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Evaluation of the Impact of Excipients and an Albendazole Salt on Albendazole Concentrations in Upper Small Intestine Using an *In Vitro* Biorelevant Gastrointestinal Transfer (BioGIT) System

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ABSTRACT

An *in vitro* biorelevant gastrointestinal transfer (BioGIT) system was assessed for its ability to mimic recently reported albendazole concentrations in human upper small intestine after administration of free base suspensions to fasted adults in absence and in presence of supersaturation promoting excipients (hydroxypropylmethylcellulose and lipid self-emulsifying vehicles). The *in vitro* method was also used to evaluate the likely impact of using the sulfate salt on albendazole concentrations in upper small intestine. In addition, BioGIT data were compared with equilibrium solubility data of the salt and the free base in human aspirates and biorelevant media. The BioGIT system adequately simulated the average albendazole gastrointestinal transfer process and concentrations in upper small intestine after administration of the free base suspensions to fasted adults. However, the degree of supersaturation observed in the duodenal compartment was greater than *in vivo*. Albendazole sulfate resulted in minimal increase of albendazole concentrations in the duodenal compartment of the BioGIT, despite improved equilibrium solubility observed in human aspirates and biorelevant media, indicating that the use of a salt is unlikely to lead to any significant oral absorption advantage for albendazole.

Introduction

New drug candidates often have low aqueous solubility to an extent that require administration in enabling formulations to promote supersaturation, to achieve luminal concentrations to get most of the dose absorbed.[1] In the case of a poorly soluble weak base, the impact of gastrointestinal transfer in the fasted state on absorption rates may be substantial, because, due to the abrupt increase in pH, precipitation in the upper small intestine is likely. The use of supersaturation promoting formulations may then be required.

Various methodologies have been proposed to evaluate the impact of gastrointestinal transfer on the performance of the dosage form and of the drug intraluminally.[2] Recently, an *in vitro* biorelevant gastrointestinal transfer (BioGIT) system was proposed for the evaluation of the impact of gastrointestinal transfer on concentrations in upper intestinal lumen during the first hour, after oral administration of dispersing/solution dosage forms of lipophilic weak bases in the fasted state.[3]

In the present study the impact of excipients and of free base versus salt on concentrations of lipophilic weak bases in upper small intestine was investigated with BioGIT by using albendazole free base (ABZ, pka 2.80,[4] logP 3.46[5]) as a model lipophilic weak base. Albendazole is one of the WHO Essential medicines and is commercially available for oral administration at a single dose strength of 400 mg with the objective being to maintain effective luminal concentrations in the entire GI lumen, due to its specific pharmacological action (prescription drug information on http://home.intekom.com/pharm/smith_kb/zentel.html). There are no albendazole salts or lipid-based formulations commercially available, no iv data in humans were ever collected and, therefore, no absolute bioavailability data are available. It has been classified as BCS Class II API based on its logP value[6] and as borderline BCS

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Class II/IV, due to inconclusive data available.[7] Albendazole undergoes extensive first-pass effect, is not substrate of p-gP and shows significantly higher BA in the fed state.[7] Albendazole solubility decreases from ~500 μg/mL in pH 1.2 to ~2 μg/mL in pH 4.1 and ~1.5 μg/mL in pH 7.0,[8] that is, albendazole solubility is low, even under conditions of extensive ionization. Based on *in vitro* data[9] and rat data,[10] albendazole is expected to precipitate fast on entering the upper small intestine.

There were 3 specific objectives of the present study:

First, to evaluate the usefulness of BioGIT in reproducing recently observed ABZ concentrations and supersaturation of contents of the upper small intestine of healthy adults after administration of various ABZ free base suspensions in absence and in presence of supersaturation promoting excipients in the fasted state.[11]

Second, to evaluate the impact of free base versus salt on ABZ concentrations and degrees of supersaturation of contents of the upper small intestine in absence and in presence of HPMC E5 using BioGIT.

Third, to compare BioGIT data with equilibrium solubility data (in human aspirates and in biorelevant media) in order to evaluate the impact of free base versus salt on ABZ behavior in the upper gastrointestinal lumen.

Because only the free base of ABZ is commercially available, a salt had to be prepared for the needs of the present investigation. Although various salts of ABZ have been prepared and characterized in the past,[8] the relatively low pKa makes ABZ salts sensitive to disproportionation.[12] After considering various counter ions, the sulfate salt of ABZ was selected to be used, based on its comparatively better crystallinity and stability characteristics.

In the present investigation, lower than the pharmacologically relevant single dose was studied (50 mg instead of 400 mg), for 2 reasons:

1. The impact of supersaturation promoting excipients, if any, is expected to be increased in presence of fewer particles and, therefore, easier to be detected.
2. To achieve the first objective of this study, that is, to evaluate *in vitro* data collected in this study versus recently reported luminal data collected at the 50-mg single dose level.[11]

Materials and Methods

Materials

Albendazole free base was from Sigma-Aldrich (99% pure; Sigma-Aldrich, St. Louis, MO). Sodium phosphate monobasic, sodium hydroxide, sodium chloride, ammonium formate, and pepsin from porcine gastric mucosa (15.8% protein) were purchased from Sigma-Aldrich. Acetonitrile and water (HPLC grade) as well as tetrahydrofuran and ethanol were also from Sigma-Aldrich. n-Butanol was purchased from Fluka (Neu-Ulm, Germany). SIF® Powder Original was kindly donated by Biorelevant.com (Surrey, UK). Hydrochloric acid was from Panreac Co. (Barcelona, Spain). Hydroxypropylmethylcellulose E5 was from JRS Pharma (Zacapu, Mexico). Miglyol 812N [Caprylic/Capric (C8-C10) triglycerides] was received from Sasol Germany. Cremophor RH 40 was from BASF (Ludwigshafen, Germany). Polysorbate 80 was from Sigma-Aldrich.

Preparation of Various ABZ Salts and Selection of the Sulfate Salt

Initially, the solubility of ABZ free base was assessed in tetrahydrofuran, ethanol, water, and n-butanol at a range of temperatures (25-80°C). To obtain albendazole hydrochloride, albendazole sulfate and albendazole mesylate, 10, 20, 30, 40, and 50 mg of ABZ free base were transferred into 2-mL HPLC vials followed by the addition of 1 mL of solvent (tetrahydrofuran, water, ethanol, or n-butanol) and the addition of an equimolar amount of acid (HCl, H_2SO_4, or methanesulfonic acid). The vials were placed in a Crystal 16 apparatus (Avantium Crystallization System, Amsterdam, The Netherlands), temperature was cycled with the maximum temperature set 10°C below the solvents boiling point and the salt was formed at the end of the experiment. Two scale-up experiments were conducted using 25 mL and 50 mL of solvent, to ensure the scale-up procedure was viable. Equimolar amounts of ABZ free base and the acids (HCl, H_2SO_4, or methanesulfonic acid) were added to 100-mL glass beakers containing either 25 or 50 mL of solvent and the mixture heated to 10 degrees below the boiling point of the solvent. At the elevated temperature, the ABZ salt was completely dissolved; it was then left at room temperature to cool down and the salt precipitated. Residual solvent was evaporated at room temperature, and the material was further dried at 40°C, over 24 hours, and weighed. The formation of ABZ salts (as hydrates, based on Karl-Fisher titration data) was confirmed using nuclear magnetic resonance, differential scanning calorimetry (30-300°C in 10 min, Mettler Toledo DSC 822e), and Elemental Analysis for carbon, hydrogen, and nitrogen (data on file). Based on X-ray diffraction data, albendazole hydrochloride was poorly crystalline (unlike with a previous report[8]) whereas albendazole mesylate crystals and crystal stability characteristics varied with the solvent used for their isolation. In contrast, albendazole sulfate salt (ABZ sulfate) samples obtained with tetrahydrofuran and ethanol were concordant (some differences in peak intensities may suggest small differences in crystallinity). In addition, based on the 1-month stability data ABZ sulfate, peaks were concordant at 2 tested conditions (40°C/75% humidity and 50°C/ambient humidity) with the initial analysis. Based on these data, ABZ sulfate obtained using tetrahydrofuran was selected to be used in the present investigation.

Experiments With BioGIT

Methodology

BioGIT is an open *in vitro* setup simulating the drug transfer from the stomach into the fasted upper small intestine (Fig. 1). The initial volume of the gastric compartment is 250 mL. The duodenal volume is maintained at 40 mL during the entire experiment. The mini paddles in gastric and duodenal compartments rotate at 75 rpm. The emptying of contents of gastric compartment (on a volume basis) follows first-order kinetics with a half-life of 15 min. Experiments are performed using a 3-channel peristaltic pump (Reglo ICC pump, part ISM 4308, Ismatec®) for 45 min, after the initiation of an experiment. Incoming flow rates are changed every 10 min, and sampling is performed at midpoint.[3] In this study, experiments were performed in triplicate at 37°C.

Contents of Gastric Compartment

To simulate ABZ administration with a glass of water, aqueous suspensions were brought in gastric compartment in the beginning of experiment in all cases.

Two aqueous suspensions of ABZ free base were tested. For the first, 50-mg ABZ free base was mixed with 200-mL table water (Nera Kritis, Heraklion, Greece) under vigorous magnetic stirring for 5 min at room temperature. Preparation of the second suspension was achieved by dissolving 5-mg HPMC E5 in 200-mL table water, before mixing 50-mg ABZ free base with the aqueous HPMC E5 solution. Regardless of the presence of HPMC E5, the suspension was mixed with 50-mL concentrated level III FaSSGF. The resulting 250-mL suspension in level III FaSSGF pH 1.6,[13] Susp or Susp-HPMC, was immediately added to the gastric compartment, and the transfer experiment was initiated.