of CSA-induced structural damage, namely irreversible interstitial fibrosis. This injury has been associated with degenerative hyaline changes in the afferent arteriole walls, consisting of endothelial swelling, nodular hyaline protein deposition, and areas of smooth muscle cell lesion and necrosis.[46] In kidney transplant recipients, this arteriolar lesion is considered the key for the discrimination between CSA chronic nephropathy and rejection.[45,46] Recently, clinical and experimental studies have shown that this arteriopathy, which was considered irreversible, can remit after CSA discontinuation, whereas the tubulointerstitial changes did not regress.[46-48] CSA-induced chronic nephrotoxicity was described in renal and nonrenal transplant recipients and in patients with autoimmune diseases receiving the drug for periods of 6 months or more.[45,46]

The lack of a suitable animal model has hampered the study of the mechanisms of chronic CSA nephrotoxicity for a long time. Using the observation that sodium depletion exacerbates CSA nephrotoxicity, Elzinga et al[49] and Rosen et al[50] developed a reproducible animal model of chronic CSA nephrotoxicity. In this model, CSA treatment in rats on a low-salt diet produced histologic changes similar to those described in patients on long-term CSA therapy accompanied by a profound decrease in GFR.[49-51] When the drug was discontinued the GFR improved, returning to baseline values, but the tubulointerstitial injury was progressive, even in the absence of CSA.[49]

Mechanisms of Injury

The well-documented effects of CSA on afferent arterioles led to the logical hypothesis that chronic afferent arteriolopathy ultimately would result in vascular occlusion causing downstream renal tissue ischemia with consequent fibrosis, nephron dropout, and tubular atrophy in the affected areas. However, recent experimental studies provided evidence that the mechanisms promoting the interstitial scarring and the hemodynamic changes in CSA nephropathy are distinct. In fact, Hunley et al[52]

showed that endothelin receptor blockers normalized renal hemodynamics but had no effect on structural lesions produced in the low-salt chronic CSA model. Conversely, blockade of the RAS by enalapril and losartan strikingly reduce the progression of CSA-induced tubulointerstitial fibrosis despite failure to normalize GFR.[53] More recently, Vieira et al[54] found that salt-depleted rats receiving low and clinically relevant dosages of CSA (5 mg/kg) for 8 weeks developed significant interstitial fibrosis without any decrease in RBF or structural afferent arteriole injury, clearly showing that the interstitial injury can occur in a totally independent way of the preglomerular vasoconstriction.

Angiotensin II

Angiotensin II seems to play a major role in CSA-induced chronic nephrotoxicity. As pointed out earlier, the intrarenal RAS is activated by CSA.[6] The salt depletion maneuver used for achievement of the chronic model enhances systemic and intrarenal RAS, and consequently angiotensin II generation. Angiotensin II activates fibroblasts and induces extracellular matrix deposition and tissue scarring.[55] Chronic infusion of angiotensin II in rats induced tubulointerstitial injury similar to that following CSA chronic nephropathy. A high concentration of angiotensin II AT_1 receptors is present in the inner zone of the outer medulla, particularly in longitudinal bands paralleling the vasa recta bundles, which is the preferential area of CSA damage. Likewise, renal outer medulla type 1 interstitial cells have a high density of angiotensin II receptors. These interstitial cells have extensive cytoplasmic processes that are related closely to the basement membrane of the vasa recta.[56]

Blockade of the RAS by an ACE inhibitor (enalapril) and/or an AT_1 angiotensin II receptor antagonist (losartan) in salt-depleted CSA-treated rats reduced blood pressure, promoted afferent arteriole vasodilatation, and significantly attenuated interstitial fibrosis generation without improving renal hemodynamics. Losartan and losartan plus enalapril, but not enalapril alone, decreased renal cortical α1 (I) procollagen messenger RNA (mRNA). Treatment with hydralazine plus furosemide reduced blood pressure to the same extent as enalapril and/or losartan, but did not prevent tubulointerstitial injury.[53] In an interesting study, Johnson et al[57] showed that enalaprilat, the active metabolite of enalapril, completely reversed the stimulatory effect of CSA on collagen synthesis by cultured renal cortical fibroblasts. This RAS blockade can prevent CSA chronic nephrotoxicity independently of hemodynamic or systemic angiotensin II effects. Angiotensin blockade by losartan also has been shown to prevent CSA-induced epidermal growth factor decrease in salt-depleted rats. Epidermal growth factor promotes kidney tubular regeneration after injury and so may be important for the prevention of apoptosis and subsequent fibrosis in this model.[58]

Aldosterone

Aldosterone, another component of the RAS, has been receiving increased attention as an individual key element in the mechanisms of progression of chronic renal disease. Aldosterone blockade prevented renal injury in hypertensive models of renal injury and aldosterone infusion reversed the protection afforded by angiotensin-converting enzyme inhibitors or losartan in hypertensive and renal ablation models of chronic renal disease. Not surprisingly, Feria et al[59] recently showed that spironolactone afforded significant structural and functional protection in the chronic CSA nephrotoxicity rat model. These effects were accompanied by prevention of TGF-β up-regulation and amelioration of renal down-regulation of epidermal growth factors mRNA levels.

TGF-β

CSA stimulates renal and systemic production of TGF-β.[54,60] The potential sources for renal interstitial TGF-β include interstitial macrophages, interstitial fibroblasts, and tubular epithelial cells. Using a double-immunolabeling technique, Pichler et al[60] suggested that the majority of CSA-induced TGF-β–expressing cells likely are fibroblasts. This cytokine plays a major role in the generation of renal fibrosis by directly stimulating extracellular matrix components and reducing collagenase production, ultimately leading to renal scarring. Data from experimental and clinical studies suggest that TGF-β overexpression may be an important factor in the development of CSA chronic nephrotoxicity. Vieira et al[54] showed, in the salt-depleted rat model, that CSA induced a progressive increase in renal TGF-β 1 immunostaining, preceding the later development of tubulointerstitial fibrosis. Interestingly, the staining was more prominent at the jux-

taglomerular arterioles. Cuhaci et al[61] found that 72% of the renal biopsy specimens from CSA-treated transplant patients with chronic allograft fibrosis expressed high levels of TGF-β. These patients had a rate of renal functional decline approximately 3 times higher than patients with minimal or no TGF-β renal expression. In heart transplant recipients the presence of TGF-β 1 codon 10-gene polymorphism was associated with a higher late prevalence of renal dysfunction.[62]

RAS activation seems to be related closely to CSA-induced TGF-β overproduction. Enalaprilat prevented the CSA-induced TGF-β secretion by cultured human proximal tubular cells, losartan and enalapril decreased TGF-β mRNA and reduced interstitial fibrosis in the salt-depleted rat model, and spironolactone normalized CSA-induced renal TGF-β up-regulation in the same model.[57,59,63] Similarly, losartan decreased plasma levels of TGF-β in renal transplant recipients treated with CSA.[9]

The use of anti–TGF-β antibodies in CSA-treated salt-depleted rats reduced renal TGF-β expression, prevented GFR impairment, and attenuated arteriolar hyalinosis but surprisingly did not change the extent of tubulointerstitial fibrosis.[64] These results suggest that TGF-β, although clearly related to CSA-induced fibrosis, is not responsible for all the effects of this drug on the mechanisms of exaggerated extracellular matrix deposition and renal scarring.

Macrophages

The presence of infiltrating mononuclear cells has been shown in the interstitial area of the cortex and outer medulla of salt-depleted rats treated with CSA. Young et al[65] and Vieira et al[54] showed that significant interstitial macrophage infiltration occurs very early in the salt-depletion model of chronic nephropathy, preceding GFR decrease and development of interstitial fibrosis. This infiltration was accompanied by an impressive interstitial and tubular cell proliferation that started in the medulla and progressed to areas of cortical fibrosis.[65] An up-regulation of the macrophage chemoattractant osteopontin was observed in proximal tubular cells of CSA-treated rats, and was correlated closely with macrophage infiltration and fibrosis development.[60,65] Intense staining for monocyte chemoattractant protein 1 was found in renal biopsy specimens from kidney transplant recipients with CSA nephrotoxicity.[66] The presence of osteopontin was confirmed in human biopsy specimens with CSA nephrotoxicity, but there was no significant inflammatory cell infiltration, suggesting that this molecule might be important in the early but not in the established phase of chronic CSA nephrotoxicity.[67] Macrophages are known sources of cytokines and other mediators of inflammation and play a key factor in several processes that lead to progressive renal fibrosis.[68] Activated infiltrating macrophages amplify the inflammatory and profibrogenic response by recruiting more immunocompetent cells, by stimulating fibroblast proliferation/migration, and increasing the synthesis of collagen. There is a close relationship between angiotensin II and macrophage function. Angiotensin II stimulates the production of monocyte chemoattractant protein 1 and osteopontin and induces the expression of adhesion molecules responsible for penetration of monocytes into the interstitial spaces.[68] Additionally, macrophages express functional components of the RAS.[69] Angiotensin not only recruits but also activates macrophages,[68] and in fact human cells activate the RAS during human monocyte/macrophage differentiation.[69]

Interventional experimental studies support a role for inflammatory cells in chronic CSA nephrotoxicity. Suppression of osteopontin expression and macrophage infiltration by colchicine improved renal interstitial fibrosis in CSA-treated rats.[70] Correction of CSA-induced hypomagnesemia abolished interstitial inflammation, up-regulation of osteopontin, and monocyte chemoattractant protein 1, and significantly attenuated interstitial fibrosis.[71]

Although there is little doubt about the relevance of the macrophage as an important participant in CSA chronic nephrotoxicity, a crucial question remains unanswered: what is (or are) the stimuli for renal macrophage infiltration and activation? Clearly, preglomerular ischemia has a role, but as already pointed out, CSA can cause significant interstitial fibrosis with normal RBF. Conceivable candidates are postglomerular ischemia caused by vasa recta constriction, sublethal tubular epithelial cell injury, and endothelial cell lesions allowing plasma leakage into the renal interstitial area or activation of resident renal interstitial cells.

Effects in Resident Renal Cells and Matrix Degradation Systems

Several experimental studies showed that CSA could act directly on resident renal cells, favoring the development of interstitial fibrosis. Indeed, Ghiggeri et al[72] showed that even very low concentrations of CSA induced collagen synthesis in a variety of cultured human and rat renal fibroblasts and mesangial and tubular epithelial cells. Addition of CSA to primary culture of human renal cortical fibroblasts and proximal tubule cells resulted in direct toxicity, release of profibrotic cytokines, and increased collagen synthesis. CSA stimulated insulin-like growth factor secretion and inhibited secretion of insulin-like growth factor I binding protein by fibroblasts. In tubular cells, CSA enhanced the secretion of TGF-β and platelet-derived growth factor.[73] CSA may have different effects on cultured human epithelial and endothelial cells and fibroblasts. Collagen production was enhanced in endothelial and epithelial cells, whereas mRNA for tissue inhibitors of metalloproteinase was up-regulated in fibroblasts.

Impairment of the proteolytic system responsible for renal matrix degradation is also an important element of CSA chronic nephrotoxicity. CSA significantly enhanced inhibitors of metalloproteinase and inhibited metalloproteinase production.[73] The CSA-induced up-regulation of tissue inhibitor of matrix metalloproteinase was abolished almost entirely by magnesium supplementation.[74] It recently has been shown that only mesangial cells from mice susceptible to glomerulosclerosis increased collagen content and inhibited matrix metalloproteinase activity after exposure to low CSA doses in contrast with mesangial cells from a strain of glomerulosclerosis-resistant mice, suggesting that the genetic background may influence CSA-induced profibrotic cellular response.[75]

Injured tubular epithelial cells may be another cause for CSA-induced fibrosis. As previously discussed, CSA causes sublethal tubular cell injury. Moreover, use of the salt-depletion model of chronic CSA nephrotoxicity showed a significant activation of apoptosis-related genes, even preceding the appearance of apoptotic cells and fibrosis.[76] Once again, angiotensin II seems partially to mediate this phenomenon because cotreatment with losartan significantly reduced the number of tubular and interstitial apoptotic cells in CSA-treated animals.[77] There is also evidence of NO participation in apoptosis induced by CSA. Thomas et al[77] found that L-arginine administration decreased the magnitude of tubulointerstitial apoptosis in salt-depleted rats treated with CSA significantly. Amore et al[78] showed that CSA-stimulated apoptosis in various renal cell lines, including human tubular cells, was related to increased inducible NO synthase mRNA via activated p53 proteins. Another possible mechanism for CSA-caused tubular injury is impairment of the P-glycoprotein system (P-GP). This transporter expels hydrophobic substances from the cell, acting as a detoxification system. CSA is a known inhibitor of P-GP, and so it can potentially promote intracytoplasmic accumulation of its own metabolites and toxic cell catabolism metabolites. Supporting this hypothesis, renal epithelial cells express P-GP in salt-depleted rats receiving CSA, which was related inversely to interstitial fibrosis and intrarenal angiotensin II deposits.[79] In renal transplant recipients, increased P-GP expression was found in infiltrating and resident cells of biopsy specimens showing acute tubular necrosis (ATN), acute rejection, and chronic rejection but not in chronic CSA nephrotoxicity, suggesting that failure to up-regulate P-GP is associated with CSA-induced apoptosis and fibrosis.[80]

Nitric Oxide

Supplementation of the NO substrate, L-arginine, ameliorated whereas use of the NO synthase inhibitor, L-NAME, aggravated CSA-induced tubulointerstitial fibrosis.[81] Likewise, CSA-induced up-regulation of TGF-β1, plasminogen activator inhibitor–1, and deposition of extracellular matrix components were aggravated by blocking NO and ameliorated by enhancing NO production.[82]

Endothelin

There is abundant evidence that CSA markedly increases endothelin production. Endothelin up-regulates TGF-β expression that is clearly involved in CSA chronic nephrotoxicity, suggesting the existence of an endothelin–TGF-β factor pathway in CSA-induced fibrosis. Supporting this hypothesis, increased tubular cell endothelin mRNA expression was found in human biopsy specimens with chronic CSA nephrotoxicity, as well as dra-

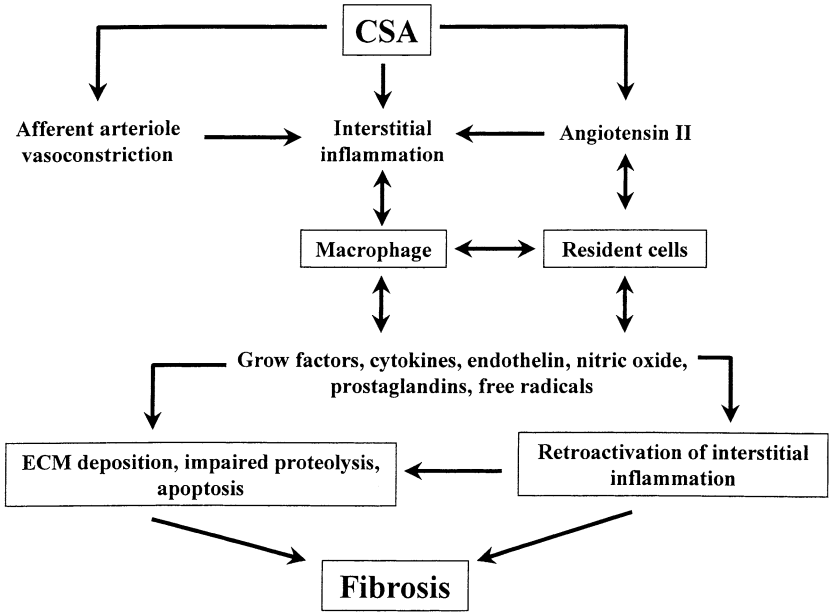

Fig 1. A working hypothesis for chronic CSA nephrotoxicity.

matic elevations in other endothelin system components in CSA-treated rats that strongly correlated with renal structural lesions.[66,83] However, experimental use of endothelin receptor antagonist did not prevent interstitial fibrosis in the salt-depleted chronic nephrotoxicity model,[52] and measurement of components of the ET system were not associated with progression of chronic graft dysfunction in renal transplant recipients.[84]

Vascular Endothelial Growth Factor

Recent studies have raised the possibility of vascular endothelial growth factor participation in CSA structural nephrotoxicity. Vascular endothelial growth factor is a potent endothelial cell mitogen that mediates endothelial cell proliferation and survival, induces angiogenesis, participates in vascular remodeling and repair, and causes vasodilation and increased vascular permeability through an increase in NO production. Shihab et al[85] found an up-regulation of vascular endothelial growth factor in salt-depleted CSA-treated rats but not in normal salt diet animals. As previously pointed out, only salt-depleted animals develop renal structural injury resembling human chronic CSA nephrotoxicity. In an interesting study, Kang et al[86] found that vascular endothelial growth factor administration to rats with established chronic CSA nephropathy resulted in improvement of interstitial fibrosis, decreased osteopontin expression, reduced macrophage infiltration, and improved blood pressure.

Other Mechanisms

Reactive oxygen species, besides its possible effects on renal function, also may mediate tissue injury in chronic CSA nephrotoxicity. Use of the antioxidant vitamin E inhibited increased TGF-β and osteopontin expression and the development of renal fibrosis in CSA-treated rats.[36] Mazzali et al[87] showed that hyperuricemia exacerbates experimental chronic CSA nephrotoxicity, apparently owing to activation of the RAS and inhibition of renal NO production. Preliminary evidence showed that prednisone altered the structural changes induced by CSA in salt-depleted rats, perhaps by enhancing tubular hypertrophy in the medulla, and tended to reduce tubulointerstitial fibrosis.[88] Johnson et al[89] showed that simvastatin, a 3-hydroxy-3-methylglutaryl CoA reductase inhibitor, completely prevented CSA-stimulated collagen synthesis and IGF-I secretion by cultured human renal cortical fibroblasts.

A WORKING HYPOTHESIS FOR CHRONIC CSA NEPHROTOXICITY

At this point it is clear that chronic CSA nephrotoxicity can be caused by preglomerular ischemia and/or direct effects independent of afferent arteriole vasoconstriction and injury. In the clinical setting and in most of the experimental models used, it is likely that interstitial fibrosis occurs through a combination of both processes.

The mechanisms of chronic CSA nephrotoxicity dissociated from preglomerular ischemia seem to be closely dependent on CSA-induced intrarenal RAS enhancement, macrophage infiltration and fibroblasts, and interstitial cell activation. Direct and indirect effects of macrophage migration, vasa recta constriction, activation of interstitial resident cells, endothelial cell injury, and subsequent leakage of plasma into the interstitial area and inflammatory stimulus originated from injured or apoptotic tubular cells may be part of this complex phenomenon. Obviously, more than one factor may be occurring at the same time. The final pathway is an inflammatory interstitial microenvironment in which angiotensin II, aldosterone, macrophages, fibroblasts, and resident cells interact to overexpress profibrotic substances (cytokines, growth factors, reactive oxygen species) while inhibiting antifibrotic components (metalloproteinase, and so forth) to enhance extracellular matrix deposition (Fig 1).

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