Renal Toxicity of Concomitant Exposure to Tenofovir and Inhibitors of Tenofovir's Renal Efflux Transporters in Patients Infected With HIV Type 1

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Background. Exposure to tenofovir disoproxil fumarate (TDF) may cause renal toxicity. Inhibitors of TDF's apical multidrug-resistance–associated protein efflux–transporters (MRPs) in the renal proximal tubule could enhance this unwanted effect.

Methods. We performed a cohort study involving patients with human immunodeficiency virus type 1 (HIV) infection. All patients had a suppressed viral load and were receiving TDF as a part of combination antiretroviral therapy. Data on mean cumulative defined daily doses (DDDs) of MRP inhibitors (NSAIDs, PDE5-i, salicylates, dipyridamole) were collected. The effects of MRP inhibitors on the estimated glomerular filtration rate (eGFR) and proximal tubular function were evaluated by generalized linear models, with adjustment for renal- and HIV-specific factors.

Results. A total of 721 HIV-infected patients were included (76.3% were male; median age, 45 years; median $CD4^+$ T-cell count, 600 cells/mm^3). The median duration of TDF exposure was 54 months, and the total cumulative exposure duration was 3484 patient-years. Three hundred twenty-one patients had MRP inhibitor exposure, ranging from 0.02 to 120 mean DDDs/month. Exposure to MRP inhibitors was associated with an additional mean eGFR change of −1.4 mL/min (95% confidence interval [CI], −2.9 to .1 mL/min) over 12 months in patients with ≥1 year of continuous TDF exposure. Associations were observed between MRP inhibitor exposure and eGFR declines of >10 mL/min (odds ratio [OR], 1.38; 95% CI, .97 to 1.95), or >25% (OR, 2.14; 95% CI, 1.19 to 3.85) since initiation of TDF therapy. Overall, no clinically significant associations were found between MRP inhibitor exposure and abnormal protein, glucose, or phosphate handling in the proximal tubule or with the presence of ≥2 of these markers.

Conclusions. Concomitant incidental exposure to MRP inhibitors and TDF did not result in major additional TDF-related renal toxicity in HIV-infected patients.

Keywords. HIV; cART; tenofovir disoproxil fumarate; drug transporters; renal toxicity.

Tenofovir disoproxil fumarate (TDF) is recommended as nucleoside reverse transcriptase inhibitor (NRTI) for treatment of human immunodeficiency virus type 1 (HIV) infection in combination antiretroviral therapy (cART) [1, 2]. Prolonged TDF exposure in clinical practice has been associated with renal impairment, characterized by glomerular filtration rate (GFR) decline and proximal tubular dysfunction [3]. Use of boosted protease inhibitors (PIs) instead of nonnucleoside reverse transcriptase inhibitors in cART increases TDF's renal toxicity [4].

The pharmacokinetic profile of the prodrug TDF includes conversion to tenofovir and excretion by glomerular filtration or proximal tubular secretion. Renal injury is probably related to intracellular tenofovir accumulation in proximal tubule cells, which results in mitochondrial DNA depletion and cytotoxicity [5]. The organic anion transporter 1 enables tenofovir influx at the basolateral side of tubular epithelial cells. Apical

multidrug-resistance–associated protein 2 (MRP-2) and, predominantly, MRP-4 regulate tenofovir's active secretion in pre-urine. Mutations in the genes ABCC2/4 (encoding MRP-2/4) can impair tenofovir's transport [6] and may contribute to renal impairment in vivo [7–9].

Nonsteroidal antiinflammatory drugs (NSAIDs), anticoagulants, and erectile-dysfunction drugs can also inhibit tenofovir's primary efflux transporter MRP-4 (and often MRP-2) in vitro [10–13]. The capacity of these frequently prescribed drugs to inhibit MRP (as defined by the half maximal inhibitory concentration [IC_{50}]) is concentration dependent. Salicylates have the highest IC_{50}, whereas the IC_{50} values of others are considerably lower. The potential interaction of MRP inhibitors with TDF has never been systematically studied in vivo, and whether concomitant exposure causes additional renal toxicity is unknown. The principle aim of this study was to evaluate whether the concurrent use of TDF and MRP inhibitors is associated with additional GFR decline or with proximal tubular dysfunction in HIV-infected patients receiving TDF-containing cART.

METHODS

Study Design

This was a cohort study, performed at the Erasmus University Medical Center, Rotterdam, the Netherlands, that involved adult HIV-infected patients receiving TDF–containing cART. Patients visited their HIV physician at least once per 6 months and were recruited between 1 February and 1 September 2014. Activities at visits included measurement of serum creatinine, phosphate, glucose, and HIV RNA levels and spot testing of urine with dipstick urinalysis to evaluate glycosuria and to quantify creatinine, total protein, albumin, and phosphate levels. All patients provided written informed consent. The study was approved by the institutional ethical board, conducted in accordance with good clinical practice, and registered (clinical trials registration NTR4618; available at: http://www.trialregister.nl).

Exposure to physician-prescribed and over-the-counter (OTC) NSAIDs (ie, ibuprofen, diclofenac, naproxen, celecoxib, etoricoxib, indomethacin, meloxicam, and aspirin), salicylates (for cardiovascular disease [CVD] prevention), dipyridamole, and phosphodiesterase-5 inhibitors [PDE5-i], ie, sildenafil, tadalafil, and vardenafil) over 6 months was determined using structured patient interviews conducted by the researchers. Anthropometric, demographic, clinical characteristics and adherence data were collected. The exposure to potential nephrotoxic drugs (ie, acyclovir, trimethoprim-sulfamethoxazole, angiotensin-converting-enzyme inhibitors [ACEi], and angiotensin-receptor blockers [ARB]) was extracted from the patients' electronic medical files. Clinical data included cART history, HIV RNA levels, CD4+ T-cell counts, HIV transmission route, and TDF treatment duration. We measured serum

creatinine levels (in μmol/L, divided by 88.4 equals mg/dL) at inclusion, at TDF initiation, and 12 months (±3 months) prior to study inclusion. Comorbidity data collected were history of hypertension (or a blood pressure of >150/100 mm Hg at study inclusion), history of diabetes (or a glucose level of >11.0 mmol/L at inclusion), and hepatitis C virus infection. Previous macrovascular complications, angina pectoris, or heart failure defined CVD. Chronic kidney disease (CKD) at inclusion was categorized on the basis of the Kidney Disease: Improving Global Outcomes (KDIGO) guidelines, as follows: (1) an eGFR of >60 mL/min and a ratio of urinary albumin level to creatinine level (ACR) of <3 mg/mmol (low risk); (2) either an eGFR of 45–59 mL/min without an ACR of >3 mg/mmol or an eGFR of >60 mL/min with an ACR of 3–30 mg/mmol (moderate risk); (3) an eGFR of 30–44 mL/min with an ACR of <3 mg/mmol, an eGFR of 45–59 mL/min with an ACR 30–300 mg/mmol, or an eGFR of >60 mL/min with an ACR of >300 mg/mmol (high risk); and (4) all other combinations of eGFR and ACR values (very high risk) [14]. The KDIGO guidelines on acute kidney injury (AKI) were used to evaluate whether patients would meet the criteria for possible AKI at study inclusion, compared with their kidney function 12 months earlier (assumed to represent the baseline eGFR) [15]. The Data Collection on Adverse Events of Anti-HIV Drugs (D:A:D) risk score was used to categorize patients with a low (<0 points), medium (0–4), or high (≥5) risk of CKD [16].

We calculated the mean monthly cumulative defined daily doses (DDDs) of OTC and physician-prescribed MRP inhibitors according to World Health Organization guidelines [17]. This value was used to categorize patients on the basis of increasing MRP exposure into 4 quartiles of comparable sizes. All GFRs were estimated from the serum creatinine level, according to the CKD Epidemiology Collaboration (CKD-EPI) formula. The CKD-EPI formula is recommended for HIV patients by the Infectious Diseases Society of America [18, 19]. The urine ACR, the ratio of protein level to creatinine level (PCR), the ratio of albumin level to protein level (APR), the fractional excretion of phosphate (FEPO), and the tubular maximum reabsorption of phosphate per liter of GFR (TmPO4/GFR) were calculated. Hypophosphatemia, increased FEPO, decreased TmPO4/GFR, glycosuria without hyperglycemia, and tubular proteinuria defined tubular dysfunction. The presence of ≥2 markers defined proximal tubulopathy [19].

Hypophosphatemia was defined as a serum phosphate concentration of <0.8 mmol/L (mmol/L divided by 0.334 equals mg/dL). The FEPO was considered abnormal if >20% or, in hypophosphatemic patients, >10%. A TmPO4/GFR of <0.8 mmol/L was considered abnormal. If the tubular reabsorption of phosphate (TRP) was ≤0.86, TmPO4/GFR was calculated by multiplying the serum phosphate level by the TRP; and if the TRP was >0.86, TmPO4/GFR was calculated by multiplying the serum phosphate level by the result of the following

equation: $[0.3 \times TRP]/[1 - (0.8 \times TRP)]$ [20]. The ACR was categorized as normal (<3 mg/mmol), moderately increased (3–30 mg/mmol), or severely increased (if >30 mg/mmol). A PCR of <15 mg/mmol was considered normal. Tubular proteinuria was defined as a urine APR of <0.4, provided that the PCR was ≥20 mg/mmol [19, 21].

Statistical Analysis

The primary outcome was the eGFR decline over 12 months. Secondary outcomes were an eGFR decrease of >10 mL/min, an eGFR decrease of >25%, and the overall eGFR decline since TDF initiation; the presence of proximal tubular dysfunction; and potential AKI at study inclusion. These outcomes were analyzed in patients with ≥12 months of continuous TDF exposure at the time of study inclusion. Comparisons were made between patients who had or had not been exposed to MRP inhibitors and between patients in the highest quartile of exposure and those without exposure. The evaluation of the following 2 subgroups was included in the protocol: an analysis of proximal tubule function in patients with <12 months of continuous TDF exposure at inclusion and an analysis of patients with NSAID exposure only. All outcomes were analyzed in patients with HIV suppression (defined as an HIV RNA load of <500 copies/mL), to minimize the influence of HIV replication on renal toxicity [22].

Baseline data are reported as medians with interquartile ranges (IQRs) or as numbers of patients with percentages. The relationship of MRP inhibitor exposure with mean eGFR changes over time was assessed by independent t tests, and the continuous markers of proximal tubulopathy (ie, PCR, APR, and FEPO) and median MRP inhibitor exposure were evaluated with Wilcoxon rank sum tests. χ^2 tests were performed to assess associations between exposure groups and eGFR declines of >10 mL/min since TDF initiation, eGFR declines of >25% since TDF initiation, and markers of proximal tubulopathy.

Multivariable generalized linear models were constructed for an adjusted analysis of the effect of MRP inhibitor exposure on eGFR decline over 12 months and to calculate adjusted odds ratios (ORs) with 95% confidence intervals (CIs) for an eGFR decline of >10 mL/min since TDF initiation, an eGFR decline of >25% since TDF initiation, and markers of proximal tubulopathy. The models were corrected for age, sex, ethnicity (African or other), HIV transmission route (male-male sex, injection drug use, and other), comorbidities, cART (PI and other), nephrotoxic medication, weight, CD4+ T-cell count, KDIGO CKD risk group, duration of TDF use, and baseline eGFR. A P value <.05 was considered statistically significant.

RESULTS

Baseline Characteristics

Of 893 eligible HIV-infected patients receiving TDF-containing cART, 731 (81.9%) consented to participation. The majority (n = 721) had a suppressed HIV RNA load (defined as <500 copies/mL) and contributed to 3484 patient-years of TDF exposure. Their characteristics at study inclusion are shown in Table 1.

A total of 627 patients had been continuously exposed to TDF for least 12 months at the time of inclusion. These participants were predominantly males (76.9%) with HIV transmission through male-male sexual contact (56.5%). The median age was 46 years. Patients were mostly of non-African origin (78.6%) and had a high CD4+ T-cell count (median, 620 cells/mm³). Patients had received TDF-containing cART for a median of 62 months, contributing to 3434 patient-years of cumulative exposure. The median D:A:D CKD risk score at TDF initiation was −1. Most patients had an eGFR of >90 mL/min (median, 93 mL/min), without albuminuria (median ACR, 0.7 mg/mmol). The KDIGO CKD classification at inclusion was low or moderate in 99.4% of patients. Four males, aged 54–66 years, had high KDIGO CKD classifications. Two of these 4 patients did not have comorbidities and were not using nephrotoxic drugs, 1 had diabetes mellitus, and 1 had hypertension and a membranous glomerulopathy for which an ARB was used. Eighty-four patients (13.4%) had a PCR of >20 mg/mmol (median, 28.8 mg/mmol; IQR, 20.8–45.2 mg/mmol), including 2 patients with a PCR of >200 mg/mmol (361.7 and 1220.0 mg/mmol). A total of 64 of these 84 patients (76.2%) had an APR of <0.4 (median, 0.17; IQR, 0.08–0.26).

Two hundred eighty-six patients with a minimum of 12 months of TDF exposure had been exposed to MRP inhibitors. The mean monthly cumulative total MRP inhibitor exposure ranged from 0.02 to 120.0 DDDs/month (median, 1.4 DDDs/month; IQR, 0.3–5.7 DDDs/month). The range within the quartiles were 0.02–0.3 DDDs/month for quartile 1 (n = 73), 0.4–1.3 DDDs/month for quartile 2 (n = 69), 1.4–5.6 DDDs/month for quartile 3 (n = 73), and 6.2–120.0 DDDs/month for quartile 4 (n = 71). The median exposure to MRP inhibitors was 30.0 DDDs/month (IQR, 10.0–33.1 DDDs/month) in the highest quartile. Patients with any MRP inhibitor exposure had received TDF for a median of 55 months, and patients without exposure had received TDF for a median of 65 months (P = .016). Patients at highest exposure and patients without exposure had received TDF for a comparable median duration (69 vs 65 months; P = .794).

At least 1 NSAID was used by 202 patients (median, 0.5 DDDs/month; IQR, 0.2–2.0 DDDs/month). This exposure consisted predominantly of diclofenac (n = 51; median, 1.0 DDDs/month; IQR, 0.2–2.4 DDDs/month) or ibuprofen (n = 141; median, 0.4 DDDs/month; IQR, 0.2–1.0 DDDs/month). Thirty-eight patients used other NSAIDs (median, 2.5 DDDs/month; IQR, 0.3 to 17.5 DDDs/month). PDE5-i was used by 116 patients (94.0% received sildenafil; median, 2.0 DDDs/month; IQR, 0.7–4.0 DDDs/month). Twenty-eight patients used salicylates (71.4% received acetylsalicylic acid 80 mg) as CVD prophylaxis, including 3 patients who were also using dipyridamole. All

Table 1. Characteristics of Patients With Human Immunodeficiency Virus Type 1 (HIV) Suppressed on Tenofovir Disoproxil Fumarate (TDF)–Containing Combination Antiretroviral Therapy

Characteristic	Overall (n = 721)	With MRP Inhibitor Exposure (n = 321)	Without MRP Inhibitor Exposure (n = 400)
Male sex	550 (76.3)	255 (79.4)	295 (73.8)
Age, y	45 (38–53)	46 (38–53)	45 (37–45)
Ethnicity			
White	434 (60.2)	218 (67.9)	216 (54.0)
African or African descent	161 (22.3)	51 (15.9)	110 (27.5)
Other	126 (17.5)	52 (16.2)	74 (18.5)
HIV transmission route			
Male-male sex	407 (56.4)	210 (65.4)	197 (49.3)
Heterosexual sex	254 (35.2)	82 (25.5)	172 (43.0)
Injection drug use	20 (2.8)	11 (3.4)	9 (2.3)
Other	5 (0.7)	3 (0.9)	2 (0.4)
Unknown	35 (4.9)	15 (4.8)	20 (5.0)
CD4$^+$ T-cell count, cells/mm^3	600 (460–780)	600 (460–815)	610 (460–750)
TDF-containing regimen			
NNRTI based	634 (87.9)	278 (86.7)	356 (89.0)
BPI based	83 (11.5)	42 (13.1)	41 (10.3)
Other	4 (0.6)	1 (0.2)	3 (0.7)
TDF exposure duration			
>1 y	627 (87.0)	286 (89.1)	341 (85.2)
Overall, mo	54 (21–87)	49 (21–81)	(58) (22–90)
Comorbidity			
Hypertension	85 (11.8)	33 (10.3)	52 (13.0)
Diabetes mellitus	32 (4.4)	11 (3.4)	21 (5.3)
Hepatitis C	28 (3.9)	17 (5.3)	11 (2.8)
Cardiovascular disease	51 (7.1)	32 (10.0)	19 (4.8)
Renal-interacting comedication			
TMP-SMZ	9 (1.2)	3 (0.9)	6 (1.5)
ACEi or ARB	52 (7.2)	23 (7.2)	29 (7.3)
Acyclovir	16 (2.2)	9 (2.8)	7 (1.8)
D:A:D CKD risk score	−1 (−2 to 1)	−1 (−2 to 1)	−1 (−2 to 1)
KDIGO CKD risk classification			
Low	581 (80.6)	255 (79.4)	326 (81.5)
Moderate	117 (16.2)	54 (16.8)	63 (15.8)
High	4 (0.6)	2 (0.6)	2 (0.5)
Insufficient information	19 (2.6)	10 (3.2)	9 (2.2)
eGFR, mL/min[a]	94 (82–106)	90 (78–103)	97 (84–109)

Data are no. (%) of patients or median value (interquartile range).

Abbreviations: ACEi, angiotensin-converting-enzyme inhibitor; ARB, angiotensin-receptor blocker; BPI, boosted protease inhibitor; CKD, chronic kidney disease; D:A:D, Data Collection on Adverse Events of Anti-HIV Drugs; KDIGO, Kidney Disease: Improving Global Outcomes; MRP, multidrug resistance protein; NNRTI, nonnucleoside reverse transcriptase inhibitor; TMP-SMZ, trimethoprim-sulfamethoxazole.

[a] Estimated glomerular filtration rate (eGFR) was calculated using the CKD-Epidemiology Collaboration formula.

patients using salicylates (30 DDDs/month) alone or in combination with dipyridamole (60 DDDs/month) were in the highest quartile. For the 4 patients with high KDIGO CKD classifications, only exposure to sildenafil (4.0 DDDs/month in 2) but not to NSAIDs or anticoagulants was observed.

Data from patients without complete quantitative urinalysis (n = 19) were not used for related interferential statistics. Twelve

of these 19 patients had negative results of urine dipstick analyses of protein and glucose. The remaining 7 patients did not undergo urinalysis and had eGFRs of 86–126 mL/min.

eGFR Decline

Exposure to MRP inhibitors did not have a major effect on the eGFR decline in patients with at least 1 year of continuous TDF

exposure at inclusion (Figure 1). Overall, eGFR changed by a mean of −1.5 mL/min (95% CI, −2.3 to −.8 mL/min) over the previous year. The kidney status in 6 patients would be classified as AKI stage 1 (≥26.5 μmol/L serum creatinine level increase), on the assumption that their creatinine level had remained stable from the time it was last measured until just prior to the observed increase in the level at inclusion. Their creatinine level increase ranged from 29 μmol/L to 54 μmol/L.
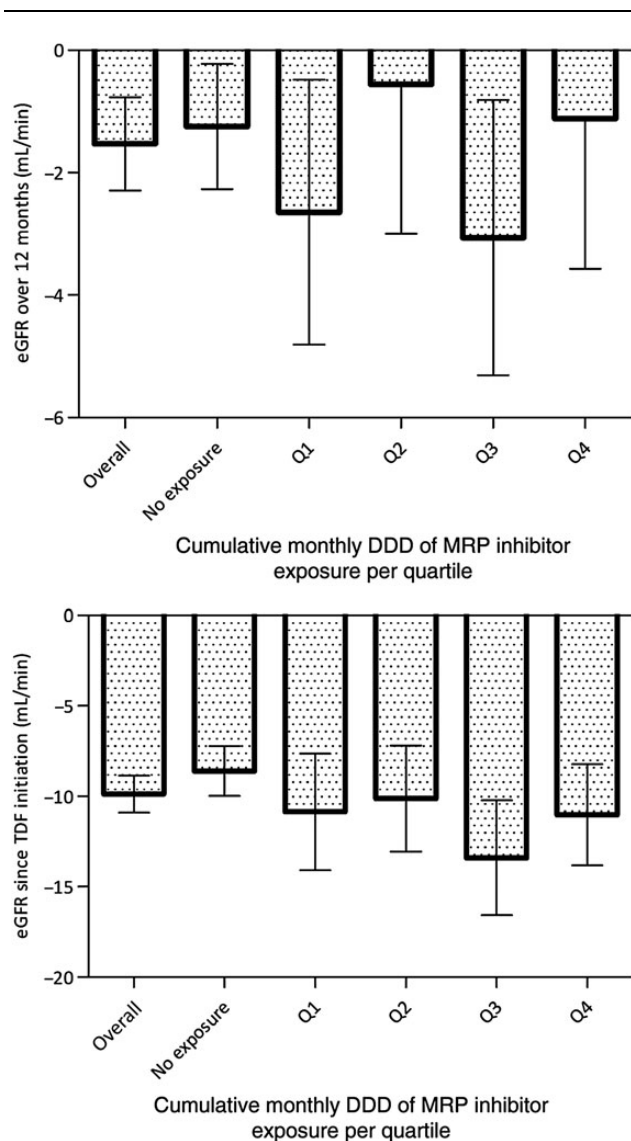
Figure 1. Bars represent the mean estimated glomerular filtration rate (eGFR) decline (with 95% confidence intervals) over 12 months and since initiation of tenofovir disoproxil fumarate (TDF) therapy. The eGFR decline for all patients and per category of cumulative monthly defined daily doses (DDDs) of multidrug resistance protein transporter (MRP) inhibitor exposure are shown. GFRs were estimated according to the Epidemiology Collaboration formula. Quartile ranges are 0.02–0.3 DDDs (quartile 1 [Q1]; n = 73), 0.4–1.3 DDDs (Q2; n = 69), 1.4–5.6 DDDs (Q3; n = 73), and 6.2–120.0 DDDs (Q4; n = 71).

Four of these 6 patients had used MRP inhibitors (0.03–45.0 DDDs/month). The mean decreases in eGFR were comparable between patients without exposure (change, −1.2 mL/min) and those with any MRP inhibitor exposure (change, −1.9 mL/min; $P = .424$) or those in the highest quartile of MRP inhibitor exposure (change, −1.1 mL/min; $P = .919$). The mean eGFR decline since TDF initiation was higher for patients with any exposure, compared with patients without exposure (change, −11.4 vs −8.6 mL/min; $P = .008$). Of all patients with any MRP inhibitor exposure, 49.7% had eGFR declines of >10 mL/min (compared with 42.8% without exposure), and 13.3% had a >25% decline in the eGFR since TDF initiation (compared with 8.2%). TDF treatment was discontinued owing to renal impairment in 17 of 66 patients with eGFR declines of >25%; the median MRP inhibitor exposure tended to be higher in these 17 patients (2.6 vs 0.03 DDDs/month; $P = .055$).

After multivariable adjustment, exposure to MRP inhibitors was not significantly associated with an additional eGFR decline over the previous 12 months (change, −1.4 mL/min; 95% CI, −2.9 to 0.1 mL/min; $P = .067$). Associations between MRP inhibitor exposure versus no exposure and eGFR declines of >10 mL/min (OR, 1.38; 95% CI, .97 to 1.95; $P = .074$) or >25% (OR, 2.14; 95% CI, 1.19 to 3.85; $P = .011$) since TDF initiation were found. Notably, these associations were predominantly driven by patients in quartile 3. For these patients, exposure to MRP inhibitors had an effect on eGFR decline (change, −2.3 mL/min; 95% CI, −4.6 to .0; $P = .053$) and increased the adjusted ORs for eGFR declines of >10 mL/min (2.06; 95% CI, 1.18 to 3.60; $P = .011$) and >25% (2.88; 95% CI, 1.29 to 6.42; $P = .010$) since TDF initiation.

Tubular Dysfunction

No major effects of MRP inhibitor exposure on the markers of proximal tubular dysfunction were observed in patients who had received TDF for at least 12 months at inclusion. These results are shown in Table 2. Overall, the median proteinuria level was 110 mg/L, and the median PCR was 9.5 mg/mmol. The median PCR was not different between patients without exposure to MRP inhibitors (9.9 mg/mmol) and patients with any (9.1 mg/mmol; $P = .094$) or the highest (9.0 mg/mmol; $P = .710$) exposure to MRP inhibitors. The patients without exposure and those with the highest exposure also had comparable median APRs (0.25 and 0.16, respectively; $P = .362$) and frequencies of an APR of <0.4 (72.9% and 78.6%, respectively; $P = .671$), both of which were assessed when the PCR was >20 mg/mmol. Multivariable adjusted models showed no increased OR yielded by tubular proteinuria for MRP inhibitor exposure overall (0.76; 95% CI, .37 to 1.56; $P = .451$) or for the highest quartile, compared with no exposure. Patients at highest exposure to MRP inhibitors had comparable rates of hypophosphatemia as compared to those without exposure. There were no significant differences in median FEPO (12.3% and 15.1%, respectively;

Table 2. Markers of Proximal Tubule Toxicity in 627 Patients With Human Immunodeficiency Virus Type 1 (HIV) Suppression During Tenofovir Disoproxil Fumarate (TDF)–Containing Combination Antiretroviral Therapy, Without and With Exposure to Inhibitors of Renal Multidrug Resistance Protein Transporters

Marker	No Exposure (n = 341)	Quartile 1 (n = 73)	Quartile 2 (n = 69)	Quartile 3 (n = 73)	Quartile 4 (n = 71)
Urine protein dipstick negative	312 (91.5)	69 (94.5)	64 (92.8)	64 (87.7)	62 (87.3)
Proteinuria level, mg/L	110 (60–175)	100 (60–160)	110 (70–153)	115 (70–203)	140 (60–273)
Urine ACR, mg/mmol	0.8 (0.4–1.8)	0.6 (0.3–1.9)	0.7 (0.4–2.2)	0.8 (0.4–1.5)	0.7 (0.4–2.8)
Urine PCR, mg/mmol	9.9 (7.2–14.5)	8.2 (6.6–12.2)	9.6 (7.0–12.9)	9.9 (7.1–13.9)	9.0 (7.4–16.2)
Urine PCR >15 mg/mmol	76 (22.3)	13 (17.8)	12 (17.4)	15 (20.5)	19 (26.8)
Urine APR[a]	0.25 (0.14–0.49)	0.15 (0.06–0.42)	0.22 (0.07–0.52)	0.22 (0.09–0.32)	0.16 (0.11–0.37)
FEPO, %	12.3 (8.0–17.3)	11.6 (7.8–16.8)	11.7 (7.4–17.2)	14.4 (9.6–21.4)	15.1 (10.2–20.0)
Hypophosphatemia	76 (22.3)	9 (12.3)	13 (18.8)	14 (19.2)	16 (22.5)
Abnormal FEPO	73 (21.4)	15 (20.5)	14 (20.3)	26 (35.6)	24 (33.8)
TmP/GFR <0.8 mmol/L	123 (36.1)	21 (28.8)	19 (27.5)	29 (39.7)	30 (42.3)
Normoglycemic glycosuria	9 (2.6%)	0 (0.0)	1 (1.5)	0 (0.0)	0 (0.0)

Data are no. (%) of patients or median value (interquartile range).

Abbreviations: ACR, albumin level to creatinine level ratio; FEPO, fractional excretion of phosphate; IQR, interquartile range; PCR, protein level to creatinine level ratio; TmP/GFR, renal tubular maximum reabsorption of phosphate per liter of GFR.

[a] The albumin level to protein level ratio (APR) in urine was calculated in patients with a PCR of > 20 mg/mmol.

$P = .083$) or frequencies of decreased TmPO4/GFR ($P = .327$). The proportion of patients with an abnormal FEPO was highest for patients in the third and fourth quartile of MRP inhibitor exposure. No increased multivariable adjusted OR associated with an abnormal FEPO was observed with overall MRP inhibitor exposure (OR, 1.24; 95% CI, .82 to 1.87; $P = .303$), although the OR associated with an abnormal FEPO was significantly increased (2.00; 95% CI, 1.13 to 3.87; $P = .019$) for patients in the third quartile. Glycosuria was rarely observed, occurring in 10 of 611 normoglycemic individuals, and no relationship with exposure to MRP inhibitors was noted ($P = .211$).

Thirty-nine patients had at least 2 markers of proximal tubulopathy. These included 21 patients (6.1%) without exposure and 9 patients (12.7%) at highest exposure to MRP inhibitors ($P = .054$). Multivariable analysis found no significant association between MRP inhibitor exposure overall (OR, 0.65, 95% CI, .26 to 1.61; $P = .350$) or per quartile and the presence of at least 2 markers of proximal tubulopathy. TDF was discontinued in 14 of 39 patients with at least 2 markers of proximal tubulopathy; median MRP inhibitor exposure duration was not significantly higher in these 14 patients (0.47 vs 0.0 DDDs/month; $P = .239$).

Subgroup Analysis

Ninety-four patients had <1 year of continuous TDF exposure at inclusion, including 35 patients with MRP inhibitor exposure. Their cumulative exposure ranged from 0.06 to 30.5 DDDs/month (median, 1.1 DDDs/month; IQR, 0.1–4.0 DDDs/month). Three of 94 patients had a PCR of >20 mg/mmol (median, 7.9 mg/mmol; IQR, 6.3–11.1 mg/mmol), including 1 patient with an APR of <0.4. Two patients had normoglycemic

glycosuria, and a comparable proportion of patients with and without exposure (20.0% and 20.3%, respectively) had an abnormal FEPO. The presence of 2 markers of proximal tubulopathy was apparent in 2 patients.

The 202 patients with NSAID exposure were categorized into 4 quartiles. The median exposure per quartile was 0.1 DDDs/month (IQR, 0.06–0.1 DDDs/month) for quartile 1, 0.25 DDDs/month (IQR, 0.22–0.33 DDDs/month) for quartile 2, 0.83 DDDs/month (IQR, 0.6–1.7 DDDs/month) for quartile 3, and 5.0 DDDs/month (IQR, 2.6–16.3 DDDs/month) for quartile 4. Patients at highest exposure had a mean additional eGFR change of −2.3 mL/min (95% CI, −4.9 to .2; $P = .076$) in the previous year and an increased OR (2.81; 95% CI, 1.21 to 6.49; $P = .016$) associated with a >25% eGFR decline, compared with patients without exposure. No association of patients with the highest NSAID exposure and the presence of tubular proteinuria ($P = .262$) or abnormal FEPO ($P = .675$) were observed. Patients with the most exposure to NSAIDs had an OR of 3.78 (95% CI, 1.00 to 14.31; $P = .05$) associated with the presence of at least 2 markers of proximal tubular dysfunction.

Diclofenac exposure in 51 patients was evaluated separately because diclofenac is the most potent MRP inhibitor and widely used. Median diclofenac exposure in those patients was 1.0 DDDs/month (range, 0.02–90.0 DDDs/month). Exposure to diclofenac was not associated with a significant eGFR decline in the previous year of TDF exposure (change, −2.2 mL/min; 95% CI, −4.9 to .5; $P = .114$), although it was associated with an increased OR (3.69; 95% CI, 1.63 to 8.36; $P = .002$) in association with a >25% eGFR decline since TDF initiation, compared with those without exposure. No significant associations between diclofenac exposure and tubular proteinuria ($P = .574$), abnormal

FEPO (*P* = .548), or at least 2 markers of proximal tubular dysfunction (*P* = .743) were observed.

One additional sensitivity analysis was performed because of the unexpected large contribution of low-dose salicylates (associated with the highest IC_{50} in vitro) to the highest quartile of total MRP inhibitor exposure. This analysis showed that, when salicylates were not used to calculate cumulative total MRP inhibitor exposure, the patients in the highest quartile had an additional annual eGFR decline of −2.7 mL/min (95% CI, −5.1 to −.4; *P* = .024) after multivariable adjustment. No associations with markers of tubular dysfunction were observed in this analysis.

DISCUSSION

This study evaluated whether frequently used and often freely available drugs that are known to inhibit tubular MRP in vitro may increase TDF-related renal toxicity in vivo. Our results do not indicate major clinically relevant additional TDF-related renal tubular injury or additional eGFR decline in the previous year due to the concomitant exposure to MRP inhibitors and TDF. However, this conclusion can primarily be made for patients with a suppressed HIV load who received TDF-containing cART for >12 months and had incidental low-dose exposure to inhibitors of MRP. Although higher exposure to MRP inhibitors was not associated with proximal tubular dysfunction or eGFR decline over the previous 12 months, we observed consistent associations between eGFR declines since TDF initiation and higher exposure to MRP inhibitors. These observations were supported by the NSAID and diclofenac subgroup analyses. This indicates that, although MRP inhibitors do not promote TDF-related tubular nephrotoxicity, they can independently accelerate CKD progression by decreasing the eGFR in patients infected with HIV. Chronic, high-dose MRP inhibitor exposure in HIV-infected patients receiving TDF-containing regimens, especially diclofenac, warrants close eGFR monitoring.

This is the first study to evaluate the renal toxicity of prescribed and OTC MRP inhibitors in HIV-infected patients receiving TDF. Large cohorts have identified patient- and HIV-related predictors for eGFR declines during TDF-containing cART [3, 4, 23]. However, MRP inhibitors were not evaluated in these studies and may have been an important confounder. Only 1 small retrospective case series described a high frequency (14.6%) of renal injury following physician-prescribed diclofenac [24]. This study did not evaluate other MRP inhibitors, did not state the HIV RNA suppression rate, and did not correct for measured covariates, and it lacked a control group of individuals receiving TDF without diclofenac. This hinders the interpretation of this small study.

Importantly, our observed annual eGFR decline and tubular injury frequency were smaller than described in the studies mentioned above. It is possible that the much longer duration of TDF exposure in our cohort (62 months), compared with that in other cohorts (median, ≤12 months) and case series (38 months), is attributable to selection bias. Patients with obvious TDF-related renal toxicity probably discontinued TDF prior to the start of our study. These patients likely included those at highest risk for TDF-related renal toxicity, comprising patients with high D:A:D risk scores or unfavorable ABCC2/4 polymorphisms. The effects of TDF and MRP inhibitors are possibly increased in these populations [16]. Moreover, only a minority received cART with a PI backbone. Therefore, our reassuring results cannot be extrapolated to patients initiating TDF or those receiving short-term TDF-containing cART, especially regimens with a PI backbone. The relationship between MRP inhibitors and renal impairment in these populations can only be evaluated in randomized clinical trials or cohorts with adequate registration of prescribed and OTC medicines.

This study has limitations. The study was designed to compare patients in the highest quartile to those without MRP inhibitor exposure. Recall bias might have influenced calculated MRP inhibitor exposure. Also, patients in the highest quartile of MRP inhibitor exposure had an unexpected relatively large contribution of salicylate exposure. Low-dose salicylates have much lower potency for MRP inhibition than NSAIDs in vitro and, possibly, in vivo [10]. The absent relationship of MRP inhibitor exposure in the highest quartile was especially surprising since some statistically significant effects of this factor on eGFR decline were observed for patients in the third quartile. Omitting low-dose salicylates from the calculation of total MRP inhibition showed that the highest quartile (not the third-highest quartile) was associated with additional eGFR decline. This may indicate that the inhibitory potency of low-dose salicylates is not of clinical relevance. The relatively small number of patients with high exposure to potent MRP inhibitors (such as diclofenac) still prevents firm conclusions. Also, the relationship between MRP inhibitor exposure and eGFR decline might also be explained by NSAID-related reduced glomerular blood flow, rather than TDF toxicity. Underlying medical conditions in HIV-infected patients warranting NSAID or PDE5-i exposure (eg, rheumatoid arthritis) may also result in renal injury promoting eGFR decline. Moreover, the relative effect of MRP inhibitors on intracellular TDF accumulation in vivo is probably not only a function of whether patients were sufficiently exposed to MRP inhibitors, but is also related to intracellular accumulation of MRP inhibitors (potentially altered by drugs or genetic variations) [25]. Furthermore, the clinical significance of the small FEPO changes remains unclear. Common conditions (eg, hypovitaminosis D) and sample collection regardless of fasting state may have influenced the interpretation of tubular dysfunction. Impaired reabsorption of other solutes in the proximal tubulus (eg, low-molecular-weight proteins, uric acid, and bicarbonate) is not observed during routine care and could have influenced the interpretation of possible proximal tubular

dysfunction. However, the presence of phosphaturia despite hypophosphatemia and normoglycemic glycosuria is particularly specific for proximal tubulopathy, were measured in this study, and used in routine care. Together, a separate evaluation of these factors by prospective collection of data on exposure to different MRP inhibitors in larger patient cohorts would provide more-specific conclusions. The inadequacy of the data collection on potential exposure to OTC NSAIDs and other MRP inhibitors in current HIV cohorts, however, makes this evaluation impossible.

In conclusion, the renal effects of TDF will continue to be relevant, especially since more patients worldwide will initiate first-line cART, including TDF. Together, the results of this study do not provide evidence for major additional TDF-related renal toxicity due to the incidental concomitant exposure to frequently used drugs that inhibit MRP in a population of individuals with HIV suppression who are receiving long-term TDF-containing cART.

Notes

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