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Letters to the Editor

Tenofovir Disoproxil Fumarate Is Not an Inhibitor of Human Organic Cation Transporter 1

Hong Shen, Wenying Li, W. Griffith Humphreys, and Yurong Lai

Pharmaceutical Candidate Optimization, Bristol-Myers Squibb Company, Princeton, New Jersey

This letter to editor is to provide commentary on results presented in "Transport of lamivudine [(−)-β-L-2',3'-dideoxy-3'-thiacytidine] and high-affinity interaction of nucleoside reverse transcriptase inhibitors with human organic cation transporters 1, 2, and 3" reported by Minuesa et al. (2009). Along with data on multiple nucleoside anti-HIV drugs, the authors reported that tenofovir disoproxil fumarate (TDF) exhibited a high-affinity interaction with human organic cation transporters (OCTs) in CHO cells stably expressing human OCT1, OCT2, and OCT3, respectively. The IC_{50} values for inhibition of [^{3}H]*N*-methyl-4-phenylpyridinium (MPP^+) uptake in OCT1-, OCT2-, and OCT3-CHO cell lines by tenofovir disoproxil fumarate (TDF) were 0.854, 0.566, and 0.005 nM, respectively (see Table 1 in the published paper) (Minuesa et al., 2009). Because OCT1 plays a key role in hepatic drug disposition and elimination, substrates of OCT1 may potentially interact with coadministered drugs that are the inhibitors. For this reason, we were interested in reproducing the results above and thus performed inhibition studies in human embryonic kidney (HEK) 293 cells stably or transiently expressing human OCT1 using TDF as an inhibitor. Our results indicated that TDF does not significantly inhibit OCT1-mediated uptake and disagree with the results published by Minuesa et al. (2009).

Minuesa et al. (2009) carried out OCT inhibition experiments using "short-time" uptake measurements to determine the IC_{50} values of nucleoside reverse transcriptase inhibitors. In their experiments, uptake in CHO-hOCTs and/or CHO-pcDNA5 (empty vector) cells was measured after 1-second (MPP^+) and 15-second (3TC) incubation. The authors commented that the use of the "short-time" uptake measurement is to reduce the passive diffusion of the radiolabeled substrates used. According to the paper, [^{3}H]MPP^+ uptake in CHO cells overexpressing hOCT1 was inhibited approximately 60% by inhibitors abacavir (ABC), azidothymidine (AZT), emtricitabine (FTC), and tenofovir disoproxil fumarate (TDF), and the IC_{50} values of hOCT1 were less than 1 nM, as stated above. Unfortunately, [^{3}H]MPP^+ uptake in Mock-CHO cells, considered as controls for passive diffusion transport and nonspecific binding, was not provided. Moreover, the authors did not provide evidence showing if the "short-time" uptake measurement provided optimal dynamic window for inhibition with a positive control known inhibitors such as pyrimethamine (PYR).

Efforts to reproduce the OCT1 inhibition of TDF reported by Minuesa et al. included incubations with HEK-293 cells either stably or transiently expressing OCT1. As shown in Fig. 1, both 1 μM TDF and 1 μM tenofovir (parent drug of TDF) have no effect on the [^{3}H]MPP^+ uptake in HEK cells stably expressing OCT1. In contrast, 50 μM PYR, a known OCT1 inhibitor (Ito et al., 2010), significantly reduced the [^{3}H]MPP^+ uptake. The results obtained from our in-house, stably expressing cells are further confirmed in HEK cells transiently expressing OCT1, purchased from Corning Life Sciences (Corning TransportoCells OCT1; Bedford, MA). As depicted in Fig. 2, the uptake of [^{3}H]MPP^+ in Corning TransportoCells OCT1 was not significantly inhibited by TDF at concentrations of 1 and 10 μM (Fig. 2). To rule out the possible substrate-dependent effects on the inhibition (Belzer et al., 2013; Izumi et al., 2013; Martinez-Guerrero and Wright, 2013), inhibitory effects on [^{14}C]metformin uptake, a well-known OCT1 substrate, in HEK cells stably overexpressing OCT1 cells were assessed with 10 μM TDF and 20 μM tenofovir, and the results showed no significant inhibition effect on the uptake of [^{14}C]metformin (Fig. 3). Furthermore, we demonstrated that both 1 μM TDF and tenofovir did not remarkably inhibit the uptake of MPP^+ in human hepatocytes (Lot: FME, LTY, and NRJ purchased from BiotrvlsmsyionIVT, Baltimore, MD), whereas positive controls 100 μM PYR or lowering temperature from 37 to 4°C dramatically decreased the hepatic uptake of MPP^+ (Fig. 4). Surprisingly, the inhibitory effect of TDF on OCT1 inhibition was not also reproduced by the same group, as the most recent publication from the group (Arimany-Nardi et al) indicated that 500 μM TDF does not inhibit OCT1-meditated lamivudine uptake in HEK-hOCT1 cells. Our findings agree with the lack of a clinical OCT-mediated drug-drug interactions of TDF and tenofovir (http://didb.druginteractioninfo.org/User/UserHome.aspx).

In contrast to Minuesa et al. (2009), we conclude that TDF is unlikely to be an inhibitor of human OCT1 up to 10 μM. The results reported by Minuesa et al. were obtained from a single-point "short" (e.g., 1 second) incubation without considering

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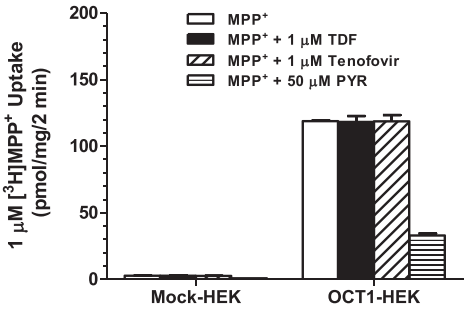

Fig. 1. Inhibitory effects of TDF (1 μM) and tenofovir (1 μM) on uptake of 1 μM [^{3}H]MPP$^+$ in HEK cells stably expressing OCT1 protein.

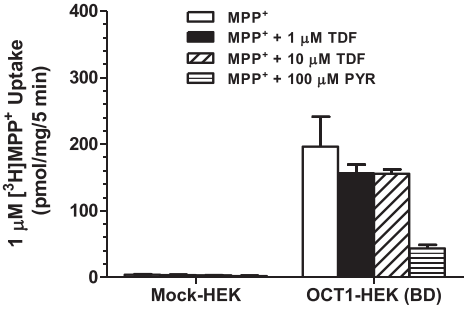

Fig. 2. Inhibitory effects of TDF (1 and 10 μM) on uptake of 1 μM [^{3}H]MPP$^+$ in HEK cells transiently expressing OCT1 protein (Corning TransportoCells OCT1).

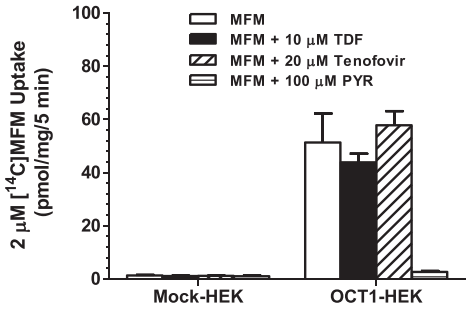

Fig. 3. Inhibitory effects of TDF (10 μM) and tenofovir (20 μM) on uptake of 2 μM [^{14}C]MFM in HEK cells stably expressing OCT1 protein.

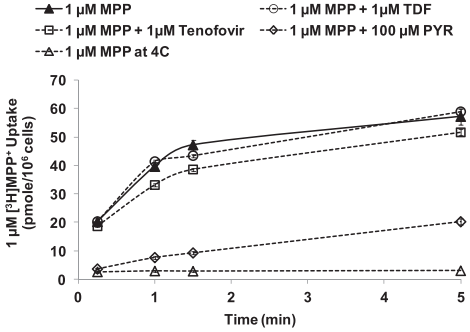

Fig. 4. Inhibitory effects of TDF (1 μM) and tenofovir (1 μM) on uptake of 1 μM [^{3}H]MPP$^+$ in human hepatocytes.

the surface binding, which could be artificial and not reproducible followed by rigorous investigations. We are concerned that conclusions made by Minuesa et al. may lead to inappropriate concern over TDF-mediated drug-drug interaction and/or unnecessary clinical trials of TDF and possibly other nucleoside reverse transcriptase inhibitors reported to be OCT1 drug inhibitors.

References

Arimany-Nardi C, Minuesa G, Keller T, Erkizia I, Koepsell H, Martinez-Picado J, and Pastor-Anglada M (2016) Role of Human Organic Cation Transporter 1 (hOCT1) Polymorphisms in Lamivudine (3TC) Uptake and Drug-Drug Interactions. *Front Pharmacol* **7**:175.

Belzer M, Morales M, Jagadish B, Mash EA, and Wright SH (2013) Substrate-dependent ligand inhibition of the human organic cation transporter OCT2. *J Pharmacol Exp Ther* **346**:300–310.

Ito S, Kusuhara H, Kuroiwa Y, Wu C, Moriyama Y, Inoue K, Kondo T, Yuasa H, Nakayama H, Horita S, et al. (2010) Potent and specific inhibition of mMate1-mediated efflux of type I organic cations in the liver and kidney by pyrimethamine. *J Pharmacol Exp Ther* **333**:341–350.

Izumi S, Nozaki Y, Komori T, Maeda K, Takenaka O, Kusano K, Yoshimura T, Kusuhara H, and Sugiyama Y (2013) Substrate-dependent inhibition of organic anion transporting polypeptide 1B1: comparative analysis with prototypical probe substrates estradiol-17β-glucuronide, estrone-3-sulfate, and sulfobromophthalein. *Drug Metab Dispos* **41**:1859–1866.

Martínez-Guerrero LJ and Wright SH (2013) Substrate-dependent inhibition of human MATE1 by cationic ionic liquids. *J Pharmacol Exp Ther* **346**:495–503.

Minuesa G, Volk C, Molina-Arcas M, Gorboulev V, Erkizia I, Arndt P, Clotet B, Pastor-Anglada M, Koepsell H, and Martinez-Picado J (2009) Transport of lamivudine [(-)-beta-L-2′,3′-dideoxy-3′-thiacytidine] and high-affinity interaction of nucleoside reverse transcriptase inhibitors with human organic cation transporters 1, 2, and 3. *J Pharmacol Exp Ther* **329**:252–261.

Address correspondence to: Y. Lai, Bristol Myers Squibb, 3551 Lawrenceville Rd., Princeton, NJ 08540. E-mail: yurong.lai@bms.comurong