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Tenofovir-induced kidney disease: an acquired renal tubular mitochondriopathy

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Tenofovir, used in combination with other antiretroviral agents, is an effective therapy for HIV infection. Although large clinical studies and post-marketing data support a benign renal profile for tenofovir, numerous cases of kidney injury raise concern for nephrotoxic potential. Early human studies and experimental evidence suggested that tenofovir itself was not associated with mitochondrial toxicity within the kidney. However, recent animal data demonstrate that tenofovir causes mitochondrial DNA depletion and mitochondrial toxicity. Herlitz *et al.* confirm the nephrotoxicity of tenofovir in humans. They describe its clinical consequences, histopathologic findings, and its mitochondrial toxicity in HIV$^+$ patients.

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Drug research and development facilitates the synthesis and release of novel therapeutics into clinical practice at a remarkable rate. Their availability has undoubtedly revolutionized the treatment of numerous diseases—in particular, devastating processes such as HIV infection. The landscape of this disease has been forever changed by the availability of highly active antiretroviral therapy (HAART) since 1996. The basic premise behind this strategy is to attack the virus at different replication steps using multiple medications. Unfortunately, most success stories have a catch; in this case, the price of success is drug-induced kidney disease, a challenging complication for HIV$^+$ patients and their caregivers.[1]

Before the widespread use of HAART, HIV-associated kidney disease was primarily due to direct and/or indirect effects of the virus. However, with successful treatment of HIV infection, drug-induced nephrotoxicity (Table 1) surfaced as an important problem. Acute kidney injury (AKI), various tubulopathies, nephrolithiasis, and chronic kidney disease were noted.[1] HAART causes these renal syndromes via multiple mechanisms, including direct tubular toxicity, allergic reactions, and precipitation of insoluble drug crystals.

Although many of the early drugs had intolerable adverse effects and difficult dosing schedules, newer agents have overcome many of these issues. One such drug, tenofovir, has gained widespread use on the basis of its efficacy, tolerability, and patient-friendly dosing schedule.[2] As it is structurally similar to the acyclic nucleotide analogs adefovir and cidofovir, which are nephrotoxic, concern about adverse renal effects existed for tenofovir as well. These two drugs cause proximal tubulopathies such as AKI as a result of acute tubular necrosis (ATN) and Fanconi's syndrome,[3] by disrupting proximal tubular mitochondrial function. A number of mechanisms underlie drug-induced mitochondriopathies, but these drugs act primarily by decreasing mitochondrial DNA (mtDNA) replication by inhibiting mitochondrial DNA polymerase-γ, which is the only enzyme capable of replicating mtDNA.[3] As a result, mtDNA and a number of the mtDNA-encoded enzymes involved in electron transport chain function and oxidative phosphorylation are depleted, resulting in disturbed mitochondrial function. This ultimately causes, among other effects, a deficit in adenosine triphosphate production, impaired cell function, and cell injury and/or death.

Early randomized clinical trials[4] and post-marketing data[5] examining tenofovir in relatively healthy HIV$^+$ subjects supported an excellent safety profile, including the absence of significant renal injury. An *in vitro* experimental study supported this clinical finding.[6] The investigators exposed various cultured human cell lines (liver, muscle, proximal renal tubule) to tenofovir. Minimal mtDNA depletion and insignificant reductions in cellular expression of the mitochondrial protein cytochrome *c* oxidase were noted with tenofovir. However, with the release of tenofovir into clinical practice and its use in HIV patients with various comorbid conditions, reports of nephrotoxicity began to surface.[7] These reports described

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Table 1 | HAART-associated kidney disease

Renal syndrome	Medication
Acute kidney injury	
Toxic acute tubular necrosis	Tenofovir, ritonavir, didanosine
Acute interstitial nephritis	Atazanavir, abacavir, indinavir
Crystal nephropathy	Indinavir, atazanavir
Tubulopathies	
Fanconi's syndrome	Tenofovir, didanosine, ritonavir
Renal tubular acidosis	Lamivudine, stavudine
Nephrogenic diabetes insipidus	Tenofovir, didanosine, indinavir
Nephrolithiasis	Indinavir, atazanavir, nelfinavir, amprenavir, saquinavir, efavirenz
Chronic kidney disease	
Chronic interstitial nephritis	Indinavir, tenofovir
Post-AKI kidney disease	Several HAART drugs

Abbreviations: AKI, acute kidney injury; HAART, highly active antiretroviral therapy.

renal consequences such as AKI from toxic ATN, Fanconi's syndrome, and rare cases of nephrogenic diabetes insipidus. Renal histopathology from these cases demonstrated acute tubular injury predominantly in the proximal tubules, as described with adefovir and cidofovir. These observations questioned the renal safety noted in the large clinical trials.

Further information about nephrotoxic potential was gleaned from a retrospective study that examined mitochondrial ultrastructure and mtDNA levels in kidney tissue obtained from biopsies performed for nephrotoxicity in HIV^- and HIV^+ patients.[8] Investigators found that HIV^+ kidneys exposed to tenofovir had altered tubular mitochondrial morphology. However, while HIV^+ kidneys had mtDNA levels lower than those in HIV^- patients, there was no difference in mtDNA levels in tenofovir-exposed versus unexposed HIV^+ kidneys. Furthermore, HIV^+/tenofovir$^+$/didanosine$^+$ kidneys had lower mtDNA levels than HIV^+-tenofovir$^+$/didanosine$^-$ kidneys, which questioned whether mitochondrial toxicity was a significant complication of isolated tenofovir exposure. More recently, two animal studies support the notion that tenofovir causes mtDNA depletion and mitochondrial dysfunction.[9,10] A study in HIV^+ transgenic mice and their wild-type littermates examined ultrastructure and mtDNA levels with tenofovir, didanosine, and vehicle exposures.[9] Only renal proximal tubules from HIV^+ transgenic mice exposed to tenofovir showed ultrastructural mitochondrial abnormalities (irregular shapes with sparse, fragmented cristae) and decreased mtDNA levels, which paralleled the ultrastructural mitochondrial abnormalities. The opposite was seen in liver cells, where didanosine depleted hepatic mtDNA and tenofovir had no effect. Another study explored the potential for mitochondrial toxicity in rats exposed to tenofovir, didanosine, or water.[10] Rats exposed to tenofovir, but not those exposed to didanosine or water, developed proximal tubular dilatation, abnormalities in mitochondrial ultrastructure, depleted mtDNA, and depressed respiratory chain enzyme expression (cytochrome *c* oxidase and nicotinamide adenyldinucleotide dehydrogenase). In contrast, didanosine induced significant hepatic mtDNA depletion, whereas tenofovir had no liver effects. These studies suggest that tenofovir causes compartmentalized mitochondrial toxicity within renal proximal tubular cells.

Herlitz and colleagues[11] (this issue) now make a strong case for tenofovir as a proximal tubular mitochondrial toxin in humans. The Columbia University group queried their renal biopsy archives in search of a histopathologic correlate to the clinical renal disease—tenofovir-associated nephrotoxicity. According to predefined diagnostic criteria, 13 patients with HIV infection were identified. All patients were on various forms of HAART; and the ten with available data had well-controlled HIV infection by CD4 count and viral load. Duration of tenofovir ranged from 3 weeks to 8 years with a median of 8 months. Biopsy was performed for AKI ($n=9$) or proteinuria with mild serum creatinine elevation ($n=4$). Mean serum creatinine at biopsy was 5.7±4.0 mg/dl, mean proteinuria was 1.6±0.3 g/d, and five patients had normoglycemic glycosuria, the latter two findings being suggestive of Fanconi's syndrome. AKI was severe enough to require hemodialysis in five patients. Of 11 cases with mean follow-up of nearly 20 months, kidney function returned to baseline in six cases, while five had partial recovery. No patient remained on dialysis. Thus, although all patients had significant recovery with tenofovir discontinuation, nearly half of the patients were left with some level of CKD.

Renal histology demonstrated proximal tubular injury and varying degrees of chronic tubulointerstitial scarring. Ten cases had findings consistent with 'toxic ATN.' Prominent eosinophilic inclusions within proximal tubular cell cytoplasm, which represented giant, abnormal mitochondria, were noted on light microscopy and constitute an interesting new finding. These inclusions are easily identifiable, as they stain brightly with hematoxylin and eosin stain or fuchsinophilic with trichrome stain. On electron microscopy, mitochondria varied widely in shape and size; some were small and rounded, while others were swollen with irregular contours. Loss and disorientation of cristae were observed in enlarged mitochondria, while the overall number of mitochondria was significantly decreased in some tubular cells.

The comprehensive clinical–histopathologic correlation described in this paper addresses a number of issues related to tenofovir nephrotoxicity. First, it confirms that tenofovir causes 'toxic ATN' of varied severity and Fanconi's syndrome in exposed HIV^+ patients. Second, patients can develop the clinical renal syndrome at any time point in tenofovir therapy, both early and late. Third, although tenofovir discontinuation is associated with significant renal recovery, many patients may suffer from CKD. Finally, histopathology confirms that tenofovir is a mitochondrial toxin in renal tubular cells and has a distinctive finding on light microscopy. However, the study does not answer one important question: Why does tenofovir cause renal injury in only a small subset of HIV^+ patients? To try to answer this question, host characteristics and drug pharmacokinetics must be considered.

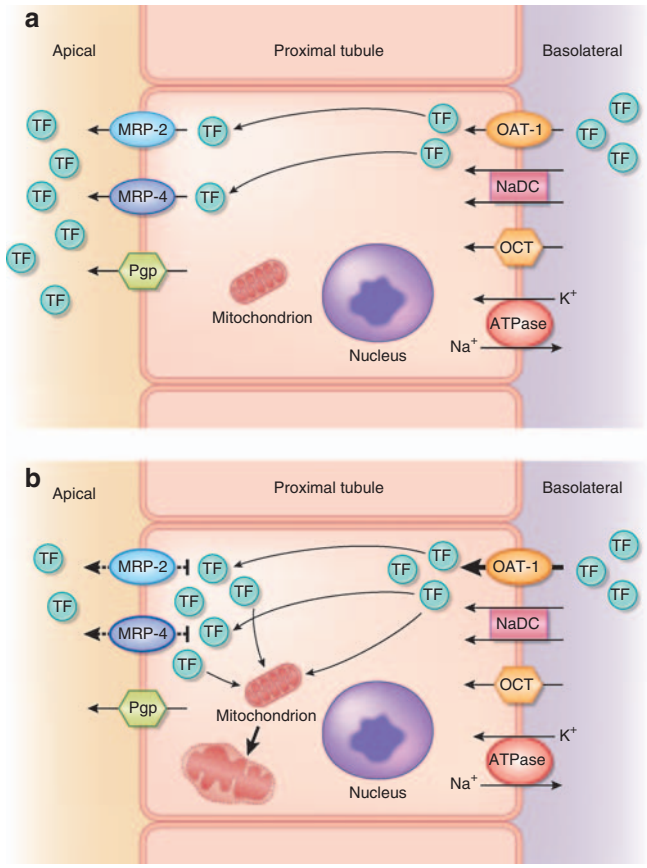

Figure 1 | Proximal tubular cell transport pathway for tenofovir. (**a**) The organic anion drug tenofovir (TF) is delivered to the basolateral membrane of proximal tubular cells and is transported into the cell by organic anion transporter-1 (OAT-1). Once within the cell, TF is transported via carrier proteins and is subsequently secreted into the urinary space by the apical efflux transporters multidrug resistance protein-2 (MRP-2) and MRP-4. (**b**) Disturbances in the secretory pathway of TF (increased OAT-1 activity or decreased MRP efflux transport activity) may lead to increased TF concentrations within the cell, which can cause mitochondrial DNA depletion and dysfunction. This can ultimately cause a proximal tubulopathy characterized by acute kidney injury and Fanconi's syndrome. Abbreviations: NaDC, sodium–dicarboxylate symporter; OCT, organic cation transporter; Pgp, P-glycoprotein.

Host factors that potentially enhance tenofovir nephrotoxicity include mtDNA depletion, a complication of HIV infection itself, which likely primes patients for more severe mitochondrial dysfunction with exposure to a drug that targets this organelle. In addition, underlying kidney disease with low glomerular filtration rate and genetic defects in certain renal drug excretion pathways also enhance risk for tenofovir-induced nephrotoxicity.[12] Tenofovir is eliminated by a combination of glomerular filtration and proximal tubular secretion, which in part explains the compartmental toxicity of tenofovir.[12] Tenofovir is transported via organic anion transporter-1 (OAT-1) from the basolateral circulation into proximal tubular cells (Figure 1), where it is eventually translocated into the urine through apical efflux transporters such as multidrug resistance protein-2 (MRP-2) and MRP-4. Renal impairment with reduced glomerular filtration rate will increase the amount of tenofovir that is secreted, increasing trafficking through the proximal tubular cells. Increased activity of OAT-1 may increase the amount of tenofovir entering proximal tubular cells, although this remains unproven. Impaired MRP-driven efflux activity can reduce tenofovir secretion and increase intracellular concentrations. A single-nucleotide polymorphism in the MRP-2 efflux transporter gene (*ABCC2*) has been documented in HIV$^+$ patients who developed tenofovir-induced nephrotoxicity, supporting this hypothesis.[13] Similarly, endogenous anions and other drugs may compete with tenofovir for these efflux transport pathways.

Ultimately, these excretory pathway defects can lead to increased tenofovir trafficking through and/or increased concentrations within proximal tubular cells, enhancing risk for mtDNA depletion and mitochondrial dysfunction. In a subgroup of patients, these energy-deficient tubular cells may manifest as the clinical renal syndromes previously described. The actual development and onset time of tenofovir-induced nephrotoxicity will likely depend on the number and severity of patient risk factors present. Genetic risk factor testing (for the single-nucleotide polymorphism in *ABCC2*) to identify high-risk patients and targeted interventions, such as probenecid to reduce OAT-1 transport of tenofovir into tubular cells, may allow HIV$^+$ patients to garner benefit from effective therapies such as tenofovir.

DISCLOSURE

The author declared no competing interests.

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Probiotics and dietary manipulations in calcium oxalate nephrolithiasis: two sides of the same coin?

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Growing evidence has assigned to oxalate a pivotal role in calcium nephrolithiasis pathophysiology. A better understanding of the mechanisms behind intestinal absorption and renal excretion has led to the identification of new treatments. Among these, diet and probiotics appear promising in terms of safety and rationale. However, the discrepancy between *in vitro* and *in vivo* results requires further studies to identify the right patient target, the correct dosage, and the real modification of natural and clinical history of nephrolithiasis.

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In recent decades, an advance in technology has led to an improvement in the efficacy of urological treatments for idiopathic calcium nephrolithiasis. From a pathophysiological point of view, in-depth studies—for example, those carried out on Randall's plaques and molecular aspects—have allowed better definition of the illness and currently weaken the hypothesis that nephrolithiasis is a simple combination of chemical–physical factors that induce crystallization.[1] Nevertheless, the prevalence of calcium oxalate nephrolithiasis is rising in industrialized countries, and we can still consider this disease an 'orphan' of effective drugs.

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This becomes crucial because the apparent role of oxalate in determining the risk and development of calcium nephrolithiasis seems to be increasing; according to some, it is more relevant than calcium itself. This is true both for simple chemical–physical and stoichiometric events in the crystallization process, which cause small variations in the concentrations of urinary oxalate to determine greater changes in calcium oxalate supersaturation values, and for our growing knowledge of other mechanisms involved. For example, the renal reabsorption of oxalate is linked to other ions (sulfate, chloride, other anions) through a specific family of transporters, solute carrier family 26A (SLC26A);[2] in fact, patients with cystic fibrosis in which there is a defect in the chloride channel CFTR, which is co-regulated with some receptors of the SLC2A family, show hyperoxaluria and an increased incidence of calcium oxalate nephrolithiasis. Moreover, oxalate itself seems to reduce renal blood flow and is responsible for microvascular damage due to an insufficient crystal clearance; it has been suggested that this is one of the possible mechanisms explaining the greater prevalence of nephrolithiasis in hypertensive subjects. In fact, it has been demonstrated that the administration of 'vasodilators' such as enalapril or losartan in rats with ethylene glycol-induced nephrolithiasis reduces tubulointerstitial lesions. Finally, interactions of oxalate with renal epithelium could induce oxidative stress with apoptotic and regenerative stimulus and greater crystal adhesion capacity.

It is therefore clear how important and at the same time how complex the medical treatment of idiopathic calcium nephrolithiasis is. An interesting study by Lieske *et al.*[3] (this issue) shows the difficulty of translating research on calcium oxalate nephrolithiasis from bench to bedside; assumptions based on excellent *in vitro* studies have proved to be inefficient in *in vivo* experiments.

Must we, therefore, conclude that probiotics are ineffective in the treatment of calcium nephrolithiasis? Probably not. The problem is rather to identify in which patients they actually work. In fact, given the variable absorption of oxalate in the gastrointestinal tract (from 7% to 72%), the amount of oxalate intake in the diet becomes crucial. It is not surprising that major proof of a fall in oxaluria following the use of probiotics derives from studies that analyzed patients with enteric hyperoxaluria[4] or after an oral load of oxalate;[5] in contrast, results with mild hyperoxaluria are not very satisfactory.[6]

Many factors are involved in determining the amount of gastrointestinal oxalate absorption. We can identify at least four pivotal points:[1,2,7] (1) the amount of soluble oxalate and/or its precursors that is naturally present in foods; (2) other foods or nutrients that can influence the absorption of oxalate because of competition or formation of non-absorbable complexes (for example, calcium, magnesium, lipids, fiber); (3) intestinal flora and the presence of microorganisms capable of metabolizing oxalate; and (4) variations of pH and density of anion transporters