Tacrolimus (FK506)-Associated Renal Pathology

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Tacrolimus (FK506) is now an accepted primary immunosuppressive agent after solid-organ transplantation (1-13). It has both short-term and long-term advantages over conventional drugs: it is associated with less frequent rejection, hypertension, and hypercholesterolemia compared with cyclosporine. It also has been used to salvage allografts with acute cellular rejection not responding to cyclosporine and OKT3 (4-6,8,14,15).

Cyclosporine and tacrolimus are structurally unrelated compounds and bind to different cytosolic proteins in target cells. Nonetheless, both drugs have a closely related mechanism of action that is related primarily to a block in the transcription of interleukin-2 mRNA (16). This basic similarity in the mechanism of action is paralleled by an overlap in their toxicity profile: both drugs are toxic principally to the kidneys, central nervous system, gastrointestinal tract, and islets of Langerhans. The overall incidence of adverse events depends on dosage and clinical experience but appears to be comparable for both agents (7,11,17). Multicenter trials report tacrolimus to have higher nephrotoxicity, neurotoxicity, and diabetogenicity (18,19), but these conclusions have been criticized (20).

The following review focuses on the nephrotoxic actions of tacrolimus with particular reference to the associated histopathological changes seen in the allograft kidney. Throughout the discussion, emphasis is on how similar these changes are to those reported with cyclosporine therapy.

MECHANISMS OF TACROLIMUS NEPHROTOXICITY

The mechanism of immunosuppression mediated by cyclosporine and tacrolimus has been investigated intensively. It is believed that these drugs first must bind to their respective intracellular immunophilin receptors, particularly cyclophilin A and FK506 binding protein 12 (FKBP12). The immunophilin drug complex inactivates a phosphatase called calcineurin, which acts on additional target proteins that ultimately prevent the nuclear import of nuclear factor of activated T cells (NF-AT), a factor known to mediate the expression of several T-cell-activation genes. The resultant effects on the transcription and mRNA degradation of several cytokine genes have been described, of which the most important is interleukin-2, because this molecule plays a critical role in T-cell proliferation associated with alloimmune reactions (21).

The molecular basis of cyclosporine and tacrolimus nephrotoxicity is less well understood, but there is evidence that it may be mediated by the calcineurin pathway as well. Thus, cyclosporine and tacrolimus binding proteins are present at a high concentration in the kidney (higher than in the liver and spleen). Calcineurin immunoreactivity and enzyme activity in the kidney can be specifically inhibited by tacrolimus or cyclosporine but not by rapamycin, a drug that blocks a different cell signal involved in the T-cell proliferative response to interleukin-2 (22,23). At the cellular level, morphologic observations (reviewed subsequently) suggest that tubular epithelial cells, vascular endothelial cells, arteriolar myocytes, and interstitial fibroblasts are all targets for cyclosporine and tacrolimus nephrotoxicity.

The occurrence of epithelial vacuolization in clinical biopsy material suggests a direct toxic effect of tacrolimus and cyclosporine on the renal tubule. The exact
that the diagnosis must be made by the process of exclusion. In allograft kidneys, it may be necessary to show an absence of acute rejection by biopsy, whereas in liver-transplant patients, hepatorenal syndrome and hepatic glomerulopathies are considerations in the differential diagnosis. Likewise, in heart and lung transplant recipients, atherosclerotic and hypertensive nephropathy need to be excluded before making a diagnosis of tacrolimus nephrotoxicity. The clinical diagnosis is most secure when there is a decrease in serum creatinine levels after a reduction in tacrolimus dosage; however, chronic tacrolimus nephrotoxicity may be nonreversible (11,44) and, indeed, may not be detected unless routine monitoring of serum creatinine is supplemented periodically by creatinine clearance measurements. The distinction of chronic tacrolimus nephrotoxicity from insidiously developing chronic rejection is difficult on clinical grounds.

The temporal evolution of acute tacrolimus nephrotoxicity and its response to reduction in drug dosage have been described. The baseline creatinine level in one series of 22 patients was $212.2 \pm 168.0 \mu mol/L$ and showed a mean rise of $40.6\% \pm 14.2\%$ during episodes of nephrotoxicity (48). Concurrent extrarenal manifestations of tacrolimus toxicity were relatively common: hyperkalemia was recorded in 41% cases, hyperglycemia in 36% of previously nondiabetic patients, and hand tremors (neurotoxicity) in 9% of subjects. The highest mean plasma and whole blood tacrolimus levels during the toxic episodes were $2.7 \pm 0.8$ ng/ml (normal range, 0.5–1.5) and $31.6 \pm 10.6$ ng/ml (normal range, 5–20), respectively. Nephrotoxicity episodes were associated with elevated plasma or whole blood in tacrolimus levels in 82% patients. In other studies, a correlation between blood tacrolimus levels and nephrotoxicity was observed by some authors (52–54) but not by others (45,55). The dose of tacrolimus was reduced stepwise in response to a diagnosis of tacrolimus nephrotoxicity until a satisfactory response in serum creatinine was obtained. The mean dose reduction was $41\% \pm 21\%$ (range, 11–89) and led to a $86\% \pm 18\%$ (range, 45–100) decrease in the serum creatinine level (48). This variation in the percent of dose reduction necessary to restore allograft function reflects the known variability of tacrolimus pharmacokinetics in individual patients (56). The drug kinetics can be altered further by hepatic dysfunction and a variety of drug interactions. Thus, in patients receiving drugs that interfere with tacrolimus metabolism by the liver (itraconazole, erythromycin, diltiazem), drastic reductions in tacrolimus dosage may be required to reverse tacrolimus nephrotoxicity, and clinical response is delayed.

MORPHOLOGIC FINDINGS OF TACROLIMUS NEPHROTOXICITY

A description of the principal morphologic findings found in renal allograft biopsies performed during clinical episodes of tacrolimus nephrotoxicity follows.

Functional toxicity

Some patients on tacrolimus therapy develop laboratory evidence of graft dysfunction without remarkable morphological findings at biopsy. The renal dysfunction recovers as the dosage of tacrolimus is reduced. A similar phenomenon has been described with cyclosporine therapy and is believed to be the result of drug-induced vasospasm (57,58).

Acute tubular necrosis

Cyclosporine has been linked to the occurrence of acute tubular necrosis in the first several weeks after transplantation. Acute tubular necrosis during this interval can, of course, be due entirely to ischemic injury associated with the harvesting and implantation of the donor organ. There is experimental evidence, however, that immunosuppressive drugs can exaggerate such ischemic injury to the tubules (59). The frequency of acute renal failure and the duration of postoperative oliguria are greater in patients immunosuppressed with cyclosporine than with azathioprine (60). These observations also may be applicable to tacrolimus-treated patients, as this drug could potentiate renal ischemia by causing vasospasm, but clinical studies confirming this have not yet been performed.

Tubular vacuolization

Biopsies performed during clinical episodes of tacrolimus nephrotoxicity frequently show tubular vacuolization. In our experience, these tubular vacuoles are seen in proximal as well as distal tubules (Fig. 1). Although typically about equal in size and shape (isometric), focal coalescence into larger vacuoles is also demonstrable (61). The Japanese FK506 study group considers tacrolimus therapy to cause a “rough and foamy” tubular vacuolation in the proximal tubules (62). Cyclosporine toxicity is also associated with isometric tubular vacuolization, which is described by some authors to be found almost exclusively in the straight part of the proximal convoluted tubules (57). Tubular vacuolization may be better appreciated on trichrome compared with routine hematoxylin and eosin stains (63). Ultrastructurally, the vacuoles reflect dilatations in the endoplasmic reticulum (Fig. 2); some contain proteinaceous material and resemble lysosomes (61,63).

In addition to cyclosporine and tacrolimus toxicity,
This afferent arteriole from a patient with clinical tacrolimus nephrotoxicity shows myocyte vacuolization. Hyaline material can be seen deposited in the intima (periodic acid Schiff's stain).

curs in cardiac myocytes exposed to chemical, metabolic, and ischemic injury (71-74).

Tubular calcifications

Focal microcalcification in the renal parenchyma has been described in experimental tacrolimus and cyclosporine nephrotoxicity (75). Similar lesions occur in human biopsies (57,61) and, in some cases, may represent calcification of tubular epithelium damaged by these drugs; however, participants of one international workshop found the frequency of microcalcifications in patients on cyclosporine therapy to be comparable to that observed with azathioprine, a drug not considered nephrotoxic (66). The differential diagnosis includes dystrophic calcification at the site of prior ischemic or immunologic tubular injury and, less commonly, calcification resulting from hypercalcemic states, such as renal hyperparathyroidism.

Giant mitochondria

Giant mitochondria within the tubular epithelium have been demonstrated in tacrolimus-treated rats (59). We material, but literature is available on cyclosporine-treated patients. Mihatsch et al. described round, oval, or cigar-shaped mitochondria up to half the size of the nucleus (57). These inclusions are rare even in cases with severe toxicity, and usually only one giant organelle is found per cell. Giant mitochondria and isometric vacuolation are said never to be observed in the same cell. Electron microscopy is needed for definite differentiation from phagolysosomes. The frequency of giant mitochondria in patients on cyclosporine therapy is higher compared with conventional immunosuppressive drugs in some series (57) but not in others (60). Giant mitochondria are not specific for cyclosporine toxicity and have been described in ischemia, glomerulonephritis, systemic lupus erythematosus, minimal change nephrotic syndrome, and patients receiving azathioprine.

Acute microvascular toxicity

Renal allografts maintained on cyclosporine show scattered thrombi in the glomerular capillaries and arterioles in that they reflect a combination of dilated endoplasmic reticulum and lysosomes.

FIG. 3. This afferent arteriole from a patient with clinical tacrolimus nephrotoxicity shows myocyte vacuolization. Hyaline material can be seen deposited in the intima (periodic acid Schiff's stain).

FIG. 4. Ultrastructurally, myocyte vacuoles resemble tubular vacuoles.
therapy: One showed peculiar eosinophilic globules in the media, and another showed rare subendothelial lymphocytes and focal medial necrosis (76). Other similar cases have been reported from Japan and France (62,92). Differentiating these lesions from vascular rejection with intimal arteritis is both difficult and critical. The presence of significant lymphocytic infiltrates in the intima, scattered interstitial hemorrhages, tubulitis, and diffuse global glomerulitis favor the diagnosis of rejection over drug toxicity.

Before leaving this subject, it is of interest to recall that the potential of tacrolimus to cause injury to blood vessels was much debated during the developmental phase of this drug. Vascular damage described as "vasculitis" involving medium-sized arteries in the liver, pancreas, and heart was reported in tacrolimus-treated dogs (93). Work done by our colleagues and others raised doubts about the significance of these findings, as vasculitis was found with equal frequency in control animals (94,95). Studies in rats, baboons, and monkeys treated with tacrolimus (59,96) also were unable to reproduce vasculitis lesions. Arteriolar-sized renal vessels in rats and dogs treated with tacrolimus develop focal medial necrosis, accumulation of eosinophilic inclusions, and juxtaglomerular transformation but not true arteritis (94,97,98). In our opinion, these arteriolar lesions are similar to those reported with dopaminergic and adrenergic drugs and are adequately explained by intense vasospasm (99,100).

Arteriolar hyalinosis

Both cyclosporine and tacrolimus therapy are associated with hyaline eosinophilic deposits (Figs. 3 and 7) within the arterioles (57,61,101). Immunofluorescence examination shows these deposits to contain several proteins including fibrin, immunoglobulin M, C3, and C1q. Hyalinosis is particularly seen in patients on prolonged drug therapy, but this change conceivably can develop rapidly after acute arteriolopathy. It is important to keep in mind that similar vascular changes can be seen as a result of aging, hypertension, and diabetes mellitus, which would explain why in some studies wherein cyclosporine is used at low dosage or the follow-up is relatively short, the incidence of hyalinosis is not significantly higher than in control biopsies (102,103). A nodular configuration to the hyalin has been considered characteristic of drug toxicity (57), but this feature has also been described in donor-transmitted nephrosclerosis and in patients dying of ischemic cardiomyopathy (104,105). Even after review of clinical data and previous biopsies, it is not always possible to ascertain definitively the underlying cause of hyalinosis in individual biopsies, and sometimes multiple factors appear to be involved. Some authors find electron microscopy (Fig. 8) useful in this setting and state that in cyclosporine toxicity the hyaline deposits are circular and replace underlying necrotic smooth muscles, whereas in diabetes or hypertension, the deposits are not accompanied by degenerative or necrotizing changes in the myocytes (57). Other authors have been unable to find myocyte necrosis in cyclosporine-associated arteriolar hyalinosis, however, possibly because they looked at biopsies at a later stage of the disease (102,106). Detailed ultrastructural evaluations of tacrolimus-associated hyalin change are not reported in the literature.

Striped fibrosis

Prolonged use of cyclosporine and tacrolimus can cause interstitial fibrosis, which is said to have a "striped" pattern (57,61) resulting from areas of ischemic tubular atrophy and interstitial fibrosis alternating with relatively well-preserved or even hypertrophic renal
Glomerular pathology

The occurrence of glomerular capillary and afferent arteriolar thrombosis as a manifestation of cyclosporine and tacrolimus nephrotoxicity has been mentioned earlier (76–82). In cases with frank hemolytic uremic syndrome, clinical resolution can be complete, but residual injury in the form of glomerular capillary basement membrane thickening and duplication may persist (109).

Experimental studies show that tacrolimus and cyclosporine can cause juxtaglomerular hyperplasia, possibly by a local activation of the renin-angiotensin system (37,98). The diagnostic utility of this finding in clinical practice is limited because a study of the juxtaglomerular apparatus requires specialized histochemical techniques. Furthermore, juxtaglomerular apparatus hyperplasia is a nonspecific finding described in other clinical settings, such as rejection, arteriosclerosis, hypertension, and reflux nephropathy. The pathogenesis in cases of drug-induced injury may reflect segmental ischemic collapse of the glomerular capillary loops secondary to the lesion of arteriolar hyalinosis (57,61,101). Resolution of intraglomerular capillary thrombosis could account for some cases (76,82). Alternatively, the lesion could reflect a compensatory response to nephron loss caused by the vascular and interstitial effects of cyclosporine and tacrolimus. Thus, it is believed that compensatory glomerular hypertrophy can result in capillary hyperfiltration, endothelial injury, mesangial dysfunction, and progressive segmental or global glomerulosclerosis (114). A possible link between glomerulosclerosis and local activation of the renin-angiotensin system is suggested by experiments demonstrating stimulation of extracellular matrix protein synthesis by rat glomerular mesangial cells exposed to angiotensin II (115). Mesangial matrix protein synthesis also can be enhanced by TGF-β, a cytokine known to be stimulated by cyclosporine (40).

**NATURAL HISTORY OF TACROLIMUS NEPHROTOXICITY**

Tacrolimus-induced tubular vacuolization, acute arteriolopathy, and thrombotic microangiopathy generally respond well to reduction in the drug dose; histologic improvement can be documented in follow-up biopsies. Studies specifically addressing the question of whether chronic tacrolimus nephrotoxicity results in progressive graft loss have not yet been published; however, data are available showing that long-term graft survival in tacrolimus-treated patients is superior to that reported for cyclosporine and azathioprine (a drug not considered nephrotoxic) (10). Hence, it is unlikely that chronic administration of tacrolimus is a frequent cause of graft loss.

The natural history of tacrolimus-associated arteriolar hyalinosis and interstitial fibrosis needs to be defined by examination of sequential biopsies from renal transplant recipients. Uncontrolled studies on cyclosporine-treated patients suggest that discontinuation of the drug leads to resolution of mild arteriolar hyalin deposits in some cases but to continued accumulation in other patients (116,117). In evaluating such data, it should be kept in mind that arteriolar hyalin deposits can be extremely focal in distribution and may involve fewer than 10% of vessels sampled at biopsy (116), leading to difficulty in distinguishing between sampling artefact and true resolution/progression of lesions in sequential biopsies (106). We have noted that striped fibrosis has a similar uneven distribution in renal allografts (61).

**SUMMARY**

A variety of renal lesions have been described in patients undergoing episodes of tacrolimus nephrotoxicity. Although helpful to the pathologist seeking morphologic clues to substantiate drug toxicity, none of these lesions is specific to the extent that it has not been reported in other conditions. This is not surprising because all organs in the human body possess only a limited repertoire of tissue reactions to deal with injurious stimuli. The diagnosis of tacrolimus nephrotoxicity is therefore best made with reference to the clinical context and after exclusion of other causes of graft dysfunction. The ultimate confirmation is a decline in serum creatinine levels following a reduction in tacrolimus dosage. Chronic tacrolimus nephrotoxicity can be nonreversible, however, and may be regarded as the "price to be paid" for maintaining continuous immunosuppression. Because the nephrotoxic and antirejection actions of tacrolimus might be mechanistically related, it may not be possible to avoid tacrolimus nephrotoxicity altogether. Fortunately, there is evidence that the effects can be minimized by the use of low-dose regimens. Observed and projected long-term graft survivals with tacrolimus now equal or exceed those obtainable with alternative immunosuppressive drugs (10).


