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Human and simulated intestinal fluids as solvent systems to explore food effects on intestinal solubility and permeability

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ABSTRACT

The mixed micelles and vesicles present in the intraluminal environment of the postprandial state exhibit suitable solubilizing capacities for lipophilic drugs. This increase in solubility, however, is accompanied by a decrease in the free fraction caused by micellar entrapment of these lipophilic compounds. In this study, both simulated and aspirated human intestinal fluids of fasted and fed state conditions were used to evaluate the influence of food on the intestinal disposition of a series of structurally related β-blockers, with varying log*P* values.

Using the *in situ* intestinal perfusion technique with mesenteric blood sampling in rats, it was demonstrated that fed state conditions significantly decreased the absorptive flux of the more lipophilic compounds metoprolol, propranolol and carvedilol, whereas the influence on the flux of the hydrophilic β-blocker atenolol was limited.

The solubility of BCS class II compound carvedilol was found to increase significantly in simulated and aspirated media of the fed state. Intestinal perfusions using intestinal media saturated with carvedilol, revealed a higher flux in the fasted state compared to the fed state, despite the higher solubility in the fed state. This study underscores the importance of addressing the complex nature of the behavior of compounds in the intraluminal environment in fasted and fed state conditions. Moreover, our data point out the value of studying the effect of food on both solubility and permeability using biorelevant experimental conditions.

1. Introduction

Unraveling the mechanisms by which the prandial state affects the absorption of drugs is of great importance to understand and predict the effect of food on the pharmacokinetics of orally administered drugs. The intake of drugs together with a meal has been shown to influence the rate and the extent of absorption of numerous drugs (Singh, 2005). Several mechanisms may be at the origin of these food effects; best known are a delayed gastric emptying time and an increase in the gastric pH in the postprandial state, which can significantly alter the solubility of drugs and, consequently, the amount of drug that will be presented to the small intestine for absorption. In general, the intestinal solubility of poorly soluble, lipophilic drugs will increase in the fed state. The vesicles and mixed micelles that are formed upon mixing of secreted bile with food constituents exhibit excellent solubilizing capacities for these lipophilic compounds (Clarysse et al., 2009a). Therefore, the resulting intraluminal concentrations are often much higher than the thermodynamic solubility of these compounds in simple aqueous buffer solutions. These findings have prompted researchers to utilize simulated intestinal fluids of fasted and fed state conditions (FaSSIF, FeSSIF) in studies on the effect of food on drug absorption. These media have been optimized and adapted in accordance with the expanding body of literature on the composition of aspirated human intestinal fluids in the fed and fasted state (Kleberg et al., 2010). Despite these efforts to approximate the composition of intestinal fluids as closely as possible, still considerable differences in experimental results are observed when comparing simulated to aspirated intestinal fluids. For instance, in a study of Holmstock et al., the solubility of indinavir in FeSSIF was found to be more than fourfold higher than its solubility in human aspirated fluids of the fed state. Moreover, the indinavir flux towards the mesenteric blood was found to be significantly lower upon perfusion with aspirated fluids of the fed state

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compared to perfusion with simulated fluids (Holmstock et al., 2013).

Several studies have highlighted the importance of studying the effect of food on both intestinal solubility and permeability. The increase in solubility in the fed state is often accompanied by a decrease in free fraction and, consequently, a less strong driving force of compounds from the intraluminal environment towards the blood, as a result of entrapment in micelles. Therefore, it may be misleading to predict the effect of food on oral bioavailability based solely on solubility profiling. Several authors have observed this solubility–permeability trade-off in micellar solvent systems (Fischer et al., 2011a; Katneni et al., 2006; Miller et al., 2011; Yano et al., 2010).

Simulated intestinal fluids of the fasted state are commonly used as solvent systems in Caco-2 experiments and the effects on solubility and permeability exerted by the micelles present in these biorelevant media can be studied using this *in vitro* model (Frank et al., 2012b; Ingels et al., 2002). However, due to the fact that simulated intestinal fluids of the fed state and human aspirated intestinal fluids are mostly incompatible with the Caco-2 model, determining the permeability for compounds in biorelevant media remains an important challenge in the evaluation of food effects. Work is ongoing to generate media that exhibit a composition that is relevant for the fed state and are compatible with Caco-2 cells (Markopoulos et al., 2013). It remains challenging, however, to render media compatible with Caco-2 cells, while maintaining substances such as bile salts within biorelevant concentration ranges. More robust experimental absorption models such as the *in situ* perfusion technique generate the possibility to study permeability for compounds in presence of biorelevant simulated intestinal fluids and aspirated human intestinal fluids (Holmstock et al., 2013).

In this study, the impact of coadministration of food on the intestinal disposition was evaluated by using simulated and aspirated human intestinal fluids of fasted and fed state conditions in the *in situ* intestinal perfusion technique with mesenteric blood sampling in rat. A series of β-blockers which are structurally related but vary significantly in lipophilicity were selected as model compounds (Table 1). Atenolol is a BCS class III compound which is hydrophilic and exhibits mostly paracellular transport across the small intestinal barrier. The more lipophilic compounds metoprolol and propranolol are highly soluble and retain favorable permeability, rendering them typical BCS class I compounds. Being a BCS class II compound, carvedilol exhibits poor solubility and dissolution characteristics. The effect of food on the intestinal solubility–permeability interplay for the four β-blockers was assessed in simulated and aspirated human intestinal fluids of the fasted and fed state.

2. Materials and methods

2.1. Chemicals

Atenolol, metoprolol tartrate, propranolol and orlistat were purchased from Sigma–Aldrich (St. Louis, MO). Talinolol was obtained from Arzneimittelwerk Dresden (Radebeul, Germany). Carvedilol was purchased from Sequoia Research Products (Pangbourne, UK). Sodium acetate trihydrate and methanol were purchased from VWR International (Leuven, Belgium). Acetonitrile and dimethylsulfoxide (DMSO) were purchased from Acros Organics (Geel, Belgium). Dichloromethane was obtained from Fisher Scientific (Leuven, Belgium). Phosphate buffered saline (PBS) and Hanks' balanced salt solution (HBSS) were provided by Lonza (Basel, Switzerland). Simulated intestinal fluid (SIF) powder was purchased from Biorelevant (Surrey, UK). Ketamine (Anesketin) and xylazin (Xyl-M 2%) were obtained from Eurovet (Heusden, Belgium) and VMD (Arendonk, Belgium), respectively. Stock solutions were prepared in DMSO. 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.

2.2. Media

Transport medium (TM) consisted of HBSS buffered with HEPES (10 mM) to pH 7.4. Fasted and fed state simulated intestinal fluid (FaSSIF and FeSSIF) were made according to the manufacturer's protocol. Shortly, FaSSIF and FeSSIF were prepared by dissolving SIF powder in a blank FaSSIF phosphate buffer (2.24 mg/ml) and a blank FeSSIF acetate buffer, respectively (11.2 mg/ml). Modified FeSSIF (pH 6.5) was prepared by dissolving SIF powder in the blank FaSSIF phosphate buffer (11.2 mg/ml).

Human intestinal fluids (HIF) from the duodenum of healthy volunteers were collected in two different nutritional states (11 volunteers for FaHIF and 9 volunteers for FeHIF) according to the method described by Bevernage et al. (2011). The human intestinal fluids 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). Lipase activity was inhibited immediately upon aspiration by addition of orlistat (final concentration of 1 μM) to the test tubes. For each nutritional state, one pooled sample was made by combining the aspirates from all volunteers. The pooled HIF were stored at −30 °C until further use. An approval for the experiments with humans was granted by the University Hospital Medical Ethics Commission of the KU Leuven.

2.3. Solubility measurements

The apparent solubility of carvedilol was determined in TM, FaSSIF, FeSSIF and in HIF of the fasted and fed state using the standard shake flask method. Upon saturation of the human intestinal fluids, the pH of FaHIF and FeHIF amounted to approximately 7.5 and 5.8, respectively. All solubility experiments were performed in triplicate. Approximately 1 mg of carvedilol was added to

Table 1
Key physicochemical and biopharmaceutical characteristics of atenolol, metoprolol, propranolol and carvedilol.

	MW	AUC ratio (fed/fasted)[a]	BCS	exp Log P[c]	Log $D_{pH5.0}$	Log $D_{pH6.5}$	Log $D_{pH7.4}$	pK_a	% Uncharged pH 5.0	% Uncharged pH 6.5	% Uncharged pH 7.4	f_a[b]
Atenolol	266.3	0.8	III	0.16	−2.8	−2.48	−1.80	9.6	0	0.07	0.54	50
Metoprolol	267.4	1.389	I	1.88	−1.47	−1.14	−0.47	9.6	0	0.07	0.54	95
Propranolol	259.3	1.521	I	3.48	−0.64	−0.32	0.36	9.5	0	0.07	0.54	90
Carvedilol	406.5	1.033	II	4.19	0.3	1.22	2.07	7.8	0.02	0.58	4.36	43

[a] AUC ratios (fed/fasted) adopted from Singh (2005).
[b] f_a: Fraction absorbed values adopted from Haslam et al. (2011).
[c] Experimental Log P values were adopted from following references: Poulin and Theil (2002), Ruell et al. (2003), Taylor et al. (1981), Wang et al. (1991).