European Journal of Pharmaceutical Sciences 49 (2013) 27–32

Contents lists available at SciVerse ScienceDirect

European Journal of Pharmaceutical Sciences

journal homepage: www.elsevier.com/locate/ejps

Exploring food effects on indinavir absorption with human intestinal fluids in the mouse intestine

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ARTICLE INFO

Article history:
Received 18 November 2012
Received in revised form 20 December 2012
Accepted 14 January 2013
Available online 9 February 2013

Keywords:
Human intestinal fluids
Mouse
in situ intestinal perfusion
Food effects
Indinavir

ABSTRACT

Food can have a significant impact on the pharmacokinetics of orally administered drugs, as it may affect drug solubility as well as permeability. Since fed state conditions cannot easily be implemented in the presently available permeability tools, including the frequently used Caco-2 system, exploring food effects during drug development can be quite challenging. In this study, we investigated the effect of fasted and fed state conditions on the intestinal absorption of the HIV protease inhibitor indinavir using simulated and human intestinal fluids in the *in situ* intestinal perfusion technique in mice. Although the solubility of indinavir was 6-fold higher in fed state human intestinal fluids (FeHIF) as compared to fasted state HIF (FaHIF), the intestinal permeation of indinavir was 22-fold lower in FeHIF as compared to FaHIF. Dialysis experiments showed that only a small fraction of indinavir is accessible for absorption in FeHIF due to micellar entrapment, possibly explaining its low intestinal permeation. The presence of ritonavir, a known P-gp inhibitor, increased the intestinal permeation of indinavir by 2-fold in FaHIF, while there was no increase when using FeHIF. These data confirm that drug–food interactions form a complex interplay between solubility and permeability effects. The use of HIF in *in situ* intestinal perfusions holds great promise for biorelevant absorption evaluation as it allows to directly explore this complex solubility/permeability interplay on drug absorption.

1. Introduction

Food intake can alter the intestinal absorption of many orally administered drugs. During early stage drug development, when the amount of available compound is limited, it is challenging to predict whether or not a drug candidate will undergo a food effect. Nevertheless, a few methods can be used to predict whether the absorption of a drug candidate will be prone to food–drug interactions. Fleisher et al. demonstrated that a food effect can be anticipated based on the compound's solubility and permeability, as defined in the Biopharmaceutics Classification System (BCS) (Fleisher et al., 1999). Briefly, this method predicts that a high fat meal will increase the bioavailability of low solubility compounds (class II and IV) and decrease the AUC of BCS class III compounds, while no effect is anticipated for class I drugs. Physiologically based pharmacokinetic modeling can also be applied to establish food–drug interactions, but this method requires extensive information on physicochemical properties, intestinal permeability and clearance of the drug (Won et al., 2012). In later stages of drug development, dissolution data obtained in simulated intestinal fluids (Dressman and Reppas, 2000) and *in vivo* methods using dogs or other animals (Lentz, 2008) can be used to predict human fed and fasted pharmacokinetics.

The approach of using biorelevant media simulating the fasted and fed state (FaSSIF and FeSSIF, respectively) or even human intestinal fluids (HIF) aspirated in fasted and fed conditions may also be taken to explore food effects during early stage drug development in drug absorption assays such as Caco-2 cell cultures. However, although the Caco-2 cell line is compatible with FaSSIF, it does not allow the use of FeSSIF or HIF because the integrity of the monolayer is not maintained during a sufficient period of time (Deferme et al., 2003; Ingels et al., 2002). Patel et al. created a modified version of FeSSIF by changing the salt composition and doubling the amount of lecithin, and showed that this modified FeSSIF was compatible with the Caco-2 model (Patel et al., 2006). Nevertheless, the question remains whether FaSSIF and FeSSIF are representative for FaHIF and FeHIF regarding their effect on intestinal drug absorption. Of all presently available absorption models, the *in situ* intestinal perfusion technique with mesenteric

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http://dx.doi.org/10.1016/j.ejps.2013.01.012

blood sampling is the closest one to the *in vivo* situation. Despite possible species differences in, e.g. P-gp expression levels (Uchida et al., 2011), this technique has been shown to better predict drug absorption in humans as compared to cell-based assays (Salphati et al., 2001), which are only predictive for passively transported drugs (Artursson et al., 2001). Because of the presence of a protective mucus layer, this model is expected to be more compatible with SIF and HIF. However, to our knowledge, HIF have never been applied in this model.

A class of drugs which is very susceptible to food effects is the class of HIV protease inhibitors: food intake increases the AUC of saquinavir (+700%), nelfinavir (+200% to 300%), lopinavir/ritonavir (+48% to 97%), atazanavir/ritonavir (+33%) and darunavir/ritonavir (+30%) (Boffito et al., 2005; Sekar et al., 2007). Since these are all BCS class II compounds, the food effect was correctly predicted with the method of Fleisher et al. For these class II compounds, food intake is expected to increase their solubility in the intraluminal environment (because of high concentrations of bile salts, phospholipids and lipid degradation products forming micelles), leading to a higher intestinal absorption. In contrast to the above mentioned HIV protease inhibitors, a negative food effect is observed for indinavir, also a BCS class II drug, for which the AUC decreases by 78% when administered with a high-fat meal (Yeh et al., 1998). These investigators proposed that this negative food effect may be attributed to a delayed gastric emptying and a possible precipitation of indinavir due to a higher gastric pH. However, Carver et al. (1999) have shown that meals that leave the gastric pH unchanged also lower indinavir plasma concentrations, suggesting that other factors are playing a role in this food effect for indinavir.

Therefore, for the first time, we used a novel combination of HIF in the mouse *in situ* intestinal perfusion technique with mesenteric blood sampling (Mols et al., 2009) and tested the hypothesis that a lower bioaccessible fraction of indinavir is responsible for a limited intestinal permeability in the fed state compared to the fasted state.

2. Materials and methods

2.1. Chemicals

Indinavir sulfate was donated by Hetero Drugs Ltd. (Hyderabad, India). Ritonavir (free base) was obtained from Xiamen Huasing Chemicals Co. (Xiamen Fujian, China). GF120918 (elacridar) was provided by GSK (London, UK). Stock solutions were prepared in DMSO. Ketamine (Anesketin) and xylazin (Xyl-M 2%) were from Eurovet (Heusden, Belgium) and VMD (Arendonk, Belgium), respectively. Sodium acetate trihydrate and methanol were purchased from VWR International (Leuven, Belgium). Phosphate buffered saline (PBS) and Hanks' balanced salt solution (HBSS) were provided by Lonza (Basel, Switzerland). Sodium taurocholic acid practical grade was purchased from MP Biomedicals (Illkirch Cedex, France). Phospholipon 90G (lecithin) was from Nattermann Phospholipid Gmbh (Köln, Germany). All other reagents were used as supplied. Water was purified with a Maxima system (Elga Ltd., High Wycombe Bucks, UK). Ensure Plus (Abbott Laboratories B.V., Zwolle, The Netherlands) was used to simulate a standard meal. One portion of 200 mL has an energy content of 1.263 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.

Table 1
Composition of FaSSIF and FeSSIF.

Component	FaSSIF	FeSSIF
Sodium taurocholate (NaTC)	3 mM	15 mM
Lecithin	0.75 mM	3.75 mM
$NaH_2PO_4 \cdot H_2O$	3.95 g	–
Glacial acetic acid	–	8.65 g
NaCl	6.19 g	30.93 g
NaOH	0.35 g	1.74 g
pH	6.5	5.0
Water	ad 1 L	ad 1 L

2.2. Media

Transport medium consisted of HBSS buffered with HEPES (10 mM) to pH 7.4. FaSSIF and FeSSIF were made according to the composition reported by Vertzoni et al. (2004) (revised standard FaSSIF and FeSSIF with practical grade taurocholate and soybean lecithin, Table 1). Human intestinal fluids (HIF) from the duodenum of four healthy volunteers (two female, two male, between 19 and 35 years old) were collected in two different nutritional states according to the method described by Bevernage et al. (2011). The HIF were collected every 15 min for up to 120 min from the duodenum (D2–D3) after the intake of 200 mL of water (fasted state) or a liquid meal (Ensure Plus 400 mL) + 200 mL of water (fed state). For each nutritional state, one pooled sample was made by combining the aspirates from all four volunteers. The pooled HIF were stored at −30 °C until further use. The characteristics of the intestinal fluids are shown in Tables 2 and 3.

2.3. Solubility measurements

The thermodynamic solubility of indinavir sulfate was determined with the standard shake flask method in transport medium, FaSSIF, FeSSIF and in HIF of the fasted and fed state. All solubility experiments were performed in triplicate. Approximately 1 mg of indinavir sulfate was added to microcentrifuge tubes containing 0.5 mL of the above mentioned media and placed in a prewarmed shaking incubator [37 °C at 200 rpm (KS 4000 i control incubator from Ika (Staufen, Germany)] for 24 h. The solid phase was separated from the dissolved part using centrifugation (15 min, 20.817*g* at 37 °C). The top layer was carefully removed by aspiration. The supernatant of the samples was diluted 1/50 with methanol:water (50:50 v/v) and quantified using an HPLC system with UV detection.

Table 2
Individual bile salt and lecithin concentrations in FaHIF and FeHIF.

Medium	Bile salt concentration (mM)									Lecithin (mM)
	TUDC	GUDC	TC	GC	TCDC	TDC	GCDC	GDC	Total	
FaHIF	0.045	0.244	0.768	2.766	0.622	0.381	2.281	1.016	8.125	2.06
FeHIF	0.057	0.232	0.789	2.585	0.748	0.383	2.371	1.250	8.415	5.81

TUDC: tauroursodeoxycholate, GUDC: glycoursodeoxycholate, TC: taurocholate, GC: glycocholate, TCDC: taurochenodeoxycholate, TDC: taurodeoxycholate, GCDC: glycochenodeoxycholate, GDC: glycodeoxycholate.