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Interaction between HIV Protease Inhibitors (PIs) and Hepatic Transporters in Sandwich Cultured Human Hepatocytes: Implication for PI-based DDIs

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Abstract

Although HIV protease inhibitors (PIs) produce profound metabolic interactions through inactivation/inhibition of CYP3A enzymes, their role as victims of transporter-based drug-drug interactions (DDIs) is less well understood. Therefore, we investigated if the PIs, nelfinavir (NFV), ritonavir (RTV), lopinavir (LPV), or amprenavir (APV) were transported into sandwich-cultured human hepatocytes (SCHH), and whether OATPs contributed to this transport. Our findings showed that except for ^3H -APV, no significant decrease in the total hepatocyte accumulation of the ^3H -PIs was detected in the presence of the corresponding unlabeled PI, indicating that the uptake of the other PIs was not mediated. Further, hepatocyte biliary efflux studies using ^3H -APV and unlabeled APV confirmed this decrease to be due to inhibition of sinusoidal influx transporter(s) and not the canalicular efflux transporters. Moreover, this sinusoidal transport of APV was not OATP-mediated. Our results indicate the hepatic uptake of NFV, RTV, or LPV was primarily mediated by passive diffusion. APV's hepatic uptake was mediated by an unidentified sinusoidal transporter(s). Therefore, NFV, RTV or LPV will not be victims of DDIs involving inhibition of hepatic influx transporters; however, the disposition of APV may be affected if its sinusoidal transport is inhibited.

INTRODUCTION

HIV protease inhibitors (PIs) are a class of antiretroviral agents that are a critical component in the management of HIV infection and Highly Active Antiretroviral Therapy (HAART). Their use in the clinic is associated with profound drug-drug interactions (DDIs) where the PIs are both victims and precipitants. To date, the study of the PI-based DDIs has been focused on CYP3A-mediated mechanisms as many of the PIs are substrates (DDI victims), inducers and potent inactivators (DDI precipitants) of these enzymes [1, 2]. Many of the PI-based DDIs may also be transporter-based as the PIs are inhibitors (DDI precipitants) of ABC transporters (e.g., P-gp, MRPs, BCRP), as well as OATPs [3–6]. In addition, the PIs may be victims of transporter-based DDI as they are substrates of several transporters such as P-gp and MRP2 [7, 8]. For example, in humans, after oral administration of a **single dose** of radiolabeled PIs, these drugs are found to be cleared from the body via extensive hepatic elimination (>80%), of which 88% is accounted by CYP3A metabolism, and ~12% by biliary excretion [9–11]. However, on **chronic administration** of the PIs, which are now almost always co-administered with ritonavir (RTV), the role of biliary excretion of the PIs may be even greater due to CYP3A inactivation [12]. Indeed, this is observed with tipranavir (TPV) after chronic administration of TPV/RTV [13]. Therefore, the role of

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hepatic transporters could become more significant in the hepatic disposition of PIs under chronic administration of PIs and/or RTV-boosted PI regimen.

While PIs' interactions with the canalicular efflux transporters (e.g., P-gp, MRP2, BCRP) as substrates and/or inhibitors have been previously studied, very little is known about their interactions with the sinusoidal influx transporters. Such characterization is important since in order for the PIs to be metabolized in the hepatocytes or be excreted into the bile, they must either diffuse or be transported across the sinusoidal membrane. If transported, DDIs at the level of the sinusoidal uptake transporters could affect the disposition of the PIs. To date, the PIs have been shown to be inhibitors of OATPs, *in vitro* [5], and possibly *in vivo* [17, 18]. However, whether they are substrates of OATPs is controversial. Some studies using primarily oocyte expression systems [14], HepG2 cells [15], or rat hepatocytes [16], have shown that selective PIs may be transported by OATPs. Others, using MDCKII cells transfected with OATP2B1 and caco2 cells [27], have concluded that they are not transported by OATPs. In addition, whether PIs are transported into **human** hepatocytes has not been investigated. It is important to note that when a drug is found to be a low affinity substrate of a transporter in an over-expressing system, the contribution of that transporter *in vivo* or in primary culture of human tissues (e.g., hepatocytes) in the disposition of the drug may be negligible. This is because the expression of that transporter *in vivo* or in the cells will likely be lower than that in the over-expressing cells, and, other processes may contribute to the disposition of the drug (e.g., passive diffusion or other transporters). Therefore, it is critical to determine the contribution of the transporter(s) *in vivo* or a representative cell culture model. Thus, we evaluated the uptake of PIs in sandwich-cultured human hepatocytes (SCHH), a physiologically relevant system that exhibits *in vivo*-like canalicular network and transporters (sinusoidal and canalicular) [28,29]. Additionally, as reported by Bi et al., 2012 and Varma et al., 2012, SCHH have also been proposed as an optimal *in vitro* system to successfully predict transporter-mediated DDIs in human [30,31]. Addressing these questions is important in delineating the role of hepatic influx transporters in the hepatic disposition of the PIs and in understanding PI-based DDIs, where PIs are the victim drugs.

Therefore, the goals of this investigation were to conduct radioactive transport studies to determine: 1) whether the hepatic uptake of the PIs is transporter-mediated in the most physiologically relevant *in vitro* model, the sandwich-cultured human hepatocytes (SCHH), 2) whether the sinusoidal influx transport (if any) is contributed by OATPs. We focused our evaluation on the PIs, RTV, nelfinavir (NFV), lopinavir (LPV), and amprenavir (APV), based on their clinical relevance and/or commercial availability of the radiolabeled compound.

MATERIALS AND METHODS

Materials

³H-RTV (37 GBq/mmol), ³H-LPV (37 GBq/mmol), and ³H-APV (18.5 GBq/mmol) were purchased from Moravek Biochemicals and Radiochemicals (Brea, CA). ³H-NFV (37 GBq/mmol), ³H-estrone-3-sulfate (ES; 2.22 TBq/mmol), and ³H-estradiol-beta-17-glucuronide (EG; 2.22 TBq/mmol) were purchased from American Radiolabeled Chemicals, Inc. (St. Louis, MO). ¹⁴C-mannitol (1.66 GBq/mmol) was purchased from PerkinElmer Life and Analytical Sciences (Boston, MA). Unlabeled PIs were obtained from the National Institutes of Health AIDS Research and Reference Reagent Program. Unlabeled ES, EG, bromosulfophthalein (BSP), 1-aminobenzotriazole (ABT) and sodium butyrate were purchased from Sigma-Aldrich (St. Louis, MO). N-(4-(2-(1,2,3,4-Tetra-hydro-6,7-dimethoxy-2-isoquinoliny)ethyl)phenyl)-9,10-dihydro-5-methoxy-9-oxo-4-acridine carboxamide (elacridar, GF120918) was a generous gift from GlaxoSmithKline (King of

Prussia, PA). 24-well BioCoated culture plates and Matrigel [high concentration (HC)] were from BD Biosciences (San Jose, CA). Hepatocyte plating and maintenance supplement packs, and CHRM® Medium, Williams E medium (WEM), Hanks balanced salt solution (HBSS) containing 10 mM Hepes and adjusted to pH 7.4, either with or without CaCl₂ and MgCl₂ (Ca²⁺ plus or Ca²⁺ minus buffer, respectively), and fetal bovine serum (FBS) were from GIBCO, Invitrogen (Carlsbad, CA). BCA protein assay reagent was from Pierce Chemical (Rockford, IL). Cell lysis buffer was prepared with 10 mM Tris-HCL, pH 8.0, 0.5% Triton X-100, and 1 mM EDTA). Freshly isolated human hepatocytes in a 24-well BioCoat plate with Matrigel overlay were purchased from Celsis (Baltimore, MD), and cryopreserved human hepatocytes were purchased from CellzDirect (Durham, NC).

SCHH tissue culture

Cryopreserved hepatocytes (0.325×10^6 viable cells/well) were thawed and plated in 24-well BioCoat culture plates overlaid with Matrigel following manufacture's protocol (Invitrogen Life Technologies). SCHH were maintained in serum-free supplemented WEM and replenished with fresh media every day until transport studies were conducted on day 6 post-plating for cryopreserved SCHH and day 2 after receipt for freshly isolated SCHH. Hepatocytes were cultured and maintained at 37°C in a humidified incubator with 95% atmospheric air and 5% CO₂.

SCHH transport studies

Due to solubility and non-specific binding issues associated with PIs, 2% FBS and 0.8% DMSO were added to all transport buffers. Positive control transport studies using OATP model substrates, 1 μM ³H-ES (uptake transport studies) with or without BSP, OATP pan-inhibitor or 1 μM ³H-EG (biliary excretion studies) with or without elacridar (P-gp, BCRP and OATP inhibitors) were performed to confirm OATP expression, the formation of canalicular network and the expression of the canalicular efflux transporters in SCHH, respectively [19–22]. All SCHH transport experiments were performed on three independent batches of hepatocytes.

1. SCHH pretreatment with ABT—To prevent significant metabolic depletion of PIs during transport studies, SCHH were pre-incubated with 5 mM ABT (pan-inactivator for CYPs) for 1 h and washed twice using Ca²⁺ plus buffer to eliminate residual ABT before initiation of all transport studies. The efficacy of ABT in preventing metabolism of 0.1 μM ³H-NFV, ³H-RTV, ³H-LPV, and ³H-APV during the transport experiments was confirmed by determining the recovery of the unchanged drug in both the transport buffer and cell lysates by LC/UV coupled with fraction collection. To confirm that ABT does not affect the OATP-mediated transport of ³H-ES, transport studies using 1 μM ³H-ES were also conducted after 1 h pre-incubation with or without 5 mM ABT.

2. SCHH uptake studies—Unlike transport experiments performed using hepatocytes in suspension, where a greater cellular surface area allows rapid uptake and thus shorter incubation period to evaluate initial uptake, transport studies performed using plated cell systems (e.g., SCHH, or cell lines) require longer incubation times [4,21,29]. Indeed, after evaluating in our pilot studies the hepatic uptake of ³H-Pis after 2, 5, 15, or 25 min of incubation, we found that a minimum of 10 min incubation period was required to provide sufficient intracellular accumulation and dynamic range of ³H-Pis radioactivity for downstream inhibition studies to evaluate potential uptake transport mechanisms. Additionally, our pilot studies demonstrated that the uptake of ³H-Pis was linear up to 25 min of incubation. To determine if the uptake of the ³H-Pis into the SCHH was temperature-dependent we first pre-incubated SCHH with Ca²⁺ plus buffer for 10 min at either 4°C or 37°C, followed by replacing the pre-incubation buffer with transport buffer (Ca²⁺ plus

buffer) containing 0.1 μM ^3H -NFV, ^3H -RTV, ^3H -LPV, or ^3H -APV, and then incubating the SCHH for 0, 10 or 20 min at 4°C or 37°C. To determine if this uptake was transporter-mediated, we pre-incubated the SCHH at 37°C for 20 min with Ca^{2+} plus buffer containing unlabeled NFV, RTV, LPV, APV (20 or 100 μM ; self-inhibition studies), or the OATP inhibitor, BSP (20 or 100 μM), followed by a 20 min incubation with transport buffers (Ca^{2+} plus buffer containing 0.1 μM ^3H -NFV, ^3H -RTV, ^3H -LPV, or ^3H -APV and the corresponding unlabeled PIs, or BSP). At the end of the incubation, the transport buffer was harvested; cells were washed twice with ice-cold PBS buffer, and then lysed with the lysis buffer (0.5 mL/well). 50 μL aliquots of the cell lysates were saved for protein quantification using the BCA assay, as per manufacturer's instructions. Radioactivity was measured in both the transport buffer and cell lysates. The cell-to-media radioactivity ratio was normalized to the protein content of the hepatocytes and used as the uptake index.

3. SCHH biliary excretion studies—To measure the biliary efflux of ^3H -APV, SCHH were first pre-incubated for 10 min with either Ca^{2+} plus buffer or Ca^{2+} minus buffer at 37°C, followed by a second pre-incubation (20 min) with Ca^{2+} plus or Ca^{2+} minus buffer containing elacridar or unlabeled APV at 20 or 100 μM . Then, the SCHH were incubated for another 20 min with Ca^{2+} plus or Ca^{2+} minus buffer containing 0.1 μM ^3H -APV, with or without the inhibitors. The Ca^{2+} plus buffer maintains tight junction integrity and bile canalicular network, allowing measurement of drug efflux across the sinusoidal and canalicular membranes and total accumulation in the cell and bile. However, the Ca^{2+} minus buffer disrupts the tight junctions and opens bile canalicular networks, and therefore measures uptake across the sinusoidal membrane only [21]. Accumulation of the radioactivity was measured at the end of the transport period, and the protein content normalized cell-to-media radioactivity ratio was determined. Biliary excretion of the ^3H -APV was calculated using the following equation:

$$\text{Biliary Excretion (\%)} = \frac{\left(\frac{^3\text{H in cell lysate}}{^3\text{H in media}} \right) \text{ in } \text{Ca}^{2+} \text{ plus buffer} - \left(\frac{^3\text{H in cell lysate}}{^3\text{H in media}} \right) \text{ in } \text{Ca}^{2+} \text{ minus buffer}}{\left(\frac{^3\text{H in cell lysate}}{^3\text{H in media}} \right) \text{ in } \text{Ca}^{2+} \text{ plus buffer}} \times 100\%$$

Statistical Analysis

Statistical assessments were performed using a paired two-tailed Student's T-test, with significance level defined as $p < 0.05$

RESULTS

After 1 h pre-treatment with 5 mM ABT, at the end of the uptake experiments, all ^3H -PIs were recovered as unchanged drug (>98%) from both the transport buffer and hepatocyte lysate, confirmed via LC-UV analysis coupled with radioactivity fractionation of ^3H -PIs or metabolites. This indicates that the metabolism of the PIs, which can potentially confound the interpretation of uptake experiments, was eliminated. The uptake of ^3H -ES, an OATP positive control substrate, was not affected by the 1 h pre-treatment of the SCHH with 5 mM ABT (Fig. 1).

During the first 20 min of transport studies, SCHH uptake of ^3H -ES or ^3H -PIs was within the linear range. The SCHH showed robust hepatic OATP activity as demonstrated by the significant decrease in the hepatic uptake of ^3H -ES in the presence of 20 or 100 μM BSP (Fig. 1). Of the four PIs, only ^3H -APV and ^3H -LPV demonstrated significant increase in 20 min SCHH uptake at 37°C vs. 4°C (Fig. 2A–D). The uptake of none of ^3H -PIs was affected by the presence of the OATP inhibitor, BSP (20 or 100 μM). Likewise, the uptake of the ^3H -

NFV, ^3H -RTV, or ^3H -LPV was unaffected by the corresponding unlabeled PI (Fig. 3A–C). However, the uptake of ^3H -APV was significantly decreased by unlabeled APV (Fig. 3D).

Of the four PIs, only ^3H -APV demonstrated both temperature-dependent uptake and self-inhibition (by the unlabeled APV) uptake in SCHH. Since our uptake data are based on radioactivity in the cell lysate plus canalicular space, these observations could be a result of potential inhibition of ^3H -APV transport into the SCHH and/or inhibition of ^3H -APV efflux at the canalicular membrane. To examine the contribution of biliary efflux in the hepatocyte uptake of APV, we evaluated the biliary excretion of ^3H -APV in the absence or presence of elacridar (P-gp and BCRP inhibitors). The hepatocyte uptake of ^3H -APV in the Ca^{2+} plus buffer was significantly different from that in the Ca^{2+} minus buffer and remained so in the presence of 20 μM elacridar (Fig. 4A). However, this difference was ablated in the presence of 100 μM elacridar due to a decrease in ^3H -APV uptake in the presence of Ca^{2+} with no effect on ^3H -APV uptake in the absence of Ca^{2+} . As a result, the biliary excretion of ^3H -APV was significantly decreased. Furthermore, in the presence of unlabeled APV (20 or 100 μM), the hepatocyte uptake of ^3H -APV remained significantly different between the Ca^{2+} plus and Ca^{2+} minus conditions, and the biliary excretion of ^3H -APV was unaffected and remained at ~35% (Fig. 4B). In the presence of 20 or 100 μM APV, the hepatocyte uptake of ^3H -APV under the Ca^{2+} plus or Ca^{2+} minus condition was significantly decreased and by similar magnitudes. To confirm the integrity of the canalicular membrane and the expression of the canalicular efflux transporters in the SCHH used for these biliary excretion studies, we measured the biliary excretion of ^3H -EG (a positive control for biliary excretion). Biliary excretion of ^3H -EG decreased from 35% in the absence of elacridar to < 5% in the presence of 100 μM elacridar (Fig. 4C).

DISCUSSION

Many of the PI-based DDIs can be explained by CYP3A-mediated mechanisms (inhibition, inactivation and/or induction). However, as presently used in the clinic (usually chronic RTV-boosted PI regimen), where CYP3A-mediated metabolism is minimized, biliary excretion is likely the predominant route of hepatic elimination for the PIs. In addition, for the PIs to be metabolized and excreted in the bile, they must first enter the hepatocytes by transport or by diffusion. To better understand PIs hepatic transport *in vivo*, and the potential role of the sinusoidal influx transporters in the transporter-based DDIs, where PIs are potential victim drugs, we investigated the hepatic transport of PIs in the SCHH model.

SCHH offers a superior and more comprehensive *in vitro* model system than other alternatives (e.g., transfected cell lines, HepG2 cells, oocyte expression systems), because they express, as *in vivo*, both the sinusoidal and canalicular membrane transporters [23]. By confirming that the hepatic uptake of ^3H -PIs is within the linear range and that the activity of known sinusoidal influx transporters (e.g., OATPs) is robust during the 10–20 min of SCHH transport studies, we were able to evaluate PIs hepatic uptake in a most *in vivo*-like model, and study the importance of the potential sinusoidal influx transporters relative to other competing processes present *in vivo* (e.g., diffusion, sinusoidal and/or canalicular efflux). However, the presence of the canalicular membrane transporters can complicate interpretation of transport data from cell lysates as the lysate measurements include the ^3H -substrate (e.g., PIs) in both the bile as well as in the hepatocytes. In addition, biliary efflux of the unchanged ^3H -substrate into the canalicular space can operate as a sink and driving force that can seemingly “accumulate” a drug in the SCHH, which includes the bile. Similarly, when the ^3H -substrate is extensively metabolized in the hepatocytes, such “accumulation” will also be observed in the absence of any sinusoidal uptake transport when only total drug radioactivity (parent plus metabolites) is monitored. Under both circumstances, this “accumulation” of the ^3H -substrate can be erroneously interpreted as

hepatic transport even when no sinusoidal transport is present. Therefore, when using the SCHH to study hepatic drug uptake, two guiding principles must be followed. First, the parent ^3H -substrate concentration must be monitored or its hepatic metabolism must be minimized. Second, sinusoidal transport must be distinguished from canalicular membrane transport. In this communication we followed these two guiding principles.

To eliminate hepatocyte metabolism of the PIs, we pre-incubated all SCHH with 5 mM ABT for 1 h before conducting our transport studies, and confirmed the lack of hepatocyte metabolism of the ^3H -PIs via LC/UV coupled with radioactivity fractionation. Previous studies conducted in human liver microsomes and hepatocytes showed that pre-incubation with ABT (>2 mM), a potent CYP inactivator, can effectively abolish the activities of multiple CYPs [24, 16]. In addition, ABT does not appear to be an inhibitor of the transporters expressed in the human hepatocytes, as Kimoto et al., 2012, found ABT (1 mM) does not inhibit the hepatocyte transport of rosuvastatin or atorvastatin, which is known to be mediated by OATPs, MRP2, BCRP, and P-gp [25]. To distinguish between biliary efflux and sinusoidal transport, we determined the hepatocyte uptake of the ^3H -PIs in the presence and absence of the canalicular network.

Of the PIs studied, only ^3H -APV and ^3H -LPV demonstrated significantly greater hepatocyte accumulation at 37°C vs. 4°C, suggesting that only these two PIs appear to be transported into the hepatocytes. However, since temperature-sensitive hepatic accumulation of ^3H -LPV can be an experimental artifact, potentially due to the temperature dependent changes in diffusion, membrane fluidity and permeability; we conducted self-inhibition studies using up to 100 μM of unlabeled PIs as inhibitors to determine whether the hepatic accumulation of these two PIs was due to transporter-mediated process. These studies showed that the hepatic accumulation of ^3H -APV was self-inhibited while that of ^3H -LPV was not. Unless LPV is a very low affinity substrate of hepatic influx transport, our results indicate that only the hepatic accumulation of ^3H -APV was transporter-mediated.

As discussed above, hepatic accumulation of ^3H -APV could be a result of canalicular efflux transporters and/or sinusoidal influx transporters. Therefore, to identify the site of transport, we determined the uptake of ^3H -APV in the presence and absence of the canalicular network. As expected, our results demonstrate that in the absence of metabolism, ^3H -APV underwent extensive biliary excretion (~40%), which was significantly decreased to ~11% in the presence of 100 μM elacridar, a P-gp/BCRP inhibitor that does not inhibit MRP2. Since APV is not a BCRP substrate [6], but a P-gp substrate [7], these data indicate that the biliary excretion of APV is likely mediated by P-gp. In contrast, the biliary excretion of ^3H -APV was unaffected in the presence of unlabeled APV. Furthermore, the uptake of ^3H -APV in SCHH with intact canalicular network (Ca^{2+} plus condition) and disrupted canalicular network (Ca^{2+} minus condition) was decreased by similar magnitudes (~8-fold in the presence of 100 μM APV). Together, these results indicate that the significant decrease in the hepatocyte uptake of ^3H -APV (Ca^{2+} plus condition) in the presence of unlabeled APV is due to inhibition of the sinusoidal uptake transporters and not due to inhibition of the biliary efflux of ^3H -APV. We speculate that APV may have higher affinity for the sinusoidal transporter(s) than for P-gp and that the intracellular concentrations of APV may not have reached the concentration sufficient to inhibit P-gp.

Based on prior studies that indicate that the PIs can be transported by OATPs, we investigated if the sinusoidal transport of APV was mediated by hepatic OATPs. It is not. Several lines of evidence indicate that sinusoidal OATPs are not involved in the uptake of APV or any of the other PIs studied. First, the hepatic uptake of all four ^3H -PIs, including ^3H -APV, was not inhibited by BSP, a classical pan-OATP inhibitor. Second, the ^3H -APV hepatic uptake in the absence of Ca^{2+} was not inhibited by elacridar. Elacridar,

besides inhibiting P-gp/BCRP, also inhibits OATPs [19]. This is consistent with our own finding that showed elacridar inhibited the uptake of ^3H -EG into the hepatocytes in the absence of Ca^{2+} (Fig. 4C). The fact that both elacridar and BSP did not inhibit the sinusoidal influx transport of APV suggests that allosterism of OATPs [26, 27], is not a likely explanation for the observed lack of inhibition. This is further supported by the fact that we did not observe self-inhibition of ^3H -PI transport in the presence of the corresponding unlabeled PIs.

The lack of transport of the PIs by OATPs is not inconsistent with data from others who have studied human OATP-mediated transport of the PIs. For example, Kis et al., 2010 confirmed that RTV is not a substrate of OATP2B1 (also expressed hepatically) using both MDCKII cells transfected with OATP2B1, and Caco-2 cells [28]. In the same study, atazanavir (ATV) was also shown to not be an OATP2B1 substrate, and both RTV and ATV were transported into Caco-2 cells by an unknown pH-dependent active influx transporter. However, a previous study that did report OATP-mediated transport of PIs (lopinavir, saquinavir or darunavir) found low or marginal affinity transport of the PIs, based on results of <2 fold difference between the ^3H -PIs accumulation in OATP overexpressing and control oocytes, compared to ~10 fold difference observed in the accumulation of ^3H -ES (standard OATP substrate) [14]. Kinetic analysis of saquinavir uptake into OATP1A2 overexpressing oocytes reported a high K_m of $36.4 \pm 21.8 \mu\text{M}$, and this value increased to $94.6 \pm 22.8 \mu\text{M}$ in Hep-G2 cells [15]. OATP1B1 genetic polymorphism (521T>C, V174A) has been reported to be associated with slightly higher LPV plasma concentration [14]. Indeed, the evaluation in LPV plasma $C_{\min}$ concentration (10–14 h postdose) was marginal (TT homozygotes: 8.5 ng/mL, TC heterozygotes: 8.7 ng/mL, and CC heterozygotes [6 out of 400 patients]: 9 ng/mL), suggesting that the contribution of OATPs (if any) to the disposition of LPV is small. Therefore, we propose that except for APV (which is transported into the SCHH by as yet unknown transporter), the PIs are unlikely to be significantly transported by OATPs *in vivo*. If they were transported, this contribution is minimal. Furthermore, due to the lower, but more *in vivo*-like OATPs expression levels in human hepatocytes than in the overexpression systems, diffusion across the cell membrane is likely the dominant mechanism for the *in vivo* hepatic uptake of the PIs studied here.

In conclusion, our results indicate that NFV, RTV, LPV and APV are not substrates (or are poor substrates) of human hepatic OATPs; however, *in vivo*, APV may be transported into the liver by an unidentified sinusoidal transporter(s) provided this transporter(s) is not saturated at the unbound plasma concentrations ($C_{ss,\max}$ and $C_{ss,av}$ of $1.35 \mu\text{M}$ and $0.28 \mu\text{M}$ respectively) at the usual clinical doses of APV (1200 mg b.i.d; APV is now administered as the prodrug, fosamprenavir). In contrast to APV that exhibits xlogP value of 1.8, NFV, RTV, and LPV have higher xlogP values (4.6, 3.9, and 3.9). Therefore, the hepatocyte uptake of NFV, RTV or LPV is likely to be primarily mediated by passive diffusion, and the contribution of sinusoidal active uptake may only be significant in the hepatic uptake of APV. Further studies are needed to identify this transporter(s). It would also be interesting to determine if PIs not studied here (e.g., darunavir) are substrates of this unidentified transporter(s). Based on our results, we propose that inhibitors and/or inducers of hepatic uptake transporters (including OATPs) will not affect the hepatic disposition of NFV, RTV or LPV. However, all four PIs are OATP inhibitors in both SCHH and OATP-transfected cell lines, and therefore have the potential to produce, and in some cases do produce, clinically significant OATP-based DDI (e.g., with statins) [17, 18].

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Nonstandard Abbreviations

DDI	drug-drug interaction
PIs	HIV Protease inhibitors
RTV	ritonavir
NFV	nelfinavir
APV	amprenavir
SQV	saquinavir
ATV	atazanavir
LPV	lopinavir
DRV	darunavir
SCHH	sandwich cultured human hepatocytes

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Figure 1.

The hepatic uptake of ^3H -ES (1 μM), an OATP positive control transport substrate, was not affected by 1 h pre-treatment of 5 mM ABT (N=3 donor sources; N=2 donor sources in the absence of ABT). However, the uptake of ^3H -ES, in the presence of ABT was decreased by 2.67 or 3.84-fold in the presence of 20 or 100 μM BSP, respectively, confirming robust OATP expression in SCHH (* $p < 0.05$; mean $\pm$ SD, N=3 donor sources).

Figure 2.

A–D. Of the four PIs, only ^3H -APV (**D**) and ^3H -LPV (**C**) demonstrated statistically significant decrease in their 20 min SCHH uptake at 4°C vs. 37°C. These results indicate that ^3H -NFV (**A**) and ^3H -RTV (**B**) are not transported into SCHH. Data are expressed as mean $\pm$ SD, N=3 donor sources. * $p < 0.05$

Figure 3.

A–D. The SCHH uptake of all of the ^3H -PIs, (A) ^3H -NFV, (B) ^3H -RTV, (C) ^3H -LPV, (D) ^3H -APV, was not affected by BSP (20 or 100 μM). Likewise, except for ^3H -APV, the uptake of the ^3H -PIs was unaffected in self-inhibition studies (in the presence of unlabeled PIs). The uptake of ^3H -APV (D), was significantly decreased (6.04 $\pm$ 1.52 fold, *** p <0.001 and 9.39 $\pm$ 1.20 fold, ** p <0.01) by unlabeled APV (20 or 100 μM , respectively). Data are expressed as mean $\pm$ SD, N=3 donor sources.

Figure 4.

A–C. (A) SCHH uptake of ^3H -APV in the presence of Ca^{2+} was significantly greater than that in the absence of Ca^{2+} under all conditions ($\dagger$: $p < 0.05$), except when elacridar was present at 100 μM concentration. As a result, biliary efflux of ^3H -APV remained unchanged in the presence of 20 μM elacridar, but was decreased from ~40% to 11% (~3.9-fold, $**p < 0.01$) in the presence of 100 μM elacridar. Data are expressed as mean $\pm$ SD, $N=4$ donor sources. (B) In the presence of unlabeled APV (20 or 100 μM), the biliary excretion of ^3H -APV was unaffected and remained at ~35%. The hepatocyte uptake of ^3H -APV under the Ca^{2+} plus or Ca^{2+} minus condition was significantly decreased in the presence of 20 μM APV (Ca^{2+} plus or Ca^{2+} minus: ~6-fold; $**p < 0.01$), or 100 μM APV (~8-fold; Ca^{2+} plus: $*p < 0.05$, or Ca^{2+} minus: $**p < 0.01$). Data are expressed as mean $\pm$ SD, $N=3$ donor sources. (C) Biliary efflux of ^3H -EG (positive control) in the absence and presence of elacridar confirmed intact canalicular network and the expression of the canalicular efflux transporters in the SCHH. In the presence of elacridar and under the Ca^{2+} minus condition, the decrease in SCHH uptake of ^3H -EG was likely due to inhibition of OATPs. Data are expressed as mean, $N=2$.