Diagnostic Microbiology and Infectious Disease 94 (2019) 202–204

Contents lists available at ScienceDirect

Diagnostic Microbiology and Infectious Disease

journal homepage: www.elsevier.com/locate/diagmicrobio

Pharmacology

Tenofovir Alafenamide and Tenofovir Disoproxil Fumarate are not transported by Concentrative Nucleoside Transporter 2

Jessica Cusato [a,*,1], Andrea Calcagno [a,1], Amedeo De Nicolò [a], Karoly Mogyorosi [b], Antonio D'Avolio [a], Giovanni Di Perri [a], Stefano Bonora [a]

[a] Department of Medical Sciences, University of Turin, Amedeo di Savoia Hospital, Turin, Italy
[b] SOLVO Biotechnology, Közép fasor 52, H-6726, Szeged, Hungary

ARTICLE INFO

Article history:
Received 29 December 2017
Received in revised form 21 May 2018
Accepted 2 July 2018
Available online 6 July 2018

Keywords:
TDF
TAF
SLC28A2
CNT2
Renal toxicity
HIV
HAART

ABSTRACT

Tenofovir-associated renal toxicity is influenced by several factors, including plasma exposure and genetic variants in transporter-encoding genes. Tenofovir plasma exposure has been associated with a polymorphism in *SLC28A2* gene (encoding the concentrative nucleoside transporter 2, CNT2): particularly, *SLC28A2* 124 CT/TT genotype patients show higher plasma tenofovir concentrations, compared to CC group.
In literature, substrate studies are lacking; for this reason, our aim was to understand if tenofovir and tenofovir-alafenamide are CNT2 substrates.
We performed an in vitro study using CNT2 expressing MDCKII cells.
We observed that tenofovir and tenofovir-alafenamide are not substrates of CNT2.
Tenofovir-alafenamide influx pathway remains to be clarified.

1. Manuscript

Overt and subclinical kidney toxicity has been associated with the administration of tenofovir disoproxil fumarate (TDF), a widely used anti HIV and HBV agent{Hill, 2018 #394}. Various strategies have been implemented in order to overcome such toxicity, including drug withdrawal, switch of concomitant drugs and the development of the new pro-drug tenofovir alafenamide (TAF){Vigano, 2018 #363;De Clercq, 2018 #365}. Renal toxicity seems to be influenced by many different factors (such as female gender, low body weight, the presence of cardiovascular disease, a low nadir CD4 count, HCV co-infection among others), including tenofovir (TFV) plasma exposure: higher plasma levels have been associated with reduced estimated creatinine clearance (eCrCl) and proximal tubular dysfunction (Calcagno et al., 2015; Calcagno et al., 2016; Rodriguez-Novoa et al., 2010).

Severity of TDF-associated renal toxicity and urinary TFV concentrations show high inter-individual variability, suggesting that host genetics may play a role. Genetic screen of variants in suspected candidate genes, including those in genes involved in TFV transport, has been associated with TDF-related renal damage. Single nucleotide polymorphisms (SNPs) in *ABCC2 (1249 G > A rs2273697* and *− 24 G > A rs717620*, gene product MRP2), *ABCC10 (526 G > A, rs9349256* and *2483 T > C, rs2125739*, gene product MRP7), *SLC22A6 (149 G > A, rs11568626*, encoding OAT1) and *ABCC4 (669 C > T, rs899494*, gene product MRP4) have been associated with kidney tubular dysfunction(Calcagno et al., 2016; Pushpakom et al., 2011; Rodriguez-Novoa et al., 2009; Rodriguez-Novoa et al., 2009). Additionally, several variants in *ABCC2* and Lowe syndrome protein *OCRL* gene have been related to decreased eGFR (Calcagno et al., 2016; Dahlin et al., 2015). The effect of SNPs on TFV exposure is not fully elucidated. In a prior study, we found that TFV urinary clearance (as measured by urinary to plasma ratios) was predicted by SNPs in *ABCC10* (*1791 + 526 G > A 138, rs9349256*), along with protease inhibitor co-administration(Calcagno et al., 2015). We also reported that plasma tenofovir concentration was associated with eCrCl, protease inhibitors co-administration and a variant in *SLC (*solute carrier*) 28A2* gene (encoding the concentrative nucleoside transporter 2, CNT2); particularly, *SLC28A2* 124 CT/TT genotype patients show higher plasma tenofovir concentrations, compared to CC group. We decided to consider CNT, since TDF is a nucleotide analog and it could be transported by this kind of carrier; in addition, CNT could indirectly interfere with TDF metabolism, acting on nucleoside pathway {Hill, 2018

* Corresponding author. Tel.: +39-011-4393841; fax: +39-011-4393996.
E-mail addresses: jessica.cusato@yahoo.it, (J. Cusato), andrea.calcagno@unito.it, (A. Calcagno), amedeo.denicolo@unito.it, (A. De Nicolò), mogyorosi@solvo.com, (K. Mogyorosi), antonio.davolio@unito.it, (A. D'Avolio), giovanni.diperri@unito.it, (G. Di Perri), stefano.bonora@unito.it. (S. Bonora).
[1] Both authors equally contributed to this manuscript.

https://doi.org/10.1016/j.diagmicrobio.2018.07.001
0732-8893/

#394}. The CNT family consists of three members, which interact with various nucleoside analog drugs. Human CNT2 is localized to the apical membranes of absorptive epithelial cells of the intestine (especially in the jejunum), liver, on the apical surface of proximal tubule cells in kidney and it is also expressed in lymphocytes. In addition, CNT2 expression in hepatocytes and macrophages has led to an understanding of the endogenous physiological role of CNT2 in the salvage of nucleosides during cell proliferation (Owen et al., 2005). The other roles of CNT2 in cell physiology, for example in modulating purinergic responses during cell activation and/or apoptosis, have also been investigated (Yee et al., 2009). It is noteworthy that lower intracellular TFV-diphosphate concentrations were found in patients harboring *CNT2* variants (*rs1060896* and *rs11854484*) (Seifert et al., 2016).

Since no data are available on TDF or TFV as substrates of CNT2, we performed an in vitro substrate feasibility study of TDF and TAF, with stable transfectant cell lines expressing the selected human uptake transporter (CNT2 for our experiment).

Cells adhere to treated surfaces, which allow the easy separation of transported and excess low permeability substrate molecules. The "substrate assessment study" was performed through three different steps: each module was planned and initiated based on the result of the previous module. The module 1 (pilot experiments to detect active transport) aimed the determination of whether or not the uptake transporter-mediated uptake of the cold compound into the cells can be measured; module 2 (detailed kinetic characterization of the transport) determined the Michaelis–Menten constant (K_M) and the maximum rate (V_{max}) values. Inhibition of transport was studied with a known substrate and inhibitor. The effect of known inhibitors/substrates on the drug transport was investigated at the optimized drug concentration and seven concentrations of the inhibitor/substrate molecule; module 3 (characterization of potential drug–drug interactions) evaluated the half maximal inhibitory concentration (IC50) of selected known or unknown perpetrator drugs on the transport.

In particolar, we used stably transfected cell lines for studying the interaction of drugs with CNT2: cells adhere to treated surfaces, which allow the easy separation of transported and intracellularly retained low permeability substrate molecules. The accumulation of TFV/TAF was investigated in MDCKII-CNT2 and control MDCKII cells, applying TFV/TAF at 0.1 and 1 μM concentrations and at 2 and 20 minutes of incubation time (Fig. 1). Usually, when transporter-specific accumulation is detected (at least 2- higher accumulation in the transfected versus the control cells), a follow-up inhibition study is performed, considering the Michaelis–Menten constant (K_M), the maximum rate (V_{max}) as parameters of the carrier-specific transport. The potential inhibitory effect of TFV/TAF can also be investigated in a competition-type assay, using a CNT2-specific probe substrate and the effect of TFV/TAF on the transport is characterized by IC_{50} values.

In Table 1, results were showed: the highest accumulations of TFV and TAF (at 1 μM, 20 minutes and 37 °C) were only 1.30 pmol/mg protein and 1.38 pmol/mg protein, respectively (considering that uridine, which is the positive control transported by CNT2, has an accumulation of 6.93 pmol/mg protein at 1 μM). Accumulation of TFV and TAF was similar in the CNT2-expressing and the control MDCKII cells (transporter specific accumulations were < 2), indicating no active accumulation of these drugs in the cells, under the tested conditions.

In conclusion, our work demonstrated that, as opposite to previous suggestions, neither TFV nor TAF are CNT2 substrates. Although clinical trials comparing TDF and TAF gave evidence of a much lesser impact of the latter on tubular physiology and bone structure, thus suggesting remarkable differences in tissue distribution between the two drugs, it is not yet clear which transporters drive the selective tissue distribution of TAF. TFV-related renal and bone diseases deserve further studies concerning aging in HIV-positive subjects as tailored treatment, through pharmacokinetic and pharmacogenetic individualizations; this might actually optimize TFV delivery improving the lifelong perspective of continuous administration.

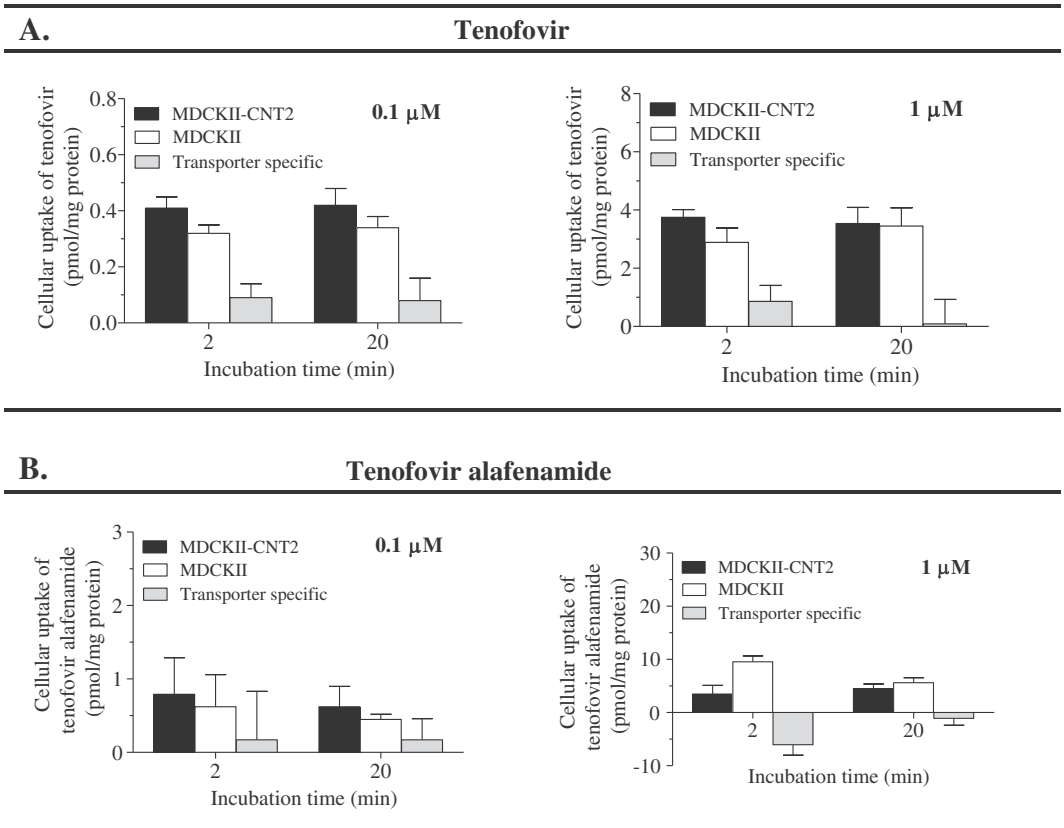

Fig. 1. Accumulation of tenofovir (A) and tenofovir alafenamide (B) in CNT2-expressing and MDCKII control cells in the uptake transporter substrate feasibility assay.

Table 1
Accumulation of tenofovir (A) and tenofovir alafenamide (B) in CNT2 expressing and MDCKII control cells measured in the uptake transporter substrate feasibility assay.

A.

Compound	Conditions		Accumulation (pmol/mg protein)			
	μM	min	In transporter expressing cells	In control cells	Active transport	Fold accumulation
Tenofovir	0.1	2	0.41 ± 0.04	0.32 ± 0.03	0.09 ± 0.05	1.27
		20	0.42 ± 0.06	0.34 ± 0.04	0.08 ± 0.08	1.23
	1	2	3.75 ± 0.27	2.89 ± 0.49	0.86 ± 0.55	1.30
		20	3.54 ± 0.55	3.45 ± 0.63	0.09 ± 0.84	1.03
Positive control (accumulation in cpm)						
Uridine	1	1	461.67 ± 21.46	66.67 ± 7.02	395.00 ± 22.58	6.93
Uridine + adenosine	1+ 300	1	70.00 ± 7.21	51.67 ± 13.43	18.33 ± 15.24	1.35

Data are expressed as mean (n = 3) ± SD.

B.

Compound	Conditions		Accumulation (pmol/mg protein)			
	μM	min	In transporter expressing cells	In control cells	Active transport	Fold accumulation
Tenofovir alafenamide	0.1	2	0.79 ± 0.50	0.62 ± 0.44	0.17 ± 0.66	1.28
		20	0.62 ± 0.28	0.45 ± 0.07	0.17 ± 0.29	1.38
	1	2	3.48 ± 1.62	9.54 ± 1.11	−6.06 ± 1.96	0.36
		20	4.52 ± 0.87	5.61 ± 0.94	−1.09 ± 1.28	0.81
Positive control (accumulation in cpm)						
Uridine	1	1	461.67 ± 21.46	66.67 ± 7.02	395.00 ± 22.58	6.93
Uridine + adenosine	1+ 300	1	70.00 ± 7.21	51.67 ± 13.43	18.33 ± 15.24	1.35

Data are expressed as mean (n = 3) ± SD.
Calibration limit: 0.51 nM (Sample LLOQ: 0.073 nM).

2. Fundings for the Research

No funding.

3. Transparency declarations' section

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-Inghelheim, BMS, MSD, Gilead, Janssen-Cilag and Viiv. K.S. is a employee of SOLVO Inc. Other authors declare no potential conflict of interest.

References

Calcagno A, Cusato J, Marinaro L, Simiele M, Lucchiari M, Alcantarini C, et al. Tenofovir clearance is reduced in HIV-positive patients with subclinical tubular impairment. AIDS 2015;30(6):915–20.

Calcagno A, Cusato J, Marinaro L, Trentini L, Alcantarini C, Mussa M, et al. Clinical pharmacology of tenofovir clearance: a pharmacokinetic/pharmacogenetic study on plasma and urines. Pharmacogenomics J 2015;16(6):514–8.

Calcagno A, Di Perri G, Bonora S. What do we know about tailoring treatment with tenofovir? Pharmacogenomics 2016;17(6):531–4.

Dahlin A, Wittwer M, de la Cruz M, Woo JM, Bam R, Scharen-Guivel V, et al. A pharmacogenetic candidate gene study of tenofovir-associated Fanconi syndrome. Pharmacogenet Genomics 2015;25(2):82–92.

Owen RP, Gray JH, Taylor TR, Carlson EJ, Huang CC, Kawamoto M, et al. Genetic analysis and functional characterization of polymorphisms in the human concentrative nucleoside transporter, CNT2. Pharmacogenet Genomics 2005;15(2):83–90.

Pushpakom SP, Liptrott NJ, Rodriguez-Novoa S, Labarga P, Soriano V, Albalater M, et al. Genetic variants of ABCC10, a novel tenofovir transporter, are associated with kidney tubular dysfunction. J Infect Dis 2011;204(1):145–53.

Rodriguez-Novoa S, Labarga P, D'Avolio A, Barreiro P, Albalate M, Vispo E, et al. Impairment in kidney tubular function in patients receiving tenofovir is associated with higher tenofovir plasma concentrations. AIDS 2010;24(7):1064–6.

Rodriguez-Novoa S, Labarga P, Soriano V. Pharmacogenetics of tenofovir treatment. Pharmacogenomics 2009;10(10):1675–85.

Rodriguez-Novoa S, Labarga P, Soriano V, Egan D, Albalater M, Morello J, et al. Predictors of kidney tubular dysfunction in HIV-infected patients treated with tenofovir: a pharmacogenetic study. Clin Infect Dis 2009;48(11):e108–16.

Seifert SM, Chen X, Clayton CW, Alford T, Meditz AL, Castillo-Mancilla J, et al. Transporter genetics and TFV-DP/FTC-TP cellular pharmacology, In Vivo. Conference on Retroviruses and Opportunistic Infections 2016. Boston, MA: Poster 445; February 2016.

Yee SW, Shima JE, Hesselson S, Nguyen L, De Val S, Lafond RJ, et al. Identification and characterization of proximal promoter polymorphisms in the human concentrative nucleoside transporter 2 (SLC28A2). J Pharmacol Exp Ther 2009;328(3):699–707.