Clearance Prediction of HIV Protease Inhibitors in Man: Role of Hepatic Uptake

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ABSTRACT: The aim of this work was to explore the contribution of the organic anion transporting polypeptide-1B (OATP1B) drug transporters in the hepatic clearance (Cl) of all marketed HIV protease inhibitors (PI) in humans. HIV PI uptake rates in OATP1B1/1B3-transfected Chinese hamster ovary cells were converted to uptake Cl values in human hepatocytes via a relative activity factor, which was determined by comparing uptake of known substrates between OATP1B1/3-transfected cells and human hepatocytes. Metabolic Cl values were determined in human liver microsomes. *In vivo* hepatic Cl values were calculated either by combining drug uptake and metabolism or based on one of these individual Cl processes and compared with published *in vivo* hepatic Cl values. Excellent *in vitro–in vivo* correlation ($R^2 = 0.85$) was observed when only uptake Cl values were used, but not when only metabolic Cl was used ($R^2 = 0.40$). The correlation did not improve when both processes were taken into account ($R^2 = 0.85$). PBPK models confirmed the remarkable sensitivity of predicted exposure to hepatic drug uptake, indicating a key role for OATP1B1/3 in hepatic disposition of several HIV PI in man. This may contribute to the interindividual variability in systemic and hepatic exposure to these drugs in the clinic. © 2015 Wiley Periodicals, Inc. and the American Pharmacists Association J Pharm Sci

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INTRODUCTION

Over the last decades, hepatic uptake transporters have been recognized to play a determining role in the hepatic disposition of many endogenous and exogenous compounds. In particular, members of the organic anion transporting polypeptide family (OATP; *SLCO* gene family) influence the hepatic Cl of many drugs. The most important members of the OATP family in the basolateral membrane of the hepatocyte are the hOATP1B1 (*SLCO1B1*) and hOATP1B3 (*SLCO1B3*) isoforms.[1] Genetic polymorphism as well as inhibition or induction of OATP activity can lead to altered plasma concentrations and clinically important drug–drug interactions.[2–4]

HIV protease inhibitors (PI) represent an important class of therapeutic agents in the currently recommended treatment (highly active antiretroviral therapy) of HIV infection. Among the HIV PI that have been approved, lopinavir (LPV), atazanavir (ATV), darunavir (DRV), and fosamprenavir are most frequently prescribed.[5,6] HIV PI are extensively metabolized by CYP3A enzymes[7,8] and their biliary excretion is mediated by the efflux transporters P-gp (*ABCB1*) and MRP2 (*ABCC2*).[8,9] In addition, it has been shown that some HIV PI (or their metabolites) are excreted via the feces for more than 75%.[8] This illustrates that hepatic elimination is much more important than renal elimination for these drugs.

Based on experiments with clinically relevant concentrations of nelfinavir (NLV), ritonavir (RTV), and saquinavir (SQV) in suspended rat hepatocytes, Parker and Houston reported that hepatic uptake rather than metabolism is the rate-limiting factor in the hepatic disposition of these compounds.[10] Moreover, in addition to a carrier-mediated process, passive transmembrane permeation significantly contributed to the hepatic uptake of SQV and RTV.[11] In general, previous results imply that a carrier-mediated process is involved in the hepatic uptake of HIV PI. It follows that the "interplay" between transport and metabolism has to be considered for these compounds.[12,13]

Several studies have illustrated that HIV PI inhibit the *in vitro* cellular uptake of OATP1B probe substrates like estradiol-17β-D-glucuronide, CGamF and sodium fluorescein.[14–17] Moreover, it has been suggested that inhibition of these membrane transporters contributes to clinically important antiretroviral drug–drug interactions, for example, with cerivastatin and atorvastatin.[18] While these examples certainly illustrate the clinical relevance of OATP modulation by HIV PI, the concept of meaningful OATP-mediated transport of HIV PI remains controversial. Current evidence in this respect includes OATP1A2-mediated transport of SQV in HepG2 cells.[19] Consistently, Hartkoorn et al.[20] showed that SQV, DRV, and LPV have affinity for both OATP1A2 and OATP1B1 and that SQV is also transported by OATP1B3. Furthermore, it has been shown that the single nucleotide polymorphism 521T>C in *SLCO1B1* is associated with significantly increased plasma concentrations of LPV.[20,21] In contrast, a recent publication showed that the hepatic uptake of NLV, LPV, and RTV is primarily mediated by passive diffusion in sandwich-cultured human hepatocytes.[22] Additionally, they suggested an

Abbreviations used: APV, amprenavir; ATV, atazanavir; CHO, Chinese hamster ovary; Cl, clearance; DMEM, Dulbecco's modified eagle medium; DRV, darunavir; FBS, fetal bovine serum; ES, estrone-3-sulfate; HBSS, Hanks' balanced salt solution; HEPES, 4-(2-hydroxyethyl)-1-piperazine ethanesulfonic acid; IDV, indinavir; LPV, lopinavir; NLV, nelfinavir; OATP/Oatp, organic anion transporting polypeptide (human/rat); OCT/Oct, organic cation transporter (human/rat); PI, protease inhibitors; PBS, phosphate buffered saline; RAF, relative activity factor; RTV, ritonavir; SQV, saquinavir; TPV, tipranavir

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unidentified sinusoidal uptake transporter for amprenavir (APV). These conflicting findings highlight the need for further exploration of the exact role of transporter-mediated hepatic uptake of HIV PI in their disposition profiles. Therefore, this study aimed to investigate the contribution of hepatic uptake for all marketed HIV PI in one study under equal conditions. To achieve this goal, *in vitro* data for uptake and/or metabolism were extrapolated to *in vivo* hepatic Cl values and retrospectively compared with published non-renal Cl values.

MATERIALS AND METHODS

Chemicals

Ritonavir, indinavir (IDV) sulfate, SQV mesylate, and NLV mesylate were obtained from Hetero Drugs Limited (Hyderabad, India). APV was kindly donated by GlaxoSmithKline (Middlesex, UK). ATV was provided by Bristol-Myers Squibb (New Brunswick, New Jersey), and DRV, LPV, and tipranavir (TPV) were obtained through the NIH AIDS Reagent Program. Dulbecco's modified eagle medium (DMEM), Hanks' balanced salt solution (HBSS), L-glutamine, fetal bovine serum (FBS), penicillin–streptomycin mixture (contains 10,000 IU potassium penicillin and 10,000 μg streptomycin sulfate per mL in 0.85% saline), and Trypsin EDTA were purchased from Lonza SPRL (Verviers, Belgium). Geneticin G418 was purchased from Invitrogen (Paisley, UK). HEPES [4-(2- hydroxyethyl)-1-piperazineethanesulfonic acid] was purchased from MP Biochemical (Illkirch, France). Triton X-100, bovine serum albumin, sodium fluorescein, L-proline, and sodium butyrate were purchased from Sigma–Aldrich (Schnelldorf, Germany). For uptake experiments with suspended human hepatocytes, cryopreserved human hepatocytes were re-suspended in Krebs-Henseleit Buffer (NaCl 130 mM, KCl 5.17 mM, $CaCl_2$ 1.2 mM, $MgCl_2$ 1.2 mM, HEPES 12.5 mM, glucose 11.1 mM, Na-pyruvate 5 mM; pH 7.4).

Culture of OATP-Transfected CHO Cells

Wild-type and OATP1B1- and OATP1B3-transfected Chinese hamster ovary (CHO) cells were cultured at passage 45–65, as described previously.[15] CHO cells were grown in 75 cm^2 T-Flasks in DMEM containing 1 g/1 D-glucose, 1 mM L-glutamine, 25 mM HEPES, and 110 mg/L sodium pyruvate, supplemented with 10% FBS, 50 μg/mL L-proline, 100 IU/mL penicillin, and 100 μg/mL streptomycin. The culture media of the transfected cell lines additionally contained 500 μg/mL geneticin. Cells were incubated at 5% CO_2 and 37°C. For uptake experiments, wild-type CHO cells were seeded in 12-well cell culture plates (Greiner-Bio-One, Wemmel, Belgium) at a density of 20,000 cells/well, whereas CHO-transfected cells were seeded at a density of 25,000 cells/well. Culture medium was replaced every other day and uptake experiments were performed on days 4–5 after seeding when cells were reaching confluency. One day before the experiment, both wild-type and transfected cells were additionally treated with 5 mM sodium butyrate to induce gene expression.

Uptake Studies in Transfected CHO Cells

Uptake experiments in transfected CHO cells were performed as described previously.[15] Briefly, cells were washed twice with 1 mL/well pre-warmed uptake buffer (HBSS with 10 mM HEPES, pH 7.4) and pre-incubated for 5 min at 37 °C. After the pre-incubation, uptake buffer was aspirated and 1 mL/well of uptake buffer, containing the desired substrate concentration was added. Cells were incubated for 30 s at one clinically relevant test concentration, corresponding to the maximum *in vivo* unbound plasma concentrations (see Table 1). Consequently, medium was quickly aspirated and cells were rinsed three times with ice-cold uptake buffer. Cells were lysed with 0.3 mL/well of a 70/30 methanol/water mixture [or 0.5% Triton X in phosphate buffered saline (PBS) for CGamF and estrone-3-sulfate (ES)] and placed on a plate shaker for 30 min at room temperature. Cell lysates were transferred to a 1.5 mL microcentrifuge tube. Samples were stored at −20°C until analysis. For ES, a 200 μL sample was transferred to a scintillation vial containing 2 mL of scintillation cocktail and radioactivity was quantified using liquid scintillation spectrometry (Wallac 1410, Finland). For CGamF, fluorescence was measured using fluorescence spectroscopy (excitation/emission wavelength; 494/520 nm) in a Tecan Infinite M200 plate reader (Tecan Benelux, Mechelen, Belgium). Mean protein content/well was determined by measuring protein content of three wells for each batch of CHO cells using a BCA Protein assay kit (Pierce Chemical, Rockford, Illinois).

Table 1. Comparison of the Experimental Concentrations Used in the Present Study to the Reported Maximum Unbound Peak Plasma Concentrations of HIV PI

Substrate	*In Vitro* Concentration (μM) (Present Study)	Fu	Unbound Peak Plasma Concentrations (μM) (Reported)	Reference
Amprenavir	1	0.1	1–3.2	23
Atazanavir	1	0.14	0.6–1.3	24
Darunavir	1	0.07	0.3–1.1	25,26
Indinavir	2.5	0.35	1.7–4.4	27
Lopinavir	0.25	0.02	0.3	28
Nelfinavir	0.25	0.02	0.1	29
Ritonavir	0.25	0.01	0.16	30
Saquinavir	0.25	0.02	0.11–0.3	31
Tipranavir	0.25	0.001	0.06–0.19	25

Uptake Studies in Cryopreserved Human Hepatocytes

Cryopreserved human hepatocytes were provided by Life Technologies (Invitrogen, Durham, North Carolina). Uptake experiments in human hepatocytes were performed as described previously.[32] After thawing the hepatocytes at 37°C, cells were re-suspended in thawing medium [consisting of DMEM, 10% (v/v) FBS, 100 IU/mL penicillin, 100 μg/mL streptomycin, 4 μg/mL insulin, and 1 μM dexamethasone] and centrifuged at 168*g* for 20 min. To wash the cells, the pellet was re-suspended in 15 mL of thawing medium and centrifuged at 50*g* for 3 min. Subsequently, hepatocytes were re-suspended in uptake buffer and cell viability and yield were determined with the Trypan blue method. The cell suspension was further diluted with uptake buffer to a cell density of 2 million cells/mL (double-concentrated cell suspension) and kept on ice until the start of the uptake experiments. Before the incubation, the double-concentrated cell suspension (500 μL) was pre-incubated for 5 min at 37°C. Subsequently, 500 μL of a pre-warmed double-concentrated CGamF solution (20 μM) was added to initiate the incubation. To determine the non-saturable uptake component,

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