Inosine 5'-triphosphatase activity is associated with TDF-associated nephrotoxicity in HIV.

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ABSTRACT

Objectives

Nucleotide reverse transcriptase inhibitors play a pivotal role in HIV-treatment. The enzyme Inosine 5'-triphosphatase (ITPase) is involved in the nucleotide metabolism and has been associated with adverse drug events. We studied the association between ITPase-activity and tenofovir disoproxil fumarate (TDF)-associated nephrotoxicity.

Design

Single center 1:2 case control cohort study, including suppressed HIV-infected patients with (cases) and without (controls) TDF-associated nephrotoxicity.

Methods

26 cases (eGFR-decline >25% and/or ≥2 proximal tubular dysfunction (PTD)-markers during TDF use) were matched to 55 controls. ITPase-activity and *ITPA* genotype were measured in all patients. The primary endpoint was the proportion of patients with normal ITPase-activity (≥4 mmol IMP/mml Hb/hour) in cases versus controls. The eGFR-improvement 48 weeks after TDF-cessation was measured in cases. McNemar's test, conditional logistic regression, and paired T-tests were used.

Results

The eGFR in cases and controls at TDF-discontinuation was 78 and 85 ml/min. 19/26 cases (73.1%) versus 28/55 controls (50.9%) had normal ITPase activity, p=0.001 (OR 2.55, 95% CI 0.89-7.31, p=0.08). 23/26 cases (88.5%) versus 40/55 controls (72.7%) had wt/wt *ITPA* genotype, p=0.26 (OR 2.59, 95%CI 0.70-9.54, p=0.15). After TDF-cessation, the eGFR increased in cases with normal ITPase activity (-5.5 to +4.4 ml/min/year, p=0.008), but remained stable in cases with reduced activity (-4.3 to -4.0, p=0.97). In cases with wt/wt *ITPA* genotype, eGFR increased from –5.0 to +3.0 ml/min/year, p=0.021. 13/16 cases with PTD had normal ITPase activity. Of cases with available data, 50% with normal activity had PTD-recovery after TDF-cessation.

Conclusions

Normal ITPase-activity is associated with nephrotoxicity during TDF use and recovery after TDF-cessation. ITPase-activity might function as a screening-tool for probable occurrence and reversibility of TDF-toxicity.

INTRODUCTION

Tenofovir disoproxil-fumarate (TDF) is a recommended nucleotide-analog reverse-transcriptase inhibitor (NRTI) in combination antiretroviral therapy (cART) for HIV-treatment. Other indications for TDF-use are chronic hepatitis B virus infection and pre-exposure prophylaxis (PrEP).[1] Use of TDF is associated with an accelerated estimated glomerular filtration rate (eGFR)-decline[2-4] and proximal tubular dysfunction (PTD).[2,5,6] In clinical trials, tenofovir alafenamide (TAF), a novel tenofovir prodrug, showed comparable virological efficacy as TDF, but caused smaller eGFR-declines and renal tubular proteinuria.[7,8] Therefore, TAF-containing cART became a recommended first-line regimen next to TDF-containing cART.[9,10] Recently, generic TDF has become available, which might favor prescribing TDF over TAF for cost-effectiveness and aid in the roll out of cART in resource-limited countries. Additionally, the use of TDF as PrEP is increasing. Therefore, it is useful to predict in which patient the risk of TDF-associated nephrotoxicity is high, and whether it would recover.

DNA consists of the canonical nucleobases adenine, cytosine, guanine, and thymidine. However, incorporation of non-canonical nucleoside triphosphates in the DNA potentially causes cyto- or genotoxicity.[11] The housekeeping enzyme Inosine 5'-triphosphatase (ITPase), encoded by the polymorphic gene *ITPA* (OMIM #147520), eliminates the nucleotide pool from non-canonical nucleoside triphosphates.[12] In HIV-infected patients, ITPase activity and enzyme expression were decreased compared to non HIV-infected controls in erythrocytes and CD4+ lymphocytes,[13,14] which could not be fully explained by the single nucleotide polymorphisms (SNPs) c.94C>A (p.Pro32Thr, NCBI rs1127354) and c.124+21A>C (NCBI rs7270101) in the *ITPA* gene. In a retrospective cross-sectional study, a normal ITPase activity was associated with broadly defined nephrotoxicity in HIV-infected patients on TDF-containing cART.[15]

We evaluated whether ITPase activity or *ITPA* genotype could be useful biomarkers to predict TDF-associated nephrotoxicity, and whether they were associated with eGFR-improvement after TDF-cessation.

METHODS

This was a 1:2 matched case-control study in a cohort of HIV-1 infected adult patients from the Erasmus Medical Center, Rotterdam, the Netherlands. The study was approved by the local ethics committee, conducted according to the Helsinki Declaration, and participants provided informed consent. Participants were selected from 2 previous studies: a cohort study on TDF-associated nephrotoxicity and a randomized clinical trial in which TDF-

containing cART was discontinued (DOMONO,NCT02401828).[16,17] Cases were patients who developed TDF-associated nephrotoxicity, and controls were patients who did not. Matching was performed for gender, age and ethnicity. Nephrotoxicity was defined as >25% eGFR-decrease during TDF-use and/or presence of ≥2 PTD markers: normoglycaemic glucosuria, hypophosphatemia <0.8 mmol/L, urine protein:creatinine ratio (UPCR) >15.0 mg/mmol, urine albumin:protein ratio (APR) <0.4 in patients with increased UPCR, or increased fractional excretion of phosphate (>20%, or >10% in hypophosphatemic patients).[18] ITPase activity was measured as described previously.[19] ITPase activity ≥4 mmol IMP/mml Hb/hour was considered normal.[20] *ITPA* genotype was determined by genotyping whole blood for the *ITPA* SNPs c.94C>A (p.Pro32Thr, rs1127354) and c.124+21A>C (rs7270101). *ITPA* genotypes without these SNPs were considered wt/wt. 47 patients (15 cases and 32 controls) were selected from the study of Rokx et al, and 34 patients (11 cases and 23 controls) from the DOMONO-study.[16,17] Data on demographics, medical history (diabetes mellitus, hypertension, hepatitis C virus infection, cardiovascular disease), nephrotoxic medications (sulphamethoxazole/trimethoprim, non-steroidal anti-inflammatory drugs, angiotensin converting enzyme-inhibitors, angiotensin-2 receptor-antagonists, and valacyclovir or ganciclovir), eGFR, and PTD markers during TDF-use were collected, as well as eGFR and PTD-markers 48 weeks after TDF-cessation.

The primary outcome of this study was the proportion of normal versus reduced ITPase activity in cases versus controls. Secondary outcomes were: 1) proportions of patients with wt/wt versus wt/94C>A or wt/124+21A>C *ITPA*-genotype in cases versus controls, and 2) improvement of eGFR and PTD 48 weeks after TDF-cessation in cases with normal versus decreased ITPase activity and wt/wt versus another *ITPA* genotype. A sample size of 87 (29 cases and 58 controls) was needed to prove with a 1:2 case-control study-design that a significantly greater proportion of patients with nephrotoxicity had normal ITPase activity (π_1=0.5) than patients without nephrotoxicity (π_2=0.2), based on previous findings, with power 1-β=80% and a 2-sided α of 0.05.[15] McNemar's Test was used to compare proportions of patients with normal ITPase activity in cases and controls. Nephrotoxicity related to both ITPase activity (normal versus reduced) and *ITPA* genotype (wt/wt versus genotype with SNP) was analyzed using conditional logistic regression, resulting in an odds ratio (OR) with 95% confidence interval (CI). Fishers' Exact Test, Unpaired T-tests, Chi Square Tests, and Mann Whitney U Tests were used for other comparisons between patients with normal versus reduced ITPase-activity, and paired T-tests were performed for comparisons on eGFR-improvement. An alfa of 0.05 was used.

RESULTS

81 Patients were included, of whom 26 patients were cases and 55 patients were controls. Although we intended to include 87 patients based on our sample size calculation, we did a preliminary analysis after including 81 patients due to repeated non-adherence to scheduled outpatient appointments of the remaining eligible patients. This analysis showed highly significant results for the primary endpoint, with a calculated power of 76%. Therefore, patient inclusion was stopped for ethical arguments, since we considered that the supporting data for our assumptions for the power calculation were limited and could deviate from the true difference. In both groups, participants were predominantly Caucasian middle-aged males. The duration of TDF-use was comparable between cases and controls (83 and 84 months), as well as use of nephrotoxic co-medication and comorbidity. The mean (SD) eGFR in cases and controls at the moment of TDF-discontinuation was 78 (19) and 85 (13) ml/min. Of the cases, 80.8% had >25% eGFR-decline since TDF initiation, 61.5% had ≥2 PTD-markers, and 42.3% had both. (Table 1).

Of the cases, 73.1% (19/26) had a normal ITPase activity versus 50.9% (28/55) of controls (p=0.001; Table 2). Wt/wt *ITPA* genotype was present in 88.5% of cases and 72.7% (40/55) of controls (p=0.26; Table 2). Conditional logistic regression showed an increased and nearly statistically significant risk for nephrotoxicity in patients with normal ITPase activity and *ITPA* genotype wt/wt: OR 2.56 (95%CI 0.89-7.31; p=0.08), and OR 2.59 (95%CI 0.70-9.54; p=0.15), respectively. The eGFR-course improved from -5.5 ml/min/year during TDF to +4.4 ml/min/year after TDF-cessation (p=0.008) in cases with normal ITPase activity (N=19), whereas it remained stable in cases with reduced activity (N=4): -4.3 versus -4.0 ml/min/year, p=0.97. (Table 2). Of 11 cases that started dolutegravir therapy after TDF-cessation, 7 had normal ITPase activity, of whom 6 had improvement of eGFR, whereas 4 had reduced activity, of whom 1 had improvement of eGFR. These results indicate that patients with a normal ITPase activity may recover from TDF-associated nephrotoxicity after TDF-cessation, but patients with reduced activity may not. In cases with wt/wt *ITPA* genotype (N=22), the eGFR-course improved from -5.0 to +3.0 ml/min/year, p=0.021 (Table 2). eGFR data from only 1 patient with SNPs in the *ITPA* genotype were available, of which no conclusions can be drawn. Of the 16 cases with PTD, 3 had decreased and 13 had normal ITPase activity. Week 48 data were available in 9 patients, of whom the only patient with decreased ITPase activity had no PTD recovery, and PTD recovered in 4 of 8 patients with normal activity.

Table 1. Clinical characteristics of the patients with (cases) and without (controls) TDF-associated nephrotoxicity.

Characteristic	Cases (n=26)	Controls (n=55)	p-value
Male gender, n (%)	23 (88.5)	49 (89.1)	1.00 (FE)[a]
Age, mean years ± SD[b]	51 ± 10	52 ± 9	0.50 (UT)[c]
Ethnicity, n (%)			0.87 (FE)[a]
Caucasian	22 (84.6)	44 (80.0)	
African	2 (7.7)	3 (5.5)	
Asian	0 (0.0)	1 (1.8)	
Latino	2 (7.7)	7 (12.7)	
Route of transmission, n (%)			0.77 (FE)[a]
MSM[d]	20 (76.9)	37 (67.3)	
Heterosexual	5 (19.2)	12 (21.8)	
IVDU[e]	1 (3.8)	3 (5.5)	
Unknown	0 (0.0)	3 (5.5)	
Smoking status, n (%)			0.60 (CS)[f]
Current	8 (30.8)	22 (40.0)	
Previous	8 (30.8)	12 (21.8)	
Never	9 (34.6)	21 (38.2)	
Unknown	1 (3.8)	0 (0.0)	
Comorbidities, n (%)			
Hypertension	5 (19.2)	10 (18.2)	1.00 (FE)[a]
Diabetes mellitus	3 (11.5)	1 (1.8)	0.10 (FE)[a]
Hepatitis C virus infection	2 (7.7)	5 (9.1)	1.00 (FE)[a]
Cardiovascular disease	3 (11.5)	4 (7.3)	0.68 (FE)[a]
TDF[g]-containing cART[h], n (%)			0.86 (FE)[a]
+ NNRTI[i]-containing, N (%)	24 (92.3)	49 (89.1)	
RPV[j]-containing	9 (34.6)	18 (32.7)	
+ bPI[k]-containing, N (%)	2 (7.7)	5 (9.1)	
+ INSTI[l]-containing, N (%)	0 (0.0)	1 (1.8)	
Median duration of TDF-use[m], months (Q1,Q3)	83 (50,117)	84 (46,115)	0.72 (MWU)[n]
Mean eGFR[o] at discontinuation of TDF[g], ml/min, ± SD[b]	78.1 ± 19.2	85.2 ± 12.9	0.09 (UT)[c]
Comedication during TDF[g]-use, n (%)			
Sulfamethoxazol/trimethoprim	0 (0.0)	1 (1.8)	1.00 (FE)[a]
ACE[p]-inhibitor	3 (11.5)	3 (5.5)	0.38 (FE)[a]
AT2[q]-antagonist	0 (0.0)	4 (7.3)	0.30 (FE)[a]
Acyclovir/gancyclovir	1 (3.8)	3 (5.5)	1.00 (FE)[a]
NSAID[r]	7 (26.9)	18 (32.7)	0.62 (CS)[f]

Table 1. Clinical characteristics of the patients with (cases) and without (controls) TDF-associated nephrotoxicity. (continued)

Characteristic	Cases (n=26)	Controls (n=55)	p-value
TDF[g]-associated nephrotoxicity, n (%)			
>25% decrease in eGFR[o]	21 (80.8)		
≥2 markers of PTD[s]	16 (61.5)		
>25% decrease in eGFR[o] + ≥2 markers of PTD[s]	11 (42.3)		

[a] FE, Fisher's Exact test; [b] SD, standard deviation; [c] UT, Unpaired T-test; [d] MSM, men who have sex with men; [e] IVDU, intravenous drug use; [f] CS, Chi Square test; [g] TDF, tenofovir disoproxil fumarate; [h] cART, combination antiretroviral therapy; [i] NNRTI, non-nucleoside reverse transcriptase inhibitor; [j] RPV, rilpivirine; [k] bPI, boosted protease inhibitor; [l] INSTI, integrase strand transfer inhibitor; [m] duration of TDF-use at inclusion and allocation to 'case' or 'control'; [n] MWU, Mann Withney U test; [o] eGFR, estimated glomerular filtration rate; [p] ACE, angiotensin converting enzyme; [q] AT2, Angiotensine 2 antagonist; [r] NSAID, Non-steroidal anti-inflammatory drug; [s] PTD, proximal tubular dysfunction.

Table 2. Renal outcomes related to ITPase-activity and *ITPA* genotype.

	TDF[a]-associated nephrotoxicity		P-value	eGFR[b]-change[c] in cases, mean ±SD[d]		P-value
	Yes (n=26)	No (n=55)		during TDF[a]-use	after TDF[a] discontinuation	
ITPase activity[e]			0.001 (MN[f])			
<4	7 (26.9%)	27 (49.1%)		-4.3 ± 5.8	-4.0 ± 9.9	0.97 (PT[g])
≥4	19 (73.1%)	28 (50.9%)		-5.5 ± 4.8	+4.4 ±13.0	0.008 (PT[g])
Mean ± SD[d]	4.41 ± 1.28	3.90 ± 1.31	0.103 (UT[h])			
***ITPA* genotype**			0.256 (MN[f])			
Wt/94C>A	2 (7.7%)	3 (5.5%)		*	*	*
Wt/124+21A>C	1 (3.8%)	12 (21.8%)		*	*	*
Wt/wt	23 (88.5%)	40 (72.7%)		-5.0 (4.7)	+3.0 (13.0)	0.021 (PT[g])

[a] TDF, tenofovir disoproxil fumarate; [b] eGFR, estimated glomerular filtration rate; [c] in ml/min/year, [d] SD, standard deviation; [e] mmol IMP/mmol Hb/hour; [f] MN, McNemar (Wt/wt versus wt/94C>A and wt/124+21A>C combined); [g] PT, Paired samples T-test; [h] UT, Unpaired T-test; * indicates that not enough data were available for analysis.

DISCUSSION

In this case-control and cohort study in HIV-patients, TDF-associated nephrotoxicity was associated with a normal ITPase activity, and in these patients their eGFR-course ameliorated after TDF-cessation. Less patients with reduced ITPase activity had and accelerated eGFR-decline, which did not recover after TDF-cessation. ITPase activity may be used as biomarker to predict which patients are at high risk for developing nephrotoxicity during TDF-use (more pronounced in normal ITPase activity), in which patients TDF-associated nephrotoxicity may be irreversible (decreased activity), and in whom TDF therefore should

be discontinued when signs of TDF-associated nephrotoxicity occur. The results of the present study confirm our previous findings that a normal ITPase activity was associated with nephrotoxicity during TDF-use.[15] However, in this study, we were able to strictly define nephrotoxicity and investigate the association between ITPase activity and TDF-associated nephrotoxicity.[16]

It is unclear why a normal ITPase activity is associated with both TDF-associated nephrotoxicity and recovery after TDF-cessation. TDF causes mitochondrial DNA (mtDNA) toxicity in tubular cells.[21-23] Imbalanced mitochondrial nucleotide pools can cause mtDNA depletion, resulting in mitochondrial dysfunction.[24,25] Furthermore, TDF leads to increased oxidative stress in mitochondria of renal tubular cells.[26] In cells with oxidative stress, the enzyme xanthine oxidase (XO) activity is relatively increased[27] and XO is a source of free radicals[28]. A normal ITPase activity, compared to decreased activity, may lead to more availability of hypoxanthine (formed from inosine), a substrate for XO. Eventually, the combination of a normal ITPase activity and TDF-use may lead to increasing oxidative stress, resulting in nephrotoxicity. Further research is warranted to clarify whether erythrocyte ITPase activity is an adequate surrogate for ITPase activity in renal cells, and what the effect of ITPase on the nucleotide pools in renal mitochondria is. Differences in ITPase activity in mitochondria, the effect of the ITPase activity on mitochondrial TDF-metabolism, and the role of TDF in oxidative stress should be studied.

This study has some limitations. First, the sample size of the study was based on previous findings,[15] but data on ITPase activity related to TDF-associated nephrotoxicity are scarce and difficult to translate to assumptions for our sample size. As the results of a preliminary analysis in the first 81 included patients were already highly significant, the final 6 patients were not included. Second, we cannot exclude that the nephrotoxicity observed in our cases was due to other, unidentified, factors, although patient characteristics were comparable between the cases and controls. Besides, data on longer follow-up were not available, and therefore we cannot exclude that patients who are included as controls, could have developed nephrotoxicity with longer use of TDF. Third, recovery of nephrotoxicity may be underestimated in patients using DTG. DTG is known for its inhibitory effect on tubular creatinine clearance, leading to an increase in serum creatinine, which decreases the eGFR without impairment of actual glomerular or tubular function.[29] 11 of the 26 cases were former DOMONO-participants, and switched to DTG monotherapy. Indeed, in some of our patients using DTG the eGFR further decreased, but this was in only 1 of 7 patients with normal ITPase activity, versus in 2/4 cases with reduced activity. So even after a switch from TDF to DTG, the distinct between normal versus reduced ITPase activity in relation to eGFR-improvement remains. Given the low numbers of patients with follow-up of PTD-

markers after 48 weeks, importantly due to the observational nature of the study of Rokx et al, we were not able to provide data on recovery of PTD.

In conclusion, ITPase activity is associated with nephrotoxicity during TDF-use for HIV-infection and could be used to predict eGFR-recovery, but the underlying mechanism needs to be elucidated. ITPase activity may be used in the decision to initiate and discontinue TDF in an individual patient, and this recommendation should be confirmed in a prospective trial.

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