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Original contribution

Decreased expression of megalin and cubilin and altered mitochondrial activity in tenofovir nephrotoxicity☆

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Summary Tenofovir disoproxil fumarate (TDF) is a commonly used antiretroviral drug for HIV, rarely causing Fanconi syndrome and acute kidney injury. We retrospectively analyzed the clinico pathological presentation of 20 cases of tenofovir-induced tubulopathy, and investigated the renal expression of the megalin and cubilin proteins, as well as the mitochondrial respiratory chain activity. Estimated glomerular filtration rate (eGFR) before TDF exposure was 92 ml/min/1.73m^2, decreasing to 27.5 ml/min/1.73m^2 at the time of biopsy, with 30% of patients requiring renal replacement therapy. Proximal tubular expression of megalin and cubilin was altered in 19 and 18 cases, respectively, whereas it was preserved in patients exposed to TDF without proximal tubular dysfunction and in HIV-negative patients with acute tubular necrosis. Loss of megalin/cubilin was correlated with low eGFR and high urine retinol binding protein at the time of biopsy, low eGFR at last follow-up, and was more severe in patients with multifactorial toxicity. Patients with additional nephrotoxic conditions promoting tenofovir accumulation showed a lower eGFR at presentation and at last follow-up, and more severe lesions of acute tubular necrosis, than those with isolated tenofovir

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toxicity. Altered mitochondrial COX activity in proximal tubules was observed and may be an early cellular alteration in tenofovir nephrotoxicity. In conclusion, altered megalin/cubilin expression represents a distinctive feature in tenofovir-induced tubulopathy, and its severity is correlated with urine retinol binding protein loss and is associated with a poor renal prognosis. Concomitant exposure to other nephrotoxic conditions severely impacts the renal presentation and outcome.

1. Introduction

Tenofovir disoproxil fumarate (TDF) is a prodrug of tenofovir (TFV), a nucleotide analog reverse-transcriptase inhibitor used for the treatment and for prophylaxis of human immunodeficiency virus (HIV) infection [1,2], and for the control of hepatitis B virus (HBV) infection [3]. TDF represents the most prescribed antiretroviral molecule worldwide, because it combines a high antiviral effectiveness and a good-safety profile. TDF is metabolized in the plasma, and TFV is predominantly eliminated via the kidney, through glomerular filtration and proximal tubular secretion. TFV is actively transported into proximal tubular cells (PTCs) by the human organic anion transporters 1 and 3 and is secreted to the tubular lumen through the apical membrane transporters MRP-4 and MRP-2 (multidrug resistance proteins) [4,5]. Although phase III clinical trials demonstrated a good renal safety of TDF [6,7], cumulative exposure was associated with significant glomerular filtration rate (GFR) decrease and an increased risk of chronic kidney disease (CKD) in several observational cohorts of HIV-infected patients [8-12]. Although more rarely observed, severe renal side effects including proximal tubular dysfunction (PTD), acute kidney injury (AKI), and nephrogenic diabetes insipidus can occur in HIV-infected patients exposed to TDF [13-18]. Kidney biopsy in patients with TDF-induced tubulopathy shows non-specific defects of the PTCs and morphological alterations of mitochondria [17,18].

In the present study, we analyzed the clinical and histological characteristics of patients with TDF-induced PTD, and we correlated the renal expression of the endocytic receptors megalin and cubilin with the renal parameters. We also demonstrated alteration of the COX-dependent mitochondrial activity in kidney biopsy specimens, from TDF-treated patients.

2. Materials and Methods

2.1. Patients

We identified all HIV-infected patients, receiving TDF and presenting PTD and/or AKI, who underwent a native kidney biopsy at our Pathology Department between 2001 and 2014. Patients with HIV-associated nephropathy were excluded. The following data were recorded: age, sex, ethnicity, hypertension, diabetes mellitus, dyslipidemia, smoking, body mass index, hepatitis B (HBV) and hepatitis C (HCV) virus infections, age at the diagnosis of HIV infection, Centers for Disease Control and Prevention staging, duration of TDF exposure, antiretroviral therapies, CD4 count cell and HIV viral load, serum parameters including creatinine to calculate eGFR using the 4-variable Modification of Diet in Renal Disease equation, potassium, uric acid, phosphorus, and fasting glucose, urine parameters including creatinine, glucose, proteinuria, albuminuria (normal <3mg/mmol creatinine), and retinol-binding protein (uRBP, normal <0.08mg/mmol creatinine). CKD was defined by eGFR <60ml/min/1.73m^2 for 3 months. AKI was classified according to the KDIGO classification [19]. Fanconi syndrome was defined by the presence of four criteria: fasting serum phosphate <2.5mg/dL, serum uric acid <3.4mg/dL, glycosuria >1mmol/l with fasting serum glucose <6mmol/l and low molecular weight proteinuria (proteinuria >50mg/mmol and albuminuria/proteinuria <0.4). PTD was defined by the presence of at least three of these four criteria.

2.2. Kidney histology

Renal biopsies were processed for light microscopy, immunofluorescence and electron microscopy (EM) according to standard techniques. Acute tubular injury (ATI), tubular atrophy and interstitial fibrosis were graded on a semi-quantitative scale based on an estimate of the percentage of renal cortex affected: 0, none; 1, 5% to 25%; 2, 26% to 50%; 3, >50%. Arteriolar hyalinosis was graded as follow: 0, no periodic acid–Schiff (PAS)–positive hyaline thickening; 1, mild-to-moderate PAS-positive hyaline thickening in at least one arteriole; 2, moderate-to-severe PAS-positive hyaline thickening in more than one arteriole; 3, severe PAS-positive hyaline thickening in many arterioles. Arteriosclerosis was graded as follow: 0, no chronic vascular changes; 1, vascular narrowing of up to 25% luminal area by fibrointimal thickening of arteries, in the most severely affected vessels; 2, fibrointimal thickening from 26% to 50% narrowing of the vascular luminal area in the most severely affected vessels; 3, fibrointimal thickening >50% narrowing of the vascular luminal area in the most severely affected vessels. Ultrastructural analysis was performed as previously described [20].