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Prediction of Ketoconazole absorption using an updated *in vitro* transfer model coupled to physiologically based pharmacokinetic modelling

Aaron Ruff, Tom Fiolka, Edmund S. Kostewicz *

Institute of Pharmaceutical Technology, Goethe University, Frankfurt/Main, Germany

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ABSTRACT

The aim of this study was to optimize the *in vitro* transfer model and to increase its biorelevance to more accurately mimic the *in vivo* supersaturation and precipitation behaviour of weak basic drugs. Therefore, disintegration of the formulation, volumes of the stomach and intestinal compartments, transfer rate, bile salt concentration, pH range and paddle speed were varied over a physiological relevant range. The supersaturation and precipitation data from these experiments for Ketoconazole (KTZ) were coupled to physiologically based pharmacokinetic (PBPK) model using Stella® software, which also incorporated the disposition kinetics of KTZ taken from the literature, in order to simulate the oral absorption and plasma profile in humans. As expected for a poorly soluble weak base, KTZ demonstrated supersaturation followed by precipitation under various *in vitro* conditions simulating the proximal small intestine with the results influenced by transfer rate, hydrodynamics, volume, bile salt concentration and pH values. When the *in vitro* data representing the "average" GI conditions was coupled to the PBPK model, the simulated profiles came closest to the observed mean plasma profiles for KTZ. In line with the high permeability of KTZ, the simulated profiles were highly influenced by supersaturation whilst precipitation was not predicted to occur *in vivo*. A physiological relevant *in vitro* "standard" transfer model setup to investigate supersaturation and precipitation was established. For translating the *in vitro* data to the *in vivo* setting, it is important that permeability is considered which can be achieved by coupling the *in vitro* data to PBPK modelling.

1. Introduction

The number of water insoluble new drug candidates progressing through pharmaceutical development over the past decade has increased considerably (Lobell et al., 2006). In order to overcome solubility limitations, a formulation approach to enhance the apparent concentration of drug in the gastrointestinal (GI) lumen can be achieved through supersaturation (Kostewicz et al., 2014). A greater amount of drug in solution means that more drug is available for absorption across the intestinal mucosa. Alternatively, supersaturation may also result from the transfer of a weak base (irrespective of the formulation used) from an acidic stomach into the more pH neutral small intestine. In both cases, the supersaturated solution is thermodynamically unstable and the drug may precipitate. Supersaturation and precipitation along the GI tract may therefore have a significant impact on the overall absorption of poorly soluble drugs administered *via* the oral route.

Whilst solubility is a key parameter that needs to be investigated during product development, the solubility data do not describe the supersaturation and precipitation characteristic of the drug. The dissolution behaviour of the formulation is also important to consider, but given that the conventional USP1 and USP2 apparatus typically utilize a single media and volume at constant pH, dissolution results also do not adequately reflect the *in vivo* situation, in which transit through the GI tract exposes the drug/formulation to a constantly changing environment (Kostewicz et al., 2002).

As supersaturation and precipitation can be influenced by a multitude of factors, these need to be considered in any *in vitro* model used. For example, supersaturation concentrations in the lumen may be influenced by gastric emptying, ionization concentrations of the drug, solubilisation by bile acid micelles and dissolution characteristics of the formulation. Factors influencing precipitation along the GI tract

Abbreviations: AUC, Area under the curve; BCS, Biopharmaceutical classification system; CSC, Critical supersaturation concentration; DOS, Degree of supersaturation; FaSSGF, Fasted state simulated gastric fluid; FaSSGF-V2(HBS), Fasted state simulated gastric fluid version 2 high bile salt concentration; FaSSIF, Fasted state simulated intestinal fluid; FaSSIF-V2(HBC), Fasted state simulated intestinal fluid version 2 high buffer capacity; f_d, Fraction dissolved; f_s, Fraction solid; GIT, Gastrointestinal tract; IVIVC, *In vitro in vivo* correlation; KTZ, Ketoconazole; C_{max}, Maximal concentration; MMC, Migrating Motor Complex; PK, Pharmacokinetic; PBPK, Physiologically based pharmacokinetic modelling; PRC, Precipitation rate constant; rpm, Revolutions per minute; SIF, Simulated intestinal fluids; T_{max}, Time at which maximal concentration is observed; V_d, Volume of distribution.

* Corresponding author at: Institute of Pharmaceutical Technology, Goethe University, Max-von-Laue Str. 9, 60438 Frankfurt/Main, Germany.
E-mail address: kostewicz@em.uni-frankfurt.de (E.S. Kostewicz).

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include the pH transition between the stomach and proximal intestine, dilution of the formulation by GI fluids and corresponding composition of the GI fluids, and characteristics of the excipients used in the formulation (Brouwers et al., 2009; Tonsberg et al., 2010; Xu and Dai, 2013). To capture these parameters appropriately, the transfer model, which was initially presented in 2004 (Kostewicz et al., 2004), is a multi-compartmental model taking into account both the stomach and intestinal compartments and has been frequently used to investigate the supersaturation and precipitation characteristics for poorly soluble weak bases (Berlin et al., 2014; Kostewicz et al., 2004; Wagner et al., 2012; Xu and Dai, 2013).

To ensure that the experimental conditions of the transfer model appropriately captures the relevant GI physiology, the following parameters were investigated: disintegration of the formulation, volume of the stomach and intestinal compartments, transfer rate, bile salt concentration, pH range and paddle speed. To evaluate the impact of varying the physiological parameters, the results from the *in vitro* transfer experiments were coupled to an updated Stella® PBPK model and the resulting simulated plasma profiles compared to literature plasma profiles.

The model drug chosen to optimize the transfer model was Ketoconazole (KTZ), a BCS II antifungal agent, which exhibits a diphasic pKa (6.5 and 2.9) (Blum et al., 1991) and a log P value of 3.9 (Ghasemi and Saaidpour, 2007). Further, given the availability of *in vivo* data with respect to luminal precipitation (Psachoulias et al., 2011) and human pharmacokinetic data after administration of different formulations of KTZ (Huang et al., 1986), enabled an *in vitro in vivo* correlation (IVIVC) to be established.

2. Materials and methods

2.1. Chemicals and reagents

KTZ powder was purchased from Caesar & Lorentz GmbH (Hilden, Germany). Nizoral® tablets containing 200 mg of KTZ were kindly donated by Janssen Pharmaceutics (Buckinghamshire, UK).

Sodium hydroxide was purchased from Merck KGaA (Darmstadt, Germany). Sodium chloride, orthophosphoric acid and hydrochloric acid 37% were purchased from VWR International (Leuven, Belgium) and maleic acid was purchased from AppliChem GmbH (Darmstadt, Germany). Organic solvents for HPLC analysis including trimethylamine and acetonitrile were all HPLC grade and purchased from Merck-Schuchardt (Hohebrunn, Germany) and Merck KGaA (Darmstadt, Germany), respectively. SIF powder original and SIF powder V2 were a kind donation from biorelevant.com (London, UK). All other chemicals were analytical grade or equivalent, and purchased commercially.

2.2. Media used for solubility, dissolution and transfer experiments

2.2.1. Composition of media

Compendial and biorelevant media were used to evaluate the solubility, dissolution, supersaturation and precipitation characteristics of KTZ. The biorelevant media were prepared using SIF-powders based on instructions from www.biorelevant.com. Detailed information on the composition of all compendial and biorelevant media used in this study is presented in Table S1 (see Supplementary Information).

To simulate the conditions in the stomach, FaSSGF-V2 (Vertzoni et al., 2007) was used at pH 2.0 rather than at pH 1.6 (Fei et al., 2013), in order to reduce the drop in pH during transfer into the intestinal compartment during the transfer experiments. Additionally, to reduce the drop in pH of the intestinal compartment following transfer of the gastric media and to maintain the pH in the acceptor compartment at approximately 5.8, FaSSIF-V2 with a higher buffer capacity was used (Maleate(HBC)/FaSSIF-V2(HBC)). To maintain the bile salt concentration of FaSSIF-V2 following transfer of FaSSGF-V2 into the intestinal compartment, for some experiments the bile salt concentration in FaSSGF-V2 was increased to the same concentration as used in FaSSIF-V2 (FaSSGF-V2(HBS)). FaSSIF-V1 was prepared using the same buffer composition which was used for FaSSIF-V2 (i.e. maleate buffer). To replicate the dilution of bile salts and reduction in pH in the intestinal compartment after transfer of the gastric compartment in the transfer experiment and it evaluate its impact on solubility, various ratios of the gastric to intestinal media including FaSSGF-V2 (pH 2.0) with FaSSIF-V2 (ratio 1:2 and 1:1.4), FaSSGF-V2 (pH 2.0) with FaSSIF-V1 (ratio 1:1.4), FaSSGF-V2 (pH 2.0) with FaSSIF-V2(HBC) (ratio 2.5:1) and FaSSGF-V2(HBS) with FaSSIF-V2 (ratio 1:1.4) were prepared.

2.3. Solubility experiments

To evaluate the solubility of KTZ in the gastric and intestinal compartments, experiments were executed using both compendial and biorelevant media. To represent the stomach HCl/NaCl buffers and FaSSGF-V2 at pH values of 1.0, 1.6 and 2.0, and FaSSGF-V2(HBS) were used. To simulate the intestine before and after transfer of the gastric compartment, maleate buffer, FaSSIF-V1, FaSSIF-V2, FaSSIF-V2(HBC) and the appropriate ratios of gastric to intestinal media as described in Section 2.2.1 were prepared.

The solubility of KTZ was evaluated using the Uniprep™ system as previously described by Glomme et al. (Glomme et al., 2005). After incubation at 37 °C for 24 h, the concentration of KTZ was measured by HPLC (Section 2.5). All solubility experiments were performed in triplicate.

2.4. Dissolution and transfer experiments

2.4.1. Equipment

The dissolution and transfer experiments were performed using an ERWEKA® DT 600 USP II dissolution tester, utilizing three 500 mL mini-vessels (stomach compartment) and three standard 1000 mL vessels (intestinal compartment), each with the respective corresponding mini-paddle or standard paddle system setup (Erweka® GmBH, Heusenstamm, Germany).

2.4.2. Experimental setup

2.4.2.1. Dissolution. The dissolution characteristics of KTZ (Nizoral® tablet) were evaluated in both 250 mL of FaSSGF-V2 and 500 mL of FaSSIF-V2. Samples were taken out to 240 min. Experiments were performed in triplicate using an agitation speed of 100 rpm at 37 °C ± 0.5 °C.

2.4.2.2. Transfer model. The *in vitro* transfer model comprises of a two compartment setup simulating the stomach (donor) and intestinal (acceptor) compartment utilizing a mini-vessel/paddle and standard vessel/paddle setup, respectively (Kostewicz et al., 2004). To simulate the transfer of gastric contents (including dissolved and undissolved particles) into the small intestine, a programmable ISMATEC® MC-Process IP5 peristaltic pump was used. For each donor and acceptor compartment pair, a 70–80 cm length of tubing (ISMATEC® neoprene) with an internal diameter of 2.06 mm was used to allow the transfer of the gastric to the intestinal compartment (IDEX Health & Science GmbH, Wertheim Germany). To position the tubing at the bottom of the mini-vessel, a glass tubing system with a length of 11 cm and a diameter of approximately 0.6 mm (I.D. 0.5 mm) was securely fixed on the dissolution vessel lid, through which the neoprene tubes were threaded into the donor compartment (Fig. 1). For the intestinal compartment, a similar glass cylinder with a length of 6.5 cm was positioned in the dissolution vessel lid through which the neopren tubing was thread and positioned just below the surface of the intestinal media.

2.4.3. Detailed evaluation of the transfer model setup

As part of the update of the transfer model, not only the disintegration of the formulation in the gastric compartment, but also the range of GI physiology with respect to relevant GI volumes, transfer rates, bile