Contributions of Cathepsin A and Carboxylesterase 1 to the Hydrolysis of Tenofovir Alafenamide in the Human Liver, and the Effect of CES1 Genetic Variation on Tenofovir Alafenamide Hydrolysis

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ABSTRACT

The prodrug tenofovir alafenamide (TAF) is a first-line antiviral agent for the treatment of chronic hepatitis B infection. TAF activation involves multiple steps, and the first step is an ester hydrolysis reaction catalyzed by hydrolases. This study was to determine the contributions of carboxylesterase 1 (CES1) and cathepsin A (CatA) to TAF hydrolysis in the human liver. Our in vitro incubation studies showed that both CatA and CES1 catalyzed TAF hydrolysis in a pH-dependent manner. At their physiologic pH environment, the activity of CatA (pH 5.2) was approximately 1,000-fold higher than that of CES1 (pH 7.2). Given that the hepatic protein expression of CatA was approximately 200-fold lower than that of CES1, the contribution of CatA to TAF hydrolysis in the human liver was estimated to be much greater than that of CES1, which is contrary to the previous perception that CES1 is the primary hepatic enzyme hydrolyzing TAF. The findings were further supported by a TAF incubation study with the CatA inhibitor telaprevir and the CES1 inhibitor bis-(p-nitrophenyl) phosphate. Moreover, an in vitro study revealed that the CES1 variant G143E (rs71647871) is a loss-of-function variant for CES1-mediated TAF hydrolysis. In summary, our results suggest that CatA may play a more important role in the hepatic activation of TAF than CES1. Additionally, TAF activation in the liver could be affected by CES1 genetic variation, but the magnitude of impact appears to be limited due to the major contribution of CatA to hepatic TAF activation.

SIGNIFICANCE STATEMENT

Contrary to the general perception that carboxylesterase 1 (CES1) is the major enzyme responsible for tenofovir alafenamide (TAF) hydrolysis in the human liver, the present study demonstrated that cathepsin A may play a more significant role in TAF hepatic hydrolysis. Furthermore, the CES1 variant G143E (rs71647871) was found to be a loss-of-function variant for CES1-mediated TAF hydrolysis.

Introduction

Tenofovir (TFV) is an acyclic nucleotide phosphonate analog that potently inhibits the hepatitis B virus (HBV) DNA polymerase, a key enzyme required for viral genome replication (Trepo et al., 2014). However, the poor bioavailability of TFV is a significant limitation (Kearney et al., 2004). Tenofovir disoproxil (TDF), a bis-carbonate ester prodrug of TFV, was subsequently developed due to its enhanced stability and improved oral bioavailability (Rautio et al., 2008). Although TDF can achieve sustained viral suppression with a high barrier to drug resistance, its long-term use has been associated with renal toxicity and reduced bone mineral density because of its high plasma TFV level (Cooper et al., 2010; Buti et al., 2015; Bedimo et al., 2016). Consequently, a new TFV prodrug, tenofovir alafenamide (TAF), was developed for more efficient delivery into target tissues and cells. TAF exhibits a higher antiviral potency at approximately 10% of the TDF dose and an improved safety profile due to the reduced systemic exposure of TFV (Babusis et al., 2013; Agarwal et al., 2015; Murakami et al., 2015). TAF was listed as one of the four first-line antiviral agents in the latest American Association for the Study of Liver Diseases guidance for chronic hepatitis B treatment (2018) (Terrault et al., 2018). Despite these improvements, significant interindividual variability in both the pharmacokinetics (PK) and antiviral activity of TAF has been consistently observed in clinical studies (Markowitz et al., 2014; Agarwal et al., 2015; Custodio et al., 2016).

As a prodrug, the antiviral effect of TAF relies on multistep activation involving several enzymes (Fig. 1) (Birkus et al., 2008; Murakami et al., 2015). Therefore, identifying the key TAF activating enzymes is critical to understand the interindividual variability in the PK and pharmacodynamics of TAF therapy. Multiple hydrolases, including cathepsin A (CatA), carboxylesterase 1 (CES1), leukocyte elastase, pancreatic elastase 1, proteinase 3, and cathepsin H, have been shown to catalyze the cleavage of the ester group, forming the intermediate

ABBREVIATIONS: ACN, acetonitrile; BNPP, bis-(p-nitrophenyl) phosphate; CatA, cathepsin A; CES1, carboxylesterase 1; DIA, data-independent acquisition; HBV, hepatitis B virus; HIV, human immunodeficiency virus; HLS9, human liver S9 fractions; LC-MS/MS, liquid chromatography-tandem mass spectrometry; PK, pharmacokinetics; RDV, remdesivir; TAF, tenofovir alafenamide; TDF, tenofovir disoproxil; TFV, tenofovir.
metabolite tenofovir-alanine, the first step in the TAF activation process (Birkus et al., 2008; Birkus et al., 2015). Unsurprisingly, this step depends on the composition of hydrolyses in different cells and tissues. For example, CatA was reported as the primary enzyme hydrolyzing TAF in peripheral blood mononuclear cells, the target cells of the human immunodeficiency virus (HIV) (Birkus et al., 2007; Birkus et al., 2015), and CES1 was considered to be the major hydrolase catalyzing the first activation step of TAF in the human liver, the target tissue of HBV (Hemingsson et al., 2005; Murakami et al., 2015). However, the contributions of individual hydrolyses in TAF hydrolysis in the liver have not been fully evaluated because the expression profiles of hepatic hydrolyses were not taken into consideration in the previous study (Murakami et al., 2015). In addition, the CES1 activity in the previous study was measured with porcine liver carboxylesterase (Birkus et al., 2008), which may differ from human CES1 in terms of catalyzing TAF hydrolysis.

CES1 is one of the most abundant proteins in the human liver and has been considered the major contributor to the hepatic hydrolysis of many ester-containing drugs, including TAF (Imai, 2006; Murakami et al., 2015; Wang et al., 2020). CES1 function can be regulated by genetic variants, such as the loss-of-function nonsynonymous variant G143E (rs71647871) (Zhu et al., 2008; Her and Zhu, 2020). However, it remains unknown whether and to what extent the G143E variant may affect TAF activation.

Therefore, the purposes of this study were to 1) evaluate the contributions of CES1 and CatA to TAF hydrolysis in the human liver and 2) determine the impact of the CES1 genetic polymorphism G143E on TAF hydrolysis. We performed an absolute quantitative proteomics analysis to determine the protein levels of CES1, CatA, and other hydrolyses in human liver S9 fractions (HLS9). We also conducted an in vitro TAF hydrolysis study and found that the activities of CatA, CES1, and HLS9 on hydrolyzing TAF were pH dependent. Based on the obtained proteomics and enzymatic activity data, it appears that, although both CatA and CES1 are capable of hydrolyzing TAF, the contribution of CatA in human hepatic hydrolysis of TAF could be substantially greater than that of CES1. Additionally, the G143E was found to be a loss-of-function variant for the CES1-mediated TAF hydrolysis.

Materials and Methods

TAF, remdesivir (RDV), bortezomib, and boceprevir were purchased from Cayman Chemical. TFV and adenosine (AFV) were obtained from MP Biomedicals. Recombinant human CatA (rhCatA) and recombinant human CES1 (rhCES1) were purchased from R&D Systems; bis-(p-nitrophenyl) phosphate (BNPP) and Tris-base (Trizma base) were purchased from Sigma-Aldrich. Tris-HCl was purchased from Fisher Scientific. Pierce BCA Protein Assay Kit and PBS were obtained from Thermo Fisher Scientific. The MES [2-(N-morpholino)ethanesulfonic acid] buffer (0.2 M, pH 5.5 at 25°C and pH 5.2 at 37°C) was purchased from Alfa Aesar. All other chemicals and reagents were of analytical grade and commercially available. Pooled HLS9 was purchased from XenoTech LLC.

Cell S9 Fractions Preparation. The Flp-In 293 cell lines stably expressing wild-type CES1 and the variant G143E were developed in our previous study, and CES1 protein expression levels are comparable between the two cell lines (Zhu et al., 2008). Cells were sonicated and centrifuged at 9,000g for 30 minutes at 4°C. The supernatant (S9 fractions) was collected and stored at −80°C until use. The S9 fraction protein concentrations were measured using a Pierce BCA Protein Assay Kit.

rhCES1 and rhCatA Activity Assays. The activity of rhCES1 and rhCatA on hydrolyzing TAF was measured using the method similar to that from a previous report (Murakami et al., 2010). A preliminary experiment was performed to determine the steady-state kinetic conditions for the enzymatic reactions (Supplemental Fig. S1). The assay buffer for the rhCES1 and rhCatA activity assays was different in composition and pH. The assay buffer for rhCatA reactions was an MES buffer (0.2 M, pH 5.2 at 37°C) containing 100 mM NaCl, 1 mM DTT, and 0.1% Nonidet (Murakami et al., 2010). The rhCatA reaction was 50 mM Tris buffer, which was prepared by dissolving 6.06 g Tris-HCl and 1.39 g Tris-base in 1000 ml water (pH 7.2 at 37°C). TAF was stable in both buffers for at least 30 minutes at 37°C (Supplemental Fig. S2).

The CatA assay was conducted in 50 μL MES pH 5.2 assay buffer, containing the activated rhCatA (0.2 ng/μL) and various concentrations of TAF (10, 20, 50, and 100 μM). The rhCatA assay mixtures were incubated at 37°C for 5 minutes. The reaction was terminated by adding a twofold volume of acetonitrile (ACN) containing the internal standard RDV (5 μM). The samples were then vortexed for 15 seconds and centrifuged at 15,000 rpm for 4°C for 10 minutes. The supernatant was collected and centrifuged again using the same condition to remove the precipitated proteins. The resulting supernatant was collected and diluted with a fourfold volume of 66.7% methanol (methanol:water, 2:1, v/v) for liquid chromatography-tandem mass spectrometry (LC-MS/MS) analysis.

The rhCES1 activity study was conducted in 10 μL Tris buffer (pH 7.2), containing 40 ng/μL rhCES1 and various concentrations (10, 20, 50, and 100 μM) of TAF. The rhCES1 reaction mixtures were incubated at 37°C for 20 minutes. The reaction was terminated by adding a fivefold volume of ACN containing the internal standard RDV (2 μM). The samples were then vortexed for 15 seconds and centrifuged at 15,000 rpm at 4°C for 10 minutes to remove the precipitated proteins. The centrifugation was repeated once, and the resulting supernatant was collected for LC-MS/MS analysis.

The TAF hydrolysis activity was calculated by dividing the reduction of TAF concentration after incubation by the protein concentrations of the recombinant enzymes or HLS9 and incubation time.

pH-Dependent TAF Hydrolysis. To evaluate the effect of pH on enzymatic activity, rhCES1 (40 ng/μL) and rhCatA (0.25 ng/μL) were incubated with 20 μM TAF at 37°C in the MES assay buffer (pH 5.2) for 20 minutes and the Tris buffer (pH 7.2) for 10 minutes, respectively. Our preliminary study confirmed that TAF hydrolysis was in a linear relationship with the enzyme concentrations (CatA: 0.1–0.5 ng/μL; CES1: 20–40 ng/μL) and the incubation duration (CatA: 0–5 minutes; CES1: 0–30 minutes). Moreover, TAF (20 μM) was incubated with HLS9 (0.5 mg/ml HLS9 protein) at 37°C for 15 minutes in both MES pH 5.2 assay buffer and Tris pH 7.2 buffer. All reactions were performed on a shaker (Benchmark, Multi-Therm) at 1,500 rpm and terminated by adding a twofold volume of ACN containing the internal standard RDV (5 μM). Samples were vortexed for 15 seconds and centrifuged twice at 15,000 rpm at 4°C to remove the precipitated proteins. The resulting supernatant was collected and diluted with a fourfold volume of 66.7% methanol. The remaining TAF after incubation was determined using an LC-MS/MS assay.

CatA and CES1 Inhibition Study. An in vitro study was conducted to evaluate the effects of telaprevir (CatA inhibitor) and BNPP (CES1 inhibitor) on
TAF hydrolysis. The remaining TAF concentration was determined after incubation of TAF (20 μM) with pooled HLS9 at 37°C for 10 minutes in the absence or presence of various concentrations of telaprevir (0.5, 5, and 50 μM) and BNPP (1, 10, and 50 μM) in the MES pH 5.2 assay buffer and Tris pH 7.2 buffer. TAF was incubated with 0.5 mg/ml HLS9 for 15 minutes in the MES pH 5.2 buffer, whereas the TAF incubation in the Tris pH 7.2 buffer was 20 minutes with 1 mg/ml HLS9. The sample preparation procedure following incubation was identical to the HLS9 activity assay described above.

**Table 1**

<table>
<thead>
<tr>
<th>TAF (μM)</th>
<th>rhCatA Activity (pmol/min/µg protein)</th>
<th>rhCES1 Activity (pmol/min/µg protein)</th>
<th>CatA to rhCES1 Activity Ratio</th>
<th>CatA Abundance in HLS9 (µg/mg total protein)</th>
<th>CES1 Abundance in HLS9 (µg/mg total protein)</th>
<th>Contribution to TAF hydrolysis in HLS9 (CatA/CES1)</th>
</tr>
</thead>
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<td>10</td>
<td>3328/7 ± 162.1</td>
<td>4.8 ± 0.4</td>
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<td>0.082</td>
<td>17.70</td>
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<td>6.7 ± 1.1</td>
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<td>4.9:1</td>
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<tr>
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<td>16221.0 ± 2564.3</td>
<td>16.2 ± 4.0</td>
<td>1061:1</td>
<td></td>
<td></td>
<td>4.9:1</td>
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<tr>
<td>100</td>
<td>33918.0 ± 6175.3</td>
<td>28.6 ± 4.2</td>
<td>1203:1</td>
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<td></td>
<td>5.6:1</td>
</tr>
</tbody>
</table>

*Data were obtained from three independent experiments (n = 3).*

**Results**

**CatA Contributed More to TAF Hydrolysis in HLS9 than CES1.** The activity of rhCatA and rhCES1 on TAF hydrolysis is summarized in Table 1. rhCatA activity was approximately 1,000-fold higher than rhCES1. Our DIA-based proteomics analysis showed that the protein concentrations of CatA and CES1 were 0.082 and 17.7 µg enzyme/mg total protein, respectively, in the pooled HLS9. We estimated the contributions of CatA and CES1 to TAF hydrolysis in HLS9 by multiplying their activity with their protein abundance in HLS9. The results indicate that the contribution of CatA to TAF hydrolysis in HLS9 was approximately 3 to 5 fold of that of CES1 when TAF concentrations were 10–100 μM (Table 1).

**Activity of rhCatA and rhCES1 and HLS9 on Hydrolyzing TAF Was Affected by pH.** A significant pH-dependent effect was observed on TAF hydrolysis following incubation with rhCES1, rhCatA, and HLS9 (Fig. 2). rhCES1 activity in its optimal pH condition (pH 7.2) was about fourfold higher than that at pH 5.2 (6.10 ± 1.11 vs. 1.19 ± 0.74 pmol/min/µg protein, n = 3; P < 0.01) (Fig. 2A). On the contrary, the activity of rhCatA at pH 5.2 was more than onefold higher than its activity at pH 7.2 (2,742.45 ± 185.48 vs. 1,187.99 ± 111.80 pmol/min/µg protein, n = 3; P > 0.01) (Fig. 2B). Moreover, TAF hydrolysis activity of HLS9 measured in the MES pH 5.2 buffer was about threefold higher than that in the Tris pH 7.2 buffer (1904.20 ± 17.38 vs. 480.58 ± 75.08 pmol/min/µg protein, n = 3; P < 0.01) (Fig. 3C). TAF was stable in both MES pH 5.2 and Tris pH 7.2 blank buffers at 37°C for 30 minutes.

**Effects of CES1 and CatA Inhibitors on TAF Hydrolysis in HLS9.** To confirm the role of CatA and CES1 in hydrolyzing TAF in human livers, we further investigated the effects of the CatA inhibitor telaprevir and the CES1 inhibitor BNPP on TAF hydrolysis in HLS9. As shown in Fig. 3, in the pH 5.2 condition, BNPP (1–50 μM) showed an inappreciable effect on TAF hydrolysis by HLS9, whereas telaprevir at only 0.5 μM could significantly inhibit TAF hydrolysis (P < 0.01). Of note, telaprevir at 50 μM could nearly abolish the TAF hydrolysis activity in HLS9. In the pH 7.2 condition, telaprevir (0.5–50 μM) significantly inhibited the TAF hydrolysis in HLS9, whereas for BNPP, no significant inhibitory effect on TAF hydrolysis was observed even when the BNPP concentration reached 50 μM (P > 0.05).

**CES1-mediated TAF Hydrolysis Was Significantly Impaired by the Genetic Polymorphism G143E.** TAF was incubated with the S9 fractions of Flp-In HEK 293 cells stably expressing wild-type CES1 and the G143E variant. The S9 fractions of the wild-type CES1 cells efficiently hydrolyzed TAF to the intermediate metabolite TFV (2.45 ± 0.30 nmol/mg S9 protein/min). The catalytic activity was reduced by 64% in the S9 fractions of both the G143E and the blank vector-transfected cells (Fig. 4), suggesting that the G143E is a loss-of-function variant for CES1-mediated TAF hydrolysis.

**Discussion**

Identifying key hepatic enzymes in TAF activation is essential to understand the interindividual variability in the PK and anti-HBV efficacy of TAF therapy. A previous study suggested that CES1 was the primary enzyme responsible for TAF hydrolysis in the human liver (Murakami et al., 2015), and the information has been included in the US Food and Drug Administration label of TAF (Vemlidy). This conclusion was based on an in vitro inhibitory study in primary human hepatocytes (Murakami et al., 2015), which showed that the CatA inhibitors telaprevir and boceprevir did not significantly affect TAF activation, whereas the CES1 inhibitor BNPP significantly reduced the formation of...
the TAF active metabolite TFV-DP. Birkus et al. reported that six hydrolases, namely porcine liver carboxylesterase [a close homolog of human CES1 (Lange et al., 2001)], CatA, leukocyte elastase, pancreatic elastase 1, proteinase 3, and cathepsin H, out of the 16 tested enzymes showed catalytic activity toward TAF hydrolysis (Birkus et al., 2008; Birkus et al., 2015). Specifically, CatA exhibited the highest hydrolytic activity (31,000 pmol/µg enzyme/min), which was approximately 35- to 1,148-fold higher than other hydrolases, when TAF was incubated at a single concentration of 100 µM. In the present study, the rhCatA activity was found to be 33,918 ± 1,185 pmol/µg enzyme/min when TAF concentration was 100 µM. Of note, Birkus and colleagues performed the CatA activity assay at pH 6.5, whereas we conducted the CatA activity study at pH 5.2 to mimic the acidic condition in the lysosomes. The enzyme manufacturer also recommended using an acidic reaction buffer (pH 5.2) to determine the CatA catalytic activity. One of the aims of this study was to determine the contributions of CES1 and CatA to the TAF hydrolysis in the liver based on the hepatic protein expression levels and the enzymatic efficiencies on catalyzing TAF hydrolysis (Birkus et al., 2008; Birkus et al., 2016). Nevertheless, their contributions appear to be negligible due to their very low activity as well as very low protein expression in the human liver.

Given that the subcellular locations of CatA and CES1 are different [i.e., CatA: lysosome (pH ~4.7) and CES1: endoplasmic reticulum lumen/cytoplasm (pH 7.1–7.4)] (Casey et al., 2010), the in vitro incubation studies were conducted in two different buffers: MES buffer (pH 5.2) and Tris buffer (pH 7.2). A significant pH-dependent effect was observed for the activity of both CES1 and CatA. It is generally accepted that CatA is an acidic pH-dependent serine protease, and the acidic condition is critical for its catalytic functions. For example, chloroquine, a common agent capable of increasing lysosomal pH, was found to inhibit the intracellular activation of GS-9191, another ester prodrug hydrolyzed by CatA (Birkus et al., 2011). The activity of CES1 was decreased at pH 5.2 compared with that at pH 7.2, which was consistent with the previous report that the CES1 activity was stable in pH 6.5–7.5 (Inoue et al., 1980). Thus, the activity measured in pH 5.2 for rhCatA and pH 7.2 for rhCES1 should reasonably reflect their in situ physiologic catalytic activities.
We conducted an in vitro inhibition study to further elucidate the involvement of CatA and CES1 in hepatic TAF hydrolysis. It has been well documented that telaprevir and BNPP are potent CatA and CES1 inhibitors, respectively (Murakami et al., 2010; Li et al., 2021). Moreover, at the tested concentrations (1–50 μM), telaprevir did not impact the CES1 activity, whereas BNPP had no effect on the activity of CatA (Murakami et al., 2010; Li et al., 2021). Telaprevir at 0.5 μM could markedly inhibit TAF hydrolysis in HLS9 at pH 5.2 and pH 7.2, whereas BNPP only showed a weak inhibitory effect at 50 μM under the pH 7.2 condition. It should be noted that 50 μM BNPP could fully impair CES1 activity (Fujiyama et al., 2010); however, a significant portion of TAF hydrolysis activity remained intact after incubation with 50 μM BNPP. Thus, the inhibitory study further supports a more significant role of CatA in hydrolyzing TAF in HLS9 relative to CES1.

Interestingly, Murakami et al. did not observe a significant inhibitory effect of the protease inhibitors telaprevir and boceprevir on TAF metabolism in primary human hepatocytes (Murakami et al., 2015). This discrepancy might be due to the limited intracellular accumulation of these protease inhibitors as both telaprevir and boceprevir are the substrates of the efflux transporter P-gp, which is highly expressed in hepatocytes (Chu et al., 2013; Fujita et al., 2013; Weiss et al., 2014; Murakami et al., 2015). The lack of inhibitory effects of telaprevir and boceprevir in primary human hepatocytes could also be caused by the potential reduction of CatA expression in hepatocytes under in vitro culture conditions (Richert et al., 2006). In the present study, we performed an in vitro inhibition study using HLS9 samples instead of hepatocytes to avoid the abovementioned two potential limitations and demonstrated a significant inhibitory effect of telaprevir on hepatic TAF hydrolysis.

Taken together, our conclusion that CatA may play a more important role than CES1 in hydrolyzing TAF in the liver is supported by the following three observations: 1) a greater contribution of CatA to TAF hydrolysis in HLS9 than CES1 based on the activity data and the hepatic protein abundances of the two enzymes; 2) a substantial increase of TAF hydrolysis in HLS9 when the incubation study was conducted at the optimal pH for CatA (i.e., pH 5.2); and 3) the CatA inhibitor telaprevir exhibited a more potent inhibitory effect on TAF hydrolysis in HLS9 than the CES1 inhibitor BNPP.

The CES1 genetic polymorphism G143E is a loss-of-function variant for many CES1 substrates (Her and Zhu, 2020). Its minor allele frequencies are approximately 3.7%, 2.0%, and 4.3% in Caucasian, African American, and Hispanic populations, respectively, but the variant is extremely rare in Asian populations (Zhu et al., 2008; Suzaki et al., 2017). The G143E variant abolishes CES1 catalytic activity and subsequently affects the PK and pharmacodynamics of various CES1 substrates of the efavirenz, telaprevir, and angiotensin-converting enzyme inhibitors (Zhu et al., 2008). The minor allele frequencies are approximately 3.7%, 2.0%, and 4.3% in Caucasian, African American, and Hispanic populations, respectively, but the variant is extremely rare in Asian populations (Zhu et al., 2008; Suzaki et al., 2017). The G143E variant abolishes CES1 catalytic activity and subsequently affects the PK and pharmacodynamics of various CES1 substrates of the efavirenz, telaprevir, and angiotensin-converting enzyme inhibitors (Zhu et al., 2008). We found that the G143E variant on the anti-HBV therapy of TAF is modest because of the significant involvement of CatA in hepatic TAF hydrolysis. A further limitation of this study is that the HLS9 system is unable to evaluate the effect of the partition of a drug in cell organelles (e.g., lysosomes) on drug metabolism. As CatA is a lysosomal protein, TAF needs to enter the lysosomes to be hydrolyzed by CatA. Although it remains unexplored regarding how efficient and to what extent TAF can be accumulated in the lysosomes, TAF appears to readily enter into the lysosomes according to a previous report, which showed that TAF was effectively hydrolyzed by CatA in peripheral blood mononuclear cells (Birkus et al., 2007). Moreover, lysosomes have been identified as the organelle involving TAF hydrolysis in MT-2 and peripheral blood mononuclear cells using organelle marker assays (Birkus et al., 2008a).

In sum, our study suggested that CatA may play a major role in hydrolyzing TAF in the human liver. For the first time, the activities of CES1, CatA, and HLS9 on TAF hydrolysis were found to be highly pH dependent, highlighting the importance of using appropriate pH in in vitro drug metabolism studies. Moreover, we showed that the CES1 genetic polymorphism G143E is a loss-of-function variant for CES1-mediated TAF hydrolysis. However, we expect that the impact of this variant on the anti-HBV therapy of TAF is modest because of the significant involvement of CatA in hepatic TAF hydrolysis. Further investigations are warranted to confirm the significance of CatA in activating TAF in human livers using in vivo models.

Authorship Contributions
Participated in research design: Li, Shi, Zhu.
Conducted experiments: Li, Shi, Xiao, Tran.
Performed data analysis: Li, Shi, Xiao, Tran, Wang, Zhu.
Wrote or contributed to the writing of the manuscript: Li, Shi, Wang, Tran, Zhu.

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