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Mechanistic investigation of the negative food effect of modified release zolpidem

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ABSTRACT

Aims: When administered orally as either an immediate or modified release dosage form, zolpidem demonstrates a negative food effect, *i.e.* decrease in C_{max} and AUC. The aim of the study was to arrive at a better understanding of the absorption of this BCS class I compound *in vivo* and to simulate the observed plasma profiles using *in vitro* and *in silico* methods.
Methods: Pharmacokinetic profiles of zolpidem are presented from a bioavailability (8 mg intravenous; 10 mg immediate release Stilnox®; 10 mg and 12.5 mg modified release Ambien® CR) and from a food effect study (12.5 mg modified release Ambien® CR). The dissolution behavior of the 12.5 mg strength was investigated using compendial methods in the USP apparatus II and using biorelevant methods in the USP apparatus III and IV. The mean plasma profiles as well as selected individual plasma profiles were simulated with Simcyp® and GastroPlus™. The absorption behavior was additionally investigated using the Q_{gut} model, which entails algebraic deconvolution of all individual profiles, incorporating both first pass gut and liver extraction.
Results: It was possible to simulate the mean plasma profiles using a "middle-out" approach, based on *in vitro* data combined with pharmacokinetic parameters obtained after intravenous administration, using PBPK software (Simcyp® and GastroPlus™), resulting in average fold error (AFE) values <1.5. Deconvolution verified that the *in vivo* absorption rate from the modified release formulation is controlled by the formulation in the fasted state, whereas in the fed state, the absorption rate is mainly controlled by gastric emptying. One-stage *in vitro* tests suggested that interactions with meal components, resulting in incomplete release, may be the source of the negative food effect for both the immediate and modified release formulations.
Conclusions: The present study demonstrated that a combination of biorelevant dissolution testing with modeling approaches enables a mechanistic understanding of the absorption of zolpidem from various formulations and can serve as a useful biopharmaceutical approach for the development of modified release solid oral dosage forms.

1. Introduction

Over the past two decades, biopharmaceutical methods such as biorelevant dissolution testing (sometimes referred to as "*in vivo* predictive dissolution testing") and physiologically based pharmacokinetic (PBPK) modeling have become increasingly important tools for gaining understanding of the absorption of solid oral drug products (Dressman, 2014; Kostewicz et al., 2014a; Kostewicz et al., 2014b). Dissolution media that are able to reflect the luminal environment of a tablet in the gut offer a better prediction of dosage forms *in vivo* release in the dynamic gastrointestinal (GI) tract environment (Andreas et al., 2015; Andreas et al., 2016). For modified or extended release formulations, multi-compartmental dissolution apparatus, such as the USP apparatus III or IV, have the advantage of being able to easily switch dissolution media throughout the experiment to better replicate the changing *in vivo* conditions. Coupling the *in vitro* release results with *in silico* tools, such as PBPK modeling, enables a forecast of *in vivo* plasma profiles to be made (Wagner et al., 2012; Wagner et al., 2013; Berlin et al., 2014; Berlin et al., 2015). If individual plasma profiles are available, the investigation of single subjects additionally allows the identification of parameters affecting the population variability (Mistry et al., 2016).

In the present study, zolpidem was selected as a model for controlled release formulations which exhibit negative food effects. Zolpidem has a

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pKa of 6.16 and is considered to be a BCS class I compound (Durand et al., 1992; Colon-Useche et al., 2015). Pharmacokinetic studies of various fast dissolving formulations have already been published (Greenblatt et al., 2013a; Greenblatt et al., 2013b; Greenblatt et al., 2014; Greenblatt et al., 2000; Olubodun et al., 2002; Olubodun et al., 2003). In the present study, the absorption behavior of a modified release tablet formulation of zolpidem was investigated in both prandial states, in order to better understand the effect of food on its absorption.

The main objective of the present work was to utilize various biopharmaceutic tools to understand the key factors that control the absorption of zolpidem. For this purpose, the pharmacokinetic behavior was investigated from an *in vitro* and *in silico* perspective using biorelevant dissolution tests and PBPK modeling. The dissolution behavior of immediate and modified release zolpidem formulations in simple, compendial paddle methods was compared with biorelevant, multi-compartmental dissolution methods using USP apparatus III and IV. Based on the *in vitro* dissolution results, the *in vivo* plasma profiles were simulated using Simcyp® and GastroPlus™, both commercially available PBPK softwares, via a "middle-out" approach, taking advantage of the disposition pharmacokinetic parameters calculated after intravenous application. In addition, selected individual plasma profiles in the fasted and fed state were simulated using a "top-down" approach in order to gain a better mechanistic understanding of the absorption process. Finally, compartment models in Excel® coupled to the Qgut model were used to better understand the relative contribution of first pass gut and liver extraction limitations to the observed bioavailability.

2. Materials and Methods

2.1. Materials

Zolpidem active pharmaceutical ingredient and formulations were kindly donated by Sanofi, including the 10 mg immediate release (Stilnox®) and 12.5 mg modified release (Ambien® CR) products. FaSSIF, FeSSIF & FaSSGF Powder® and FaSSIF-V2 Powder® were kindly donated by biorelevant.com (Surrey, United Kingdom). Glycerol monooleate (GMO) was also donated to the Goethe University by biorelevant.com, while Danisco (Copenhagen, Denmark) donated GMO to the University of Athens. Sodium cholate and egg-phosphatidylcholine (Lipoid E PC®) were donated by Prodotti Chimici E Alimentari S.p.A. (Basaluzzo, Italy) and Lipoid GmbH (Ludwigshafen, Germany), respectively, to both universities. Lipofundin® MCT 20% was purchased from B. Braun Melsungen (Melsungen, Germany). The other materials used at the Goethe University were obtained commercially: maleic acid, sodium dihydrogen phosphate dehydrate, dodecyl sulfate sodium, acetonitrile, dichloromethane, and methanol were obtained from Merck KGaA (Darmstadt, Germany) and acetonitrile and methanol were of HPLC grade; sodium acetate trihydrate, sodium chloride, potassium dihydrogen phosphate, sodium hydroxide, di-sodium hydrogen phosphate dodecahydrate, tris-(hydroxymethyl) aminomethane, D(+)-glucose, acetic acid 100%, hydrochloric acid 37%, hydrochloric acid 1 N, orthophosphoric acid 85%, sodium hydroxide 1 N were purchased from VWR Prolabo (Leuven, Belgium) and were of analytical grade; sodium oleate (>82% fatty acids) was obtained from Sigma-Aldrich (Steinheim, Germany). The other materials used at the University of Athens were purchased from Sigma-Aldrich (Saint Louis, MO, USA), except glass wool, which was purchased from Panreac (Barcelona, Spain).

2.2. Solubility

The solubility of zolpidem was investigated in selected biorelevant dissolution media after 24 h of incubation at 37 °C using the Uniprep ™ system (Whatman®, Piscataway, NJ, USA). The samples in biorelevant dissolution media were incubated on an orbital mixer, after which they were filtered through the 0.45 μm PTFE membrane (Whatman®, Piscataway, NJ, USA) integrated in the Uniprep™ and analyzed by HPLC. The solubility measurements were performed in triplicate. Solubilities in other media were determined at room temperature (25 °C) after 24 h using strong acid and bases to adjust the pH.

2.3. Permeability

Permeability was measured in CaCo-2/TC7 cell monolayers using Hank's balanced salt solution (HBSS) at pH 6.5 with 0.5% BSA (fasted state) or 5% BSA (fed state) in the apical compartment. This 5% value of BSA in the donor compartment is designed to represent physiological fed conditions in the upper intestinal tract with respect to the possibility of zolpidem binding to proteins present in the meal. In a high fat breakfast according to 2002 FDA guidance "Food-Effect Bioavailability and Fed Bioequivalence Studies", around 150 kcal are derived from protein, corresponding to 37.5 g of protein (Food and Drug Administration Center for Drug Evaluation and Research, 2002). Assuming a meal volume of 600–900 mL, this results in a protein concentration of 4–6%. The basal compartment contained HBSS at pH 7.4 with 5% BSA. Zolpidem was placed into the apical compartment at a concentration of 20 μM and the cells were incubated for 2 h to attain steady state. The amount of zolpidem permeating through the Caco-2/TC7 cells as a function of time was quantified by HPLC and the apparent drug permeability (P_{app}) was calculated.

The human jejunal permeability (P_{eff}) was calculated on the basis of a P_{app} to P_{eff} correlation established for this CaCo-2/TC7 cell line with 17 compounds at Sanofi under fasted state conditions. These compounds and their associated P_{app} (10^{-7} cm × s^{-1}) were furosemide (0.7), nadolol (0.8), methyldopa (1.0), terbutaline (1.1), atenolol (1.5), fexofenadine (2.1), lisinopril (2.5), cimetidine (3.3), mannitol (3.9), cephalexin (13.6), metoprolol (60.2), fluvastatine (65.5), verapamil (74.3), propranolol (128), phenylalanine (137.3), antipyrine (250), piroxicam (399.8). The equation for the correlation was $P_{eff} = 0.1378 + 0.1295 \times P_{app}^{0.6714}$, where P_{eff} is expressed in 10^{-4} cm × s^{-1} and P_{app} is expressed in 10^{-7} cm × s^{-1}.

2.4. Compendial Dissolution Method for Zolpidem Modified-release Tablets

Compendial dissolution testing was performed in the USP apparatus II (paddle) using 500 mL of 0.01 N HCl as the dissolution medium at a paddle speed of 100 rpm. Samples were drawn periodically and experiments were conducted at least in triplicate ($n = 3$) (The United States Pharmacopeia Convention and United States Pharmacopeia and National Formulary (USP 35-NF 30), 2012).

2.5. Biorelevant Dissolution Tests

Biorelevant dissolution tests were performed with the USP apparatus III (RRT 10, Caleva Ltd., Dorset, England) and USP apparatus IV (Erweka DFZ60, HKP60 piston pump, Heusenstamm, Germany) using the biorelevant gradient settings shown in Table 1, as previously described (Andreas et al., 2015; Andreas et al., 2016). The dissolution media were prepared according to recently published procedures (Markopoulos et al., 2015). Level II biorelevant media contain bile components, dietary lipids and lipid digestion products, as well as osmolality adjusting agents. Level I biorelevant media can be considered as the corresponding buffers to the Level II media, taking only the physiological pH and buffer capacity into account (Markopoulos et al., 2015). The USP apparatus III experiments were run using 235 mL of dissolution media per vessel/simulated gastrointestinal segment with 420 μm polypropylene mesh as the top and bottom sieves. For the flow-through cell experiments, a 5 mm-sized glass bead was placed in the tip of each dissolution cell and a total of 1.7 g of 1 mm-sized glass beads were added above the 5 mm glass bead. Ambien® CR tablets were mounted on a holder. On top of the cell, a combination of two filters (MNGF-5, 0.4