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Original Citation:
Tenofovir clearance is reduced in hiv-positive patients with subclinical tubular impairment / Calcagno, A; Cusato, J; Marinaro, L; Simiele, M; Lucchiari, M; Alcantarini, C; Tettoni, M C; Trentini, L; Mengozzi, G; D'Avolio, A; Di Perri, G; Bonora, S. - In: AIDS. - ISSN 0269-9370. - 30:6(2016), pp. 915-919.

Availability:
This version is available http://hdl.handle.net/2318/1588286 since 2016-08-03T13:52:50Z

Published version:
DOI:10.1097/QAD.0000000000000995

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This is a pre-copyedited, author-produced PDF of an article accepted for publication in AIDS following peer review. The version of record 2016 Mar 27;30(6):915-20. doi: 10.1097/QAD.0000000000000995. is available online at: http://journals.lww.com/aidsonline/pages/articleviewer.aspx?year=2016&issue=03270&article=00011&type=abstract
Tenofovir Clearance is Reduced in HIV-positive Patients with Subclinical Tubular Impairment

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Running Head: Tenofovir urinary PK and RBP

Type of article: Concise Communication

Word count: 1787 (abstract 246)

Figures: 2

Supplementary table: 1

Funding: This work was supported by a research grant from Gilead (Gilead Fellowship Program 2012, c2b35f7351).

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Key words: tenofovir, pharmacokinetics, urinary, tubular dysfunction, retinol binding protein.
Abstract

Objective: The primary objective of the study was to assess if tenofovir (TFV) plasma and urinary concentrations were associated with urinary retinol binding protein (RBP) in HIV-positive patients with normal estimated filtration rate.

Design: A cross-sectional diagnostic study.

Methods: HIV-positive patients with estimated creatinine clearance (eCrCl) >60 ml/min, on tenofovir disoproxil fumarate (TDF)-containing combination since at least 6 months, taking TDF at night, without significant co-morbidities (diabetes, untreated hypertension, known renal malformations, recurrent nephrolithiasis) and nephrotoxic drugs were included. TFV plasma and urinary concentrations were measured 12 hours after drug intake (C_{12}). RBP was measured through enzyme immunoassay kit on spot urines and corrected per urinary creatinine (uRBP/uCr); normality ranges were <130 µg/g (in patients aged <50 years) and <172 µg/g (in patients aged ≥50 years).

Results: 289 patients were included (median age of 45.8 years, 71.6% male and 85.4% Caucasians); patients were concomitantly treated with non-nucleoside reverse transcriptase inhibitors (155, 53.6%), protease inhibitors (118, 40.8%) or integrase inhibitors (16, 5.5%) containing regimens. eCrCl was 89.4 ml/min (78.6-105.9). uRBP and uRBP/uCr were respectively 204.6 ng/mL (92-380) and 169.7 µg/g (85.8-318.3); abnormally high uRBP/uCr was observed in 157 patients (54.3%). A multivariate binary logistic regression confirmed that both ethnicity (p=0.004, Beta 2.93, 95CI 1.41-6.10) and TFV urinary C_{12} <21 mg/mL (p=0.006, Beta 2.04, 95CI 1.12-3.41) were significantly associated with abnormal uRBP/uCr.

Conclusions: HIV-positive TDF-treated patients showed a high prevalence of proximal tubular impairment: ethnicity (Caucasians) and low urinary TFV concentrations were significantly associated with elevated urinary RBP.
Background

Tenofovir disoproxil fumarate (TDF) is a safe antiviral although increases in serum creatinine and proportional reductions in estimated glomerular filtration rate have often been reported. [1] However, as real GFR seems unaltered [2], creatinine and renal functional measures based on it are poorly representative of the actual kidney dysfunction attributable to TDF. Several clinical studies including urinary markers of proximal tubular function (e.g. retinol-binding protein (RBP) and β2microglobulin) found a much more strict correlation between TDF intake and tubular dysfunction [3-5]. Tubular dysfunction is thought to result from TDF accumulation into proximal tubular cells; this may explain serum creatinine elevation (as proximal tubule secretion accounts for 15-20% of creatinine clearance) and might provide a plausible link with the reduction in bone mineral density (BMD) consistently seen in TDF recipients [6,7] Clearance of tenofovir (TFV) occurs by both glomerular filtration and secretion from the proximal tubule, in an approximate ratio of 2:1. [8] Although the relationship between TDF intake and proximal tubular dysfunction has been repeatedly confirmed its effects on TFV clearance has never been reported. Therefore we performed a cross-sectional pharmacokinetic study on both plasma and urinary TFV in HIV-positive patients with normal eGFR, whose tubular function was evaluated by urinary markers.

Material and Methods

A cross-sectional study was carried forward at the Unit of Infectious Diseases (Torino, Italy) after Ethics Committee approval; all patients signed a written informed consent before being enrolled. A pre-specified analysis was carried in patients aged >50 years. Inclusion criteria were adult age, confirmed HIV positivity, estimated creatinine clearance (eCrCl, evaluated through the Cockcroft-Gault equation) above 60 ml/min, being on TDF-containing triple combination antiretroviral treatment since at least 6 months, evening intake of TDF.
Exclusion criteria were diabetes, untreated hypertension, known renal malformations, recurrent nephrolithiasis and the concomitant use of nephrotoxic drugs (gancyclovir, cyclosporine).

Blood (18 mL) and urine (4 mL) samples were withdrawn twelve hours after TDF intake (+o- 2 hours). Blood, withdrawn in heparinised tubes, was centrifuged at 4°C, 3000 rpm for 10 minutes and plasma stored at -20°C for pharmacokinetic analysis. Spot urine sampling was done at the clinic before 10 a.m. as second urination after wake up (with a time lapse after the first urination of 1 to 3 hours), and was concomitant to blood sampling.

Urine samples were directly frozen at -20°C for pharmacokinetic measurements. Plasma and urinary concentrations were determined by a validated HPLC coupled with mass spectrometry: the urinary method was recently validated and it was shown to be accurate and reproducible and to hold a limit of quantification and of detection of 391ng/mL and 195ng/mL, respectively. [9,10]

Retinol binding protein (uRBP) was measured on spot urines using an enzyme immunoassay kit (Arbor Assays, Michigan, USA) with a limit of detection of 4.09 ng/mL. uRBP was corrected for spot urinary creatinine (uCr, measured through automated Jaffè methods on Roche Diagnostics clinical biochemistry analysers). uRBP/uCr normality ranges were provided by the kit manufacturer: <130 µg/g (in patients aged <50 years) and <172 µg/g (in patients aged ≥50 years).

24-hour urine analysis were available (within 4 weeks) in some patients: proteinuria >80 mg and >300 mg, glycosuria >300 mg and total phosphorus excretion >1200 mg (phosphaturia * urine volume) were deemed abnormal.

Demographic, therapeutic, clinical and pharmacological characteristics were tested for correlation with uRBP/uCr levels (Spearmen’s and Mann-Whitney tests) and with abnormal uRBP/uCr (Chi-square, Fisher’s exact test or Mann-Whitney tests). A step-wise multivariate binary logistic regression was performed including age, gender, eCrCl and variables with a p value <0.20 at univariate analysis. All statistical analyses were performed with SPSS 20.0 (IBM Corporation,
Armonk, NY, USA). All data are reported as medians (interquartile ranges) and numbers (percentages).

Results

289 patients were included. Median age was 45.8 years (39.5-51.8) and median body mass index was 23.4 kg/m² (21.6-26); most of the subjects were male (207, 71.6%) and Caucasian (246, 85.4%; 9.7% African, 4.2% South American). Median CD4+ T-lymphocytes were 547 cells/uL (417-699); plasma HIV RNA was <50 copies/mL in 271 patients (93.8%). Patients were treated with NNRTI-based (155, 53.6%), PI-based (118, 40.8%) or INSTI-based (16, 5.5%) regimens; TDF had been administered for 52.4 months (24-87.1). eCrCl was 89.4 ml/min (78.6-105.9).

TFV plasma and urinary concentrations (12±2 hours after drug intake, C₁₂) were 69 ng/mL (51.5-95) and 24.4 mg/mL (14.3-37.7); urinary to plasma ratio (upR) was 384 (209-560). 12 subjects (4.2%) had plasma C₁₂ above 160 ng/mL.

uRBP and uRBP/uCr were respectively 204.6 ng/mL (92-380) and 169.7 µg/g (85.8-318.3). A significant correlation was observed between uRBP/uCr and age (rho=0.20, p=0.001), ethnicity [higher in Caucasian (183.6 mg/g) vs. other (96.4 mg/g), p=0.001], TFV urinary C₁₂ (rho=-0.17 p=0.006), TFV upR (rho=-0.20, p=0.001, Fig. 1), eCrCl (rho=-0.16, p=0.011), serum phosphate levels (rho=-0.16, p=0.010) but not with duration of TDF administration (rho=0.02, p=0.727).

Abnormally high uRBP/uCr was observed in 157 patients (54.3%). Patients with abnormal uRBP/uCr were Caucasian (58% vs. 33.3%, p=0.004) and showed lower serum phosphate levels (2.9 vs. 3.2 mg/dL, p=0.003), TFV urinary C₁₂ (20.9 vs. 27.2 mg/mL, p=0.002) and upR (338 vs. 401, p=0.004). A ROC analysis was performed showing that TFV urinary C₁₂ (p=0.002, area 0.61, 95% confidence interval, 95%CI 0.54-0.68) and upR may moderately predict uRBP/uCr abnormality (p=0.004, area 0.60, 95%CI 0.53-0.67); a urinary C₁₂ threshold of 21.0 mg/mL showed
sensitivity and specificity values of 66% and 50%. A multivariate binary logistic regression confirmed that both ethnicity (p=0.004, aOR 2.93, 95% CI 1.41-6.10) and urinary C_{12} <21 mg/mL (p=0.006, aOR 2.04, 95% CI 1.12-3.41) were significantly associated with abnormal uRBPU/Cr (Fig. 2); the results of uni- and multivariate analysis including concentrations as continuous variables are shown in supplementary table 1. Results of available 24-hour urine analysis (n=92) are shown in supplementary table 2 and 3.

In patients aged >50 years [n=90, 86 Caucasian (95.6%) median age 55 years, (range 50-75)] abnormal uRBPU/Cr was observed in 50 subjects (55.6%) and was only associated with TFV urinary C_{12} <21 mg/mL (p=0.002, OR 4.52, 95CI 1.65-12.38).

Discussion

In this cross-sectional evaluation, we found that 54.3% of TDF recipients with eGFR >60 ml/min had abnormal renal tubular function, as defined by appropriate urinary markers. [11] Due to the cross-sectional design of the study and the significant duration of TDF exposure, it can be reasonably inferred that our patients represent a selection of TDF-tolerant intakers, as patients with lesser tolerance to the drug had been switched to alternative options before the study was carried and only those with clinically acceptable values of conventional renal function parameters were included. Tubular dysfunction was found to be associated with reduced urinary output of TFV, as testified by both TFV urinary concentration and, more importantly, urinary clearance (urinary to plasma concentration ratio). As TFV clearance occurs by both glomerular filtration and tubular secretion, and that the former is thought to be unaltered when true GFR is measured, we can hypothesize that the responsibility of this reduction in TFV clearance lies in the reduced tubular secretion of the drug. According to the available knowledge, a sort of entrapment of TFV inside
tubular cells might actually explain the subclinical tubular dysfunction, with variable loss of reabsorbing capacity. [12] In our study, the reduced tubular function was also associated to lower plasma phosphate levels, which further to confirm the dysfunctional tubular status also provides a pathophysiologial link with the described reduction of BMD in patients under TDF treatment. In a subgroup of patients we found that both uRBP/UCr and reduced tenofovir clearance were associated with increased phosphate wasting and low-level proteinuria: these findings are in agreement with other studies in which plasma TFV concentrations were associated with such abnormalities, [13] as well as with the recent data by Hamzah et al that reported a lower prevalence of abnormal uRBP/uCr (22.5%) in male patients with a shorter duration of TDF administration; notably this abnormality was associated with lower BMD of the spine. [14]

It is worth noting that the presence of tubular dysfunction was also associated with age and ethnicity. While in case of age the association with reduced tubular function reasonably points toward the physiological reduction of renal function, in case of ethnicity some contribution to such association might come from variations in human genetics. [15-19] Although the observed lower prevalence of elevated urinary RBP in non-Caucasian patients may be explained by the small sample size of this subgroup, possible differences in genetic polymorphisms prevalence might be of some relevance here, as African descendants were found to have a lower prevalence of loss of function genotype in ABCC2 (previously associated with tubular dysfunction and included in an extended haplotype along with ABCC10). [20]

TAF is the novel, highly effective pro-drug that is associated with TFV plasma concentrations corresponding to 13.9% of those observed with TDF intake. [21] The data here reported, along with those from the studies comparing TDF and TAF suggest that, a sizeable proportion of TDF recipients do actually have an impaired tubular function that might chronically impact on bone structural properties. [22] It must however be admitted that TDF has been in use for more than a decade but no overt clinical evidence has been provided on the long-term consequences of chronic
TDF intake. However such an effect might become of greater clinical significance in the next years as the HIV-infected population is aging and concurrent factors may synergize with TDF-associated BMD reduction. Beyond the uncertainty still surrounding the perspective view on the association between TDF intake, tubular dysfunction and BMD reduction, some action to get rid of these potentially harmful consequences can be taken. Further to the next availability of TAF (which was found to be remarkably safe in this setting) as a suitable candidate to replace TDF, the complementary role of companion drugs should also be considered (e.g PI/r) [23], as well as the possibility of reducing TDF dose. [24]. All these data might support active screening for tubular dysfunction in HIV-positive patients and specifically in those at risk of severe bone complications such as elder subjects, post-menopausal women and patients with metabolic and cardiovascular comorbidities. In this context the next loss of TDF patent should also be considered as generic manufacturing will offer the opportunity of cost-reducing strategies.

In conclusion, the lower TFV clearance here described in TDF recipients with normal creatinine-based renal function parameters but with altered tubular function contribute to the ongoing debate on the TDF safety profile by adding the notion of a reduced capacity to clear TFV by a substantial proportion of patients who might otherwise be considered to have a normal renal function. Although the actual long-term consequences of chronic TDF retention are not yet clear, in view of the increasing complexity of HIV therapeutic management with advancing age (and related increase in co-morbidities), the opportunity to limit or avoid such chronic renal tubular injury seems actually both rationale and feasible.

Transparency Declarations

A. C. has received grants from Gilead and Bristol-Myers Squibb (BMS), travel grants and speaker’s honoraria from Abbvie, BMS, Merck Sharp & Dohme (MSD), Gilead, Janssen-Cilag and Viiv. G.D.P and S. B. has received grants, travel grants, and consultancy fees from Abbvie, Boehringer-Ingelheim, BMS, MSD, Gilead, Janssen-Cilag and Viiv. Other authors declare no potential conflict of interest.
Authors’ contribution

AC, SB and GDP designed the study. ML, AlC, TM, TL enrolled the patients and followed the study procedures. CJ, SM, LM, MG and DAA performed the plasma and urine analysis. AC and SB analysed the data and wrote the draft of the manuscript. CJ, SM, DAA and DPG reviewed the paper. All authors made substantial contributions to this manuscript and agreed on its preparation in its present form.

References


Figure and Figure Legends
Figure 1. Scatter-dot plot of tenofovir urinary to plasma ratios according to urinary retinol binding protein (corrected for urinary creatinine). Both axes are in logarithmic scale (Log$_{10}$); continuous and dotted lines respectively represent trend lines and 95% confidence intervals.
Figure 2. Bars representing the prevalence of subclinical tubular dysfunction according to ethnicity and tenofovir urinary concentrations. “TFV”, tenofovir; “urinary C12”, 12 hour urinary concentration (mg/mL); “uRBP/uCr”, urinary retinol binding protein/urinary creatinine. Tubular function was categorized using age-defined uRBP/uCr thresholds (<130 µg/g in patients aged <50 years and <172 µg/g in patients aged ≥50 years).