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Research paper

Precipitation in the small intestine may play a more important role in the *in vivo* performance of poorly soluble weak bases in the fasted state: Case example nelfinavir

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ABSTRACT

The aim of this study was to evaluate the utility of biorelevant dissolution tests coupled with *in silico* simulation technology to forecast *in vivo* bioperformance of poorly water-soluble bases, using nelfinavir mesylate as a model compound.

An *in silico* physiologically based pharmacokinetic (PBPK) model for poorly water-soluble, weakly basic drugs was used to generate plasma profiles of nelfinavir by coupling dissolution results and estimates of precipitation with standard gastrointestinal (GI) parameters and the disposition pharmacokinetics of nelfinavir. *In vitro* dissolution of nelfinavir mesylate film-coated tablets was measured in biorelevant and compendial media. Drug precipitation in the small intestine was estimated from crystal growth theory. GI parameters (gastric emptying rate and fluid volume) appropriate to the dosing conditions (fasting and fed states) were used in the PBPK model. The disposition parameters of nelfinavir were estimated by fitting compartmental models to the *in vivo* oral PK data. The *in vivo* performance in each prandial state was simulated with the PBPK model, and predicted values for AUC and C_{max} were compared to observed values.

Dissolution results in FaSSIF-V2 and FeSSIF-V2, simulating the fasting and fed small intestinal conditions, respectively, correctly predicted that there would be a positive food effect for nelfinavir mesylate, but overestimated the food effect observed in healthy human volunteers. In order to better predict the food effect, an *in silico* PBPK simulation model using STELLA® software was evolved. Results with the model indicated that invoking drug precipitation in the small intestine is necessary to describe the *in vivo* performance of nelfinavir mesylate in the fasted state, whereas a good prediction under fed state conditions is obtained without assuming any precipitation. *In vitro–in silico–in vivo* relationships (IVISIV-R) may thus be a helpful tool in understanding the critical parameters that affect the oral absorption of poorly soluble weak bases.

1. Introduction

Food ingestion is known to induce various physiological changes in the GI environment. Poorly soluble compounds are especially prone to higher systemic exposure when given in the fed state, with the main effect being faster and more extensive dissolution under fed conditions, due to higher levels of native surfactant, presence of lipophilic meal components and the products of fat digestion, larger volumes of fluids available to dissolve the drug, and the longer upper GI residence time [1–3]. The *in vivo* dissolution behavior of weak bases is additionally influenced by variations in upper GI pH, making these drugs especially prone to food effects.

Different systemic exposure between the prandial states is often problematic in terms of ensuring safe and efficient medication. These problems have been addressed by the Food and Drug Administration (FDA, US), which provides guidance to pharmaceutical companies about conducting food-effect bioavailability (BA) studies for orally administered dosage forms with respect to study design, data analysis, and product labeling [4]. In some cases, oral administration of the drug with a meal may be required to attain sufficient oral bioavailability of a poorly soluble, lipophilic drug [5,6]. Nelfinavir mesylate, which is a potent and highly selective inhibitor of human immunodeficiency virus (HIV) protease, is a representative example of drugs that are recommended to be taken with food to enhance bioavailability and minimize pharmacokinetic variability. When nelfinavir mesylate 1250 mg is given to

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healthy humans with a high-fat meal, it exhibits a significant increase in AUC and C_{max}, with about a 5-fold advantage over fasted state administration in both parameters [7].

For the above-stated reasons, prediction of food effects for poorly soluble, lipophilic drugs would be highly advantageous during oral formulation development. The Biopharmaceutics Classification System (BCS) criteria [8] can be a trigger to initiate early assessment of food effects [2]. Animal studies, usually in dogs, are often applied to predict food effects early in oral formulation development since differences in GI physiology between dogs and humans are well characterized [9]. Although several research groups have successfully applied the dog model [10,11], it is far from a perfect surrogate to predict *in vivo* performance in humans due to limitations in terms of dosing conditions, diet, the specific GI parameters, etc. *In vitro* dissolution studies are a useful means of determining the dissolution characteristics of oral solid dosage forms and to control their quality. Biorelevant media, which can simulate the human gastric, small intestinal, and colonic fluid in each prandial state [12–15], can also be a useful tool to predict *in vivo* dissolution behavior in the GI tract for lipophilic drugs in humans [16,17]. *In vitro* biorelevant dissolution testing combined with *in silico* human PBPK modeling (IV–IS–IV) has the potential to predict food effects on the pharmacokinetics of poorly soluble compounds, as shown in our previous reports using celecoxib and aprepitant as model compounds [18,19], as well as in reports from other researchers using similar strategies [20–23].

In order to explore the IV–IS–IV approach further, nelfinavir mesylate, which is a poorly soluble weak base and exhibits a significant food effect, was chosen as a model compound. Due to its highly pH-dependent solubility [24], nelfinavir mesylate dissolves well in the stomach, whereas it is poorly soluble at the more neutral pH found in the small intestine. Nelfinavir mesylate could thus partially precipitate in the small intestinal fluid, with a negative impact on its bioperformance. Therefore, the aims of this study were to

(1) evolve the PBPK simulation model to consider the transfer of basic compounds from the stomach to the intestine, including generation of supersaturation and possible precipitation in the small intestine,
(2) forecast *in vivo* oral absorption of nelfinavir mesylate in the pre- and post-prandial states, and hence its food effect, by coupling *in vitro* biorelevant dissolution testing with PBPK modeling and
(3) determine whether biorelevant media offer an advantage over simple buffer media for predicting *in vivo* performance of weak bases.

2. Materials and methods

2.1. Chemicals and reagents

Both nelfinavir and its methanesulfonic salt were kindly donated by F. Hoffmann-La Roche AG (Basel, Switzerland). Viracept® 250 mg film-coated tablets (lot E0028E1) were purchased commercially from German market. Long-life, heat-treated, and homogenized milk (UHT milk) containing 3.5% fat (Milfina Hochwald, Kaiserslautern, Germany) was purchased commercially. Glyceryl monooleate (GMO, Rylo M19 Pharma®, 99.5% monoglyceride, lot 173403-2202/107) was kindly donated by Danisco Specialities, Brabrand, Denmark. Egg phosphatidylcholine (Lipoid E PC®, 99.1% pure, lot 108015-1/42) was kindly donated by Lipoid GmbH, Ludwigshafen, Germany. Eighty-five percentage orthophosphoric acid (H_3PO_4), 37% hydrochloric acid (conc. HCl), and pepsin (Ph. Eur., 0.51 U/mg, lot 1241256) were obtained from Fluka Chemie AG, Buchs, Switzerland. Maleic acid (99% pure, lot 4039128) was purchased from Sigma–Aldrich Chemie GmbH (Steinheim, Germany). Sodium oleate (82.7% pure, lot 51110) was obtained from Riedel-de Haën, Seelze, Germany. Sodium taurocholate (NaTC, 97% pure, lot 2007100274) was purchased from Prodotti Chimici Alimentari SpA, Basaluzzo, Italy. Sodium hydroxide solution (0.1 N NaOH) and hydrochloric acid solution (0.1 N HCl) were purchased from VWR International GmbH (Darmstadt, Germany). Dichloromethane, acetonitrile, glacial acetic acid, sodium acetate trihydrate, sodium chloride, potassium dihydrogen phosphate triethylamine, and sodium hydroxide pellets were all of analytical grade and purchased from Merck KGaA (Darmstadt, Germany).

2.2. Media preparation

The compositions and the preparation procedures of the media used for dissolution tests and solubility determination have been described previously [12–14]. Fasted State Simulated Gastric Fluid (FaSSGF), Fed State Simulated Gastric Fluid (FeSSGF), and compendial Simulated Gastric Fluid without pepsin (SGF_{sp}) (USP 33) were used for the gastric media. For the upper small intestine, updated versions of Fasted State Simulated Intestinal Fluid (FaSSIF-V2), Fed State Simulated Intestinal Fluid (FeSSIF-V2), and compendial Simulated Intestinal Fluid without pancreatin (SIF_{sp}) (USP 33) were used in this study.

2.3. Analytical methods

2.3.1. The high-performance liquid chromatography (HPLC) system

The samples obtained from solubility and dissolution testing were quantitatively analyzed for nelfinavir concentration using an isocratic HPLC system, modified and validated based on a literature method [24]. The HPLC system consisted of a pump (Merck Hitachi L7100), an autosampler (Merck Hitachi L-7200), and a UV detector (Merck Hitachi L-7400). The chromatograms were evaluated with EZChrom Elite™ Version 2.8 Software (Biochrom Ltd., Cambridge, UK). The analytical column used was Capcell Pak C18 MGII (4.6 mm-i.d. × 50 mm, 5 μm, Shiseido Co., Ltd., Japan). The mobile phase was a mixture of acetonitrile and 1% triethylamine (40:60) adjusted pH to 3 with phosphoric acid. The injection volume was 10 μL. The detection wavelength was set at UV 240 nm. The analysis was performed under ambient conditions.

2.4. Solubility measurements

The shake-flask method was used to determine the solubility of nelfinavir mesylate in each medium. Measurements were performed by adding an excess amount of the drug substance to a medium in a glass vial. The vial was incubated in a water bath at 37 °C and shaken vigorously at appropriate intervals. Samples were taken after at least 6 h and filtered through PVDF membrane filters having a pore size of 0.45 μm (25 mm GD/X, Whatman GmbH, Dassel, Germany). The filtrate was diluted immediately with the mobile phase and then analyzed by HPLC. For FeSSGF, with milk as a major component, the procedure described in a previous report was applied [18].

2.5. Dissolution testing of nelfinavir mesylate film-coated tablets

2.5.1. Dissolution testing

A scaled-down apparatus (the mini-paddle assembly produced by Erweka GmbH, Heusenstamm, Germany) was used to measure the dissolution profile as well as the initial dissolution rate. This apparatus is based upon the USP paddle setup but scaled down geometrically with respect to all dimensions, so that hydrodynamics remain essentially similar at a given paddle rotation speed [25]. The dissolution conditions consisted of a medium volume of