Solubility Profiling of HIV Protease Inhibitors in Human Intestinal Fluids

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ABSTRACT: The present study pursued to profile the intestinal solubility of nine HIV protease inhibitors (PIs) in fasted- and fed-state human intestinal fluids (FaHIF, FeHIF) aspirated from four volunteers. In addition, the ability of fasted- and fed-state simulated intestinal fluids (FaSSIF, FeSSIF) to predict the intestinal solubility was evaluated. All PIs were poorly soluble in FaHIF (from 7 μM for ritonavir to 327 μM for darunavir) and FeHIF (from 15 μM for atazanavir to 409μM for darunavir). For four of nine PIs, food intake significantly enhanced the solubilizing capacity of intestinal fluids (up to 18.4-fold increase for ritonavir). The intersubject variability (average coefficient of variance $CV_{fed} = 60.6\%$, $CV_{fasted} = 40.4\%$) was higher as compared with the intrasubject variability ($CV_{fed} = 41.3\%$, $CV_{fasted} = 20.5\%$). PI solubilities correlated reasonably well between FaSSIF and FaHIF ($R = 0.817$), but not between FeSSIF and FeHIF ($R = 0.617$). To conclude, postprandial conditions increased the inter- and intrasubject variability of the PIs. The inability of FeSSIF to accurately predict the FeHIF solubility emphasizes the need for a multivariate approach to determine solubility profiles, taking into account solid-state characteristics, pH, mixed bile acid/phospholipid micelles, and digestive products. © 2013 Wiley Periodicals, Inc. and the American Pharmacists Association J Pharm Sci

Keywords: HIV protease inhibitors; solubility; human intestinal fluids; FaSSIF; FeSSIF; food interactions; *in vitro* models; poorly water-soluble drugs; intestinal absorption

INTRODUCTION

Treatment of HIV infection involves the use of first- and second-generation protease inhibitors (PIs). Despite their tremendous success worldwide, their low oral bioavailability remains a major drawback.[1] The low bioavailability of the PIs led to the high intersubject variability, increasing the likelihood of subtherapeutic concentrations and thus affecting the *in vivo* performance of the PIs. The low and variable drug exposure could have serious clinical implications, given the dependence of the treatment effectiveness on consistent and adequate drug exposure.

The variable bioavailability of the PIs can be addressed by an indepth understanding of the underlying reasons. These involve both intestinal absorption issues and first-pass elimination of PIs. All PIs were identified as substrates of the enzyme cytochrome P450 3A4 (CYP3A4) present in intestinal epithelial cells and hepatocytes, thus limiting the concentration of PIs reaching the systemic circulation.[2–6] Furthermore, all PIs were determined to be substrates of the efflux transporter P-glycoprotein, resulting in a limited absorption of the PIs.[7–9] Several PIs were also identified as potent inhibitors of hepatic uptake transporters (including organic anion transporter polypeptide) and multidrug-resistance-associated protein-2, leading to important drug–drug interactions and deranged pharmacokinetic profiles.[10–12] Altogether, these various mechanisms contribute to the variable bioavailability.

In addition to the first-pass elimination, intraluminal dissolution and solubility issues may contribute to the variability in the oral bioavailability. The PIs are well known for their low aqueous solubility because of their inherently high lipophilicity.[13] However, in clinical practice, high doses of PIs are needed to reach effective therapeutic concentrations and compensate for the significant first-pass effect (Table 1). The poor solubility of PIs has been addressed by using formulation strategies including cosolvency (i.e., amprenavir, APV), salt formation (i.e., atazanavir sulfate, AZV; indinavir sulfate, IDV; nelfinavir mesylate, NFV; and saquinavir mesylate, SQV), lipid-based formulations (lopinavir, LPV; ritonavir, RTV; and tipranavir, TPV), and solid dispersions[14,15] (LPV and RTV). Darunavir (DRV) is used as ethanolate. Unfortunately, these absorption-enabling strategies do not completely resolve the wide interindividual pharmacokinetic variability for the different PIs, stressing the need for additional profiling.[16] To date, drug solubilities were mainly determined using plain phosphate buffers resulting in a lack of biorelevant solubility data. Recently, fasted- and fed-state simulated intestinal fluids (FaSSIF and FeSSIF) were introduced as promising solvent systems mimicking the intraluminal environment more closely by the presence of mixed micelles of taurocholate (TC) and lecithin.[17] However, these simulated media still insufficiently capture the complex and variable composition of human intestinal fluids (HIF). For instance, the absence of lipid-degradation products may result in an inadequate prediction of the intraluminal solubility in fed-state conditions.

Therefore, the aim of the present study was an in-depth profiling of the solubility of PIs in HIF aspirated from healthy volunteers. This thorough and biorelevant assessment of PI solubility could lead to new insights on the variable bioavailability in clinical practice. Moreover, HIF solubilities are important to have a general idea of the PI concentrations found *in vivo*, enabling the use of relevant concentrations for *in vitro* tests

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Table 1. Properties of the HIV Protease Inhibitors

HIV Protease Inhibitor	BCS[a]	Ionization Behavior[a]	pKa[a]	MW	log P[a]	Formulation[b]	Dose (mg)[b]	Enabling Strategy[b]	Clinical Food Effect on Bioavailability[b,14,34,35]	Susceptibility to Micellar Solubilization[c]
APV	IV	Weak base	2.4	505.6	2.4	Agenerase	1200	Cosolvent + surfactant	–	0.84 (0.49)
AZV	II	Weak base	4.3	802.9	4.5	Reyataz	300	Salt	+	1.85 (0.02)
DRV	II	Weak base	2.4	593.7	2.8	Prezista	600	/	+	1.02 (0.33)
IDV	IV	Weak base	7.2	711.9	2.8	Crixivan	800	Salt	–	1.64 (0.02)
LPV	II	Neutral[d]	/	628.8	4.7	Kaletra	400	Lipid based/solid dispersion	+	92.46 (0.0002)
NFV	IV	Weak base	8.2/9.2	663.9	4.7	Viracept	1250	Salt	+	18.9 (0.000006)
RTV	IV	Weak base	2.8	720.9	5.2	Norvir	100–200	Cosolvent/solid dispersion	+	1.28 (0.27)
SQV	IV	Weak base	8.5	766.9	3.2	Invirase	1000	Salt	+	5.92 (0.0004)
TPV	II	Weak acid	6.0/7.8	602.7	7.8	Aptivus	500	Lipid based	+	11.8 (0.001)

[a] Data obtained using Marvin sketch.
[b] Data obtained from European Medicines Agency.
[c] Solubility ratio FeSSIF/blank FeSSIF (Student's *t*-test, significance level *p*).
[d] Lopinavir: considered neutral in pH range 2–12.

including drug–drug interaction studies at the level of the intestine. An overview is generated of the solubility of first- and second-generation PIs in pooled HIF. Possible food effects on solubility were evaluated by comparing the solubilizing capacity of fluids aspirated in both fasted- and fed-state. In addition, inter- and intrasubject variability were characterized by evaluating fluids originating from different volunteers as well as fluids originating from one volunteer but aspirated at three different days. Finally, the capability of FaSSIF and FeSSIF to predict the intraluminal solubility was determined by using HIF solubility as the reference. Two versions of simulated media were used, one based on crude bile and one using SIF powder comprising the sole bile acid TC.

MATERIALS AND METHODS

Chemicals

The HIV protease inhibitors APV, AZV, DRV ethanolate, and RTV were provided by the National Institutes of Health AIDS Research and Reference Reagent Program (Germantown, Maryland). SQV, IDV, NFV, and LPV were donated by Hetero Drugs Ltd. (Hyderabad, India). TPV was provided by Boehringer Ingelheim (Rheinland Pfalz, Germany). Water was purified with a Maxima system (Elga Ltd., High Wycombe, Buckinghamshire, UK). Dimethyl sulfoxide and sodium dihydrogen phosphate were obtained from Acros Organics (Geel, Belgium). Hydroxide pellets were purchased from Merck (Darmstadt, Germany). Sodium acetate trihydrate, methanol, and sodium chloride were from VWR International (Leuven, Belgium). Sodium taurocholic acid practical grade was purchased from MP Biomedicals (Illkirch Cedex, France). Phospholipon 90G (lecithin) was from Nattermann Phospholipid Gmbh (Köln, Germany). Simulated intestinal fluid (SIF) powder was purchased from Biorelevant (Croydon, UK). Orlistat was obtained from Sigma–Aldrich (St. Louis, Missouri). Ensure Plus (Abbott Laboratories B.V., Zwolle, The Netherlands) was used to simulate a standard meal. One portion of 400 mL has an energy content of 2.528 kJ, of which lipids, carbohydrates, and proteins constitute 29%, 54%, and 17% on energy basis, respectively; the osmolality amounts to 670 mOsm/kg; the pH is 6.6.

Solubility Media

Simulated Intestinal Fluids

Fasted- and fed-state SIF were prepared according to the composition reported by Vertzoni[18] (revised standard FaSSIF and FeSSIF with practical grade TC and soybean lecithin). Throughout this article, the terms $FaSSIF_{crude}$ and $FeSSIF_{crude}$ are used to indicate the use of crude TC.[19] For comparative purposes, solubility experiments were also performed with $FaSSIF_{SIF}$ and $FeSSIF_{SIF}$ based on SIF powder. The different media compositions are listed in Table 2.

Human Intestinal Fluids

Fasted- and fed-state HIF (FaHIF and FeHIF) were aspirated from four healthy volunteers (two males, two females, between 23 and 27 years). HIF were collected from the duodenum (D2–D3) with a double-lumen polyvinyl catheter [Salem Sump Tube 14 Ch (external diameter 4.7 mm), Covidien, Dublin, Ireland] every 10 min for up to 120 min for the fasted-state and after intake of a liquid meal (Ensure plus, 400 mL) every 10 min for

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