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Prediction of pH dependent absorption using *in vitro*, *in silico*, and *in vivo* rat models: Early liability assessment during lead optimization

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ABSTRACT

Weakly basic compounds which have pH dependent solubility are liable to exhibit pH dependent absorption. In some cases, a subtle change in gastric pH can significantly modulate the plasma concentration of the drug and can lead to sub-therapeutic exposure of the drug. Evaluating the risk of pH dependent absorption and potential drug–drug interaction with pH modulators are important aspects of drug discovery and development. In order to assess the risk around the extent of decrease in the systemic exposure of drugs co-administered with pH modulators in the clinic, a pH effect study is carried out, typically in higher species, mostly dog. The major limitation of a higher species pH effect study is the resource and material requirement to assess this risk. Hence, these studies are mostly restricted to promising or advanced leads. In our current work, we have used *in vitro* aqueous solubility, *in silico* simulations using GastroPlus™ and an *in vivo* rat pH effect model to provide a qualitative assessment of the pH dependent absorption liability. Here, we evaluate ketoconazole and atazanavir with different pH dependent solubility profiles and based on *in vitro*, *in silico* and *in vivo* results, a different extent of gastric pH effect on absorption is predicted. The prediction is in alignment with higher species and human pH effect study results. This *in vitro*, *in silico* and *in vivo* (IVISIV) correlation is then extended to assess pH absorption mitigation strategy. The IVISIV predicts pH dependent absorption for BMS-582949 whereas its solubility enhancing prodrug, BMS-751324 is predicted to mitigate this liability. Overall, the material requirement for this assessment is substantially low which makes this approach more practical to screen multiple compounds during lead optimization.

1. Introduction

Oral bioavailability of weakly basic drugs which exhibit pH dependent solubility is susceptible to gastric pH change. Multiple reasons such as age (Russell et al., 1993, 1994), disease (Herzlich et al., 1992; Lake-Bakaar et al., 1988; McColl et al., 1998), demographics (Morihara et al., 2001) or co-administration of medication such as antacid, proton-pump inhibitor and H2 receptor antagonist (DeVault and Talley, 2009; Lahner et al., 2009) can alter the gastric pH which can lead to substantial decrease in oral bioavailability and sub-therapeutic exposure of a drug.

In drug discovery, if a potential lead compound exhibits pH dependent solubility, then an early assessment of gastric pH effect on oral absorption is desirable before the compound progresses further into the drug development. Several *in vitro*, *in silico* and preclinical *in vivo* models have been developed at the discovery-development interface to assess pH dependent absorption liability (Gu et al., 2005; Muenster et al., 2011; Takano et al., 2006, 2008). *In silico* modeling with input of pH-solubility data has been used along with preclinical *in vivo* studies to determine the sensitivity of the input parameters which can help medicinal chemist during Structure–Activity Relationship (SAR) exploration. In one such example, an *in silico* model along with a preclinical *in vivo* study has been used to evaluate impact of gastric pH on exposure of weakly basic drug (Bhattachar et al., 2011). Similarly, *in silico in vivo* approach has been used to evaluate different formulation approaches to mitigate the potential pH dependent absorption liability (Kuentz et al., 2006; Mitra and Kesisoglou, 2013; Mitra et al., 2011). *In silico* modeling which connects compound

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properties and physiological parameters with pharmacokinetics has been applied to predict any bioavailability risks as a function of gastric pH (Kuentz et al., 2006; Mathias and Crison, 2012).

Amongst the preclinical models, dog has been a species of choice to determine the pH dependent absorption of weakly basic compounds (Akimoto et al., 2000; Fancher et al., 2011; Zhou et al., 2005). In this model, pretreatment with pentagastrin (administered intramuscularly, to stimulate acid secretion and thus reduce inter animal variability) and famotidine or ranitidine (administered orally) results in reduced and elevated gastric pH, respectively. Gastric aspirates are analyzed to measure the gastric pH levels (Fancher et al., 2011). The advantage of this model is that dog is relatively easy to handle and allows the evaluation of dosage forms intended for subsequent use in humans. However, the canine model has its own limitations. For example, studies have shown that oral bioavailability in dog is not predictive of that in humans mainly due to significant difference in drug absorption and first-pass liver metabolism (Dressman, 1986). Additionally, physiological factors such as slower gastric emptying in the fed state, faster small intestine transit and higher and more variable intestinal pH in dogs compared with humans (Dressman, 1986; Lin, 1995; Lui et al., 1986).

Recently, a cost effective and relatively higher throughput rat pH dependent absorption model has been reported (Lubach et al., 2013). In this model, effect of pentagastrin (0.25 mg/kg, sc) and famotidine (10 mg/kg, iv) pretreatment on rat gastric pH was evaluated and compared with control rats. The gastric pH of control rats was approximately 2.0; whereas after pentagastrin pretreatment gastric pH was ranging from 1.3 to 2.9 over the 6 h time period. Famotidine increased the gastric pH, ranging from 4.0 to 5.2 (Chen et al., 2006).

The selection of an appropriate dose for *in vivo* rat pH effect model is very critical. In retrospective studies where clinical efficacious dose is known, approaches like rat dose equivalent to human dose based on body surface area calculation or normalized to differences in gastric fluid volume can be adopted. However early in discovery where higher species or clinical precedence is not available an *in silico* predicted dose where maximum pH effect is anticipated, can be used to select a relevant dose (Lubach et al., 2013).

In this work, we have tried to correlate *in vitro* (pH-solubility data), *in silico* (GastroPlus™ simulation) and *in vivo* (rat pH effect model) models to evaluate pH-dependent absorption and mitigation strategies to reduce this liability. Atazanavir (ATV) and Ketoconazole (KET) (Fig. 1) were used as tool compounds to establish a qualitative *in vitro*, *in silico* and *in vivo* (IVISIV) correlation. The IVISIV correlation was then extended to BMS-582949 and its ester prodrug, BMS-751324 (Fig. 1). BMS-751324 was prepared to increase solubility in the neutral pH range and mitigate pH dependent absorption observed with BMS-582949.

Overall, the objective of this correlation is to have an early assessment of pH dependent absorption and minimize this risk during lead optimization.

2. Materials and methods

2.1. Materials

Ketoconazole (KET) was purchased from Sigma Aldrich (St Louis, MO). Atazanavir (ATV), BMS-582949 and BMS-751324 were sourced from Bristol-Myers Squibb (New York, NY). All excipients to prepare buffer system were purchased from Merck (Mumbai, India). Methyl cellulose A4 M was purchased from Dow chemical (Michigan, USA) and Tween-80 was purchased from Fluka, Sigma Aldrich (Switzerland). Pentagastrin was sourced from Sigma Aldrich (USA) and Famotidine was purchased from Sigma Aldrich (Italy). Polyethylene glycol 400 (PEG-400) was purchased from Sigma Life Sciences (Belgium).

2.2. Methods

2.2.1. In vitro model – pH-solubility profile

Excess of ATV, KET, BMS-582949 and BMS-751324 powder was added separately to 2 mL aliquots of aqueous hydrochloride (0.1 N/0.01 N)/citrate/acetate/phosphate buffers (50 mM) with pH values ranging from 1 to 7.4. Samples were allowed to equilibrate at 37 °C with continuous shaking for 48 h. In case compound is unstable at any pH condition then sample was equilibrated till the time 90% of the compound is stable. After the incubation period, pH of each sample was measured using a pH meter (Mettler Toledo, Switzerland). Each sample (0.5 mL) was filtered through 96 well pre-saturated PTFE filter (0.45 microns) plate by centrifugation at 4000 rpm for 5 min. The filtrate was then sampled and diluted appropriately using 0.1 N HCl, followed by UPLC analysis (Acquity UPLC-H class system, Waters, Milford, MA) to determine concentrations. The crystal form of the starting material and solid in contact after equilibration with buffers was characterized by Powder X-ray diffraction (PXRD) to ensure no form conversion occurred during equilibration (data not shown).

2.2.2. In silico model

The commercially available software, GastroPlus™, version 7.0 (Simulations Plus, Lancaster, CA, USA) was used to simulate rat *in vivo* exposure. The input parameters were physicochemical, pharmacokinetic and physiological. The measured physicochemical properties such as molecular weight, partition coefficient, ionization constant, pH-solubility profile, permeability and particle size were used as input parameters. The effective rat intestinal permeability was calculated from an internal correlation of Caco-2 permeability and intestinal permeability established for literature compounds. Pharmacokinetic parameters (clearance, volume of distribution and compartmental rate constants, where applicable) were determined from PKPlus module by fitting experimentally determined mean plasma concentration–time profile following intravenous administration. GastroPlus™ default rat fasted physiology (Opt–log$D$ model) was used, except where low (pH 1 to simulate pentagastrin pretreatment), moderate (pH 2 to simulate control without any pretreatment) and high (pH 5 to simulate famotidine pretreatment) gastric pH were required to understand its impact on exposure. The model developed with these input parameters was validated by simulating a solution or suspension oral profile. With the validated model, one parameter was changed at a time, for example gastric pH, to understand its impact on the maximum drug concentration in plasma (C_{max}) and area under the drug concentration–time profile (AUC) (Mathias and Crison, 2012).

2.2.3. Formulations

Pentagastrin and Famotidine formulations were prepared in 10% PEG-400 in normal saline and 100 mM phopshate buffer, pH 6.5, respectively. Suspensions of ATV (10 mg/kg), KET (56 mg/kg), BMS-582949 (14 mg/kg) and BMS-751324 (14 mg/kg equivalent to parent) were prepared in 0.5% w/v Methyl cellulose, 0.1% w/v Tween-80 and 99.4% w/v water. In suspension, an average particle size diameter was less than 20 microns.

2.2.4. Animals

Male Sprague–Dawley rats weighing 280 ± 40 g were obtained from the Syngene in-house breeding facility (Bangalore, India). All animal experiments were conducted in the animal research facility of Syngene International Ltd. (Bangalore, India) which is registered by the Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA) and accredited