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Gastrointestinal transfer: *In vivo* evaluation and implementation in *in vitro* and *in silico* predictive tools

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ABSTRACT

Introduction: The purpose of this study was to explore the transfer of drug solutions from stomach to small intestine and its impact on intraluminal drug concentrations in humans. The collected intraluminal data were used as reference to evaluate simulations of gastrointestinal transfer currently implemented in different *in vitro* and *in silico* absorption models.

Methods: Gastric and duodenal concentrations of the highly soluble and non-absorbable compound paromomycin were determined following oral administration to 5 healthy volunteers under the following conditions: fasted state, fed state and fed state in the presence of a transit-stimulating (domperidone) or transit-inhibiting (loperamide) agent. Based on the obtained intraluminal concentration–time profiles, gastrointestinal transfer (expressed as the half-life of gastric emptying) was analyzed using physiologically-based parameter estimation in Simcyp®. Subsequently, the observed transfer profiles were used to judge the implementation of gastrointestinal transfer in 2 *in vitro* simulation tools (the TNO Intestinal Model TIM-1 and a three-compartmental *in vitro* model) and the Simcyp® population-based PBPK modeling platform.

Results: The observed duodenal concentration–time profile of paromomycin under fasting conditions, with a high average C_{max} obtained after 15 min, clearly indicated a fast transfer of drug solutions from stomach to duodenum (estimated gastric half-life between 4 and 13 min). The three-compartmental *in vitro* model adequately reflected the *in vivo* fasted state gastrointestinal transfer of paromomycin. For both TIM-1 and Simcyp®, modifications in gastric emptying and dilutions were required to improve the simulation of the transfer of drug solutions. As expected, transfer from stomach to duodenum was delayed in the fed state, resulting in lower duodenal paromomycin concentrations and an estimated gastric half-life between 21 and 40 min. Administration of domperidone or loperamide as transit-stimulating and transit-inhibiting agent, respectively, did not affect the fed state gastric half-life of emptying.

Conclusion: For the first time, the impact of gastrointestinal transfer of solutions on intraluminal drug concentrations was directly assessed in humans. *In vitro* and *in silico* simulation tools have been validated and optimized using the *in vivo* data as reference.

1. Introduction

After oral intake, the absorption of a drug depends on several intraluminal processes and physiological variables. Following release from its dosage form, a drug has to dissolve in the gastrointestinal fluids and, subsequently, permeate the intestinal mucosa. These intraluminal processes are influenced by different physiological variables including gastrointestinal hydrodynamics, secretions of gastric and intestinal fluids and gastrointestinal transit. An adequate implementation of these physiologic factors in *in vitro* and *in silico* simulation tools is of crucial importance to accurately predict the behavior of orally administered drugs. However, this implementation often remains an issue, hampering the predictive value of multiple simulation models (Kostewicz et al., 2014).

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For low solubility compounds, the rate and extent of absorption will mainly be determined by the intestinal concentrations that can be reached upon oral intake. Predicting the *in vivo* performance of absorption-enabling formulation strategies for this type of compounds therefore relies on the accurate simulation of intraluminal concentrations. This requires, *inter alia*, the adequate implementation of drug transfer from stomach to duodenum and subsequent dilution with mucosal secretions, bile and pancreatic fluid. The importance of gastrointestinal transfer is exemplified in the performance prediction of enabling strategies that rely on the creation of supersaturation, i.e. intraluminal concentrations exceeding thermodynamic solubility. Supersaturation can be created through formulation strategies, but, for basic compounds, also upon transit from stomach to the intestine (Brouwers et al., 2009). The degree of intestinal supersaturation and, consequently, the tendency for precipitation, can be significantly affected by the rate of gastrointestinal transfer and the extent of duodenal dilution. While the biorelevant evaluation of intraluminal supersaturation has recently been progressing (Bevernage et al., 2010, 2011), the adequate integration of gastrointestinal transfer remains an issue.

Knowledge of gastrointestinal transfer is presently based on indirect methodologies such as the use of plasma concentration–time profiles of high solubility, high permeability compounds, e.g. paracetamol (Heading et al., 1973). Another widely reported technique is scintigraphy (Madsen, 2013) which translates the radiation profile of a specific marker compound into the transfer of the marker from stomach to duodenum. To avoid exposure to ionizing radiation, magnetic resonance imaging (MRI) is gaining more attention (Schiller et al., 2005). The use of water-sensitive magnetic resonance imaging can be used to monitor the transit of dosage forms, along with the movement of gastrointestinal fluids. This technique has recently demonstrated very short gastric residence times of a magnetically-marked capsule: below 3 min upon swallowing for five volunteers with the fastest emptying of the capsule out of the stomach being 15 s (Weitschies et al., 2005). Similar techniques have been used to study gastric emptying of liquid solutions. By use of MRI, the gastric emptying of a 300 ml aqueous solution was investigated: the observed gastric half-life of emptying ($t_{1/2,G}$) amounted to 15.8 min (Steingoetter et al., 2006). In addition, Oberle et al. designed a study to correlate interdigestive motility and gastric emptying for 200 and 50 ml aqueous solutions of phenol red. The authors observed that a 200 ml aqueous solution leaves the stomach with a half-life of 11.8 min, being less dependent on gastric motility than a 50 ml aqueous solution (Oberle et al., 1990). Compared to gastric emptying of solid dosage forms, both studies demonstrated a trend for faster emptying of an aqueous solution (Culen et al., 2013).

Complementary to these techniques, the purpose of this study was to provide a more direct approach to evaluate the impact of gastrointestinal transfer of drugs in solution on their intraluminal concentrations. To exclude the confounding effects of absorption, dissolution and/or precipitation, we selected the non-absorbable, highly soluble paromomycin as a model drug. Following oral intake of a solution, gastric and duodenal paromomycin concentrations were simultaneously monitored in healthy volunteers. The observed concentration–time profiles directly reflect the gastrointestinal transfer and simultaneous dilution by gastrointestinal secretions. Gastrointestinal transfer was explored in the fasted and fed states. In addition, the impact of the transit-stimulating agent Motilium® (domperidone) and the transit-inhibiting agent Imodium® (loperamide HCl) was assessed. In a next step, the generated human data set was used as reference to judge the implementation of gastrointestinal transfer in two existing *in vitro* tools (TNO Intestinal Model-1 (Blanquet et al., 2004) and a simpler three-compartment *in vitro* model (Psachoulias et al., 2012)) and an *in silico* PBPK model (Simcyp®) (Jamei et al., 2009; Rostami-Hodjegan, 2012).

2. Materials and methods

2.1. Chemicals

Paromomycin sulfate, glycine, orlistat and 9-fluorenyl methoxycarbonyl chloride (FMOC-CL) were obtained from Sigma Aldrich (St. Louis, MO, USA). Acetonitrile and NaCl were purchased from Fisher Scientific (Leicestershire, UK), while $NaH_2PO_4{\cdot}H_2O$ was received from Acros Organics (Geel, Belgium). Merck (Overijse, Belgium) supplied boric acid. BDH Laboratory Supplies (Poole, UK) provided HCl and NaOH. KOH was obtained from Riedel-De Haën (Seelze–Hannover, Germany). Sodium acetate and acetic acid were supplied by VWR (Leuven, Belgium). Simulated intestinal fluid (SIF) powder was purchased from Biorelevant (Croydon, UK). Lonza (Verviers, Belgium) was the supplier of Hanks' balanced salt solution (HBSS), Dulbecco's modified Eagle's medium, penicillin–streptomycin (10,000 IU/ml), nonessential amino acid medium (100×), trypsin–EDTA solution, fetal bovine serum, and HEPES. Purified water was obtained by using a Maxima system (Elga Ltd., High Wycombe Bucks, UK).

For the Caco-2 experiments, the cell culture medium consisted of Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum, 1% nonessential amino acid, and 100 IU/ml penicillin–streptomycin. Transport medium consisted of HBSS containing 25 mM glucose and 10 mM HEPES (pH 7.4). Fasted state simulated intestinal fluid (FaSSIF), including sodium taurocholate (3 mM) and lecithin (0.75 mM) was made by dissolving 2.24 mg SIF powder per milliliter FaSSIF buffer (pH 6.5), which contains NaOH (10.5 mM), Na_2HPO_4 (28 mM) and NaCl (106 mM).

2.2. Paromomycin: luminal solubility & intestinal permeability

The solubility of paromomycin was determined by incubating an excess of powder (5 mg paromomycin sulfate) to 0.5 ml of human gastric and intestinal fluids for 28 h at 37 °C in a prewarmed shaking incubator (175 rpm) (KS4000i incubator, Ika, Staufen, Germany). Samples were centrifuged for 10 min at 20.817 g and the supernatant was analyzed for paromomycin concentration (see Section 2.6). To confirm the non-absorbable properties of paromomycin, a Caco-2 experiment was set up. Caco-2 cells were cultured as described previously (Bevernage et al., 2012). On the day of the experiment the cell culture medium was refreshed one hour before the experiment. After rinsing the cells twice, the transepithelial electrical resistance (TEER) was measured after 25 min. Only monolayers with TEER values higher than 300 $\Omega \times cm^2$ were used. After aspirating the apical and basolateral media, 1.5 ml of transport medium (pH 7.4) containing 0.2% D-α-tocopheryl polyethylene glycol 1000 succinate (to create sink conditions) was added to the basolateral compartment; the apical compartment consisted of 0.5 ml of transport medium (pH 7.4) or FaSSIF (pH 6.5) containing paromomycin at different concentrations (1400 μM in transport medium, 200 and 50 μM in FaSSIF). Samples were taken from the donor and acceptor compartment after 60 min and analyzed for paromomycin. After completion of the experiment, TEER values were measured to check the final integrity of the monolayer.

2.3. In vivo study

To evaluate the gastrointestinal transfer *in vivo*, gastric and duodenal concentrations of paromomycin were monitored in five healthy volunteers (two men, three women; aged between 22 and 24 years). Exclusion criteria included gastrointestinal disorders, HIV, hepatitis B or C infection, use of medication, pregnancy and frequent X-ray exposure. The procedure followed the tenets