Journal of Pharmaceutical Sciences 105 (2016) 996–1005

Contents lists available at ScienceDirect

Journal of Pharmaceutical Sciences

journal homepage: www.jpharmsci.org

Pharmacokinetics, Pharmacodynamics and Drug Transport and Metabolism

Effect of Common Excipients on the Oral Drug Absorption of Biopharmaceutics Classification System Class 3 Drugs Cimetidine and Acyclovir

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ARTICLE INFO

Article history:
Received 15 July 2015
Revised 19 August 2015
Accepted 26 August 2015
Available online 16 September 2015

Keywords:
Biopharmaceutics Classification System (BCS)
bioequivalence
excipients
oral absorption
permeability
cimetidine
acyclovir

ABSTRACT

The objective was to assess the impact of larger than conventional amounts of 14 commonly used excipients on Biopharmaceutics Classification System (BCS) class 3 drug absorption in humans. Cimetidine and acyclovir were used as model class 3 drugs across three separate four-way crossover bioequivalence (BE) studies ($n = 24$ each) in healthy human volunteers, denoted as study 1A, 1B, and 2. In study 1A and 1B, three capsule formulations of each drug were manufactured, collectively involving 14 common excipients. Capsule formulations that incorporated hydroxypropyl methylcellulose (HPMC) or magnesium stearate exhibited lower absorption. The cimetidine commercial solution contained sorbitol and also resulted in lower absorption. Hence, in study 2, two capsule formulations with lower amounts of HPMC and magnesium stearate, the sorbitol-containing commercial solution, and a sorbitol-free solution were assessed for BE. Overall, 12 common excipients were found in large amounts to not impact BCS class 3 drug absorption in humans, such that these excipients need not be qualitatively the same nor quantitatively very similar to reference, but rather simply be not more than the quantities studied here. Meanwhile, for each HPMC and microcrystalline cellulose, BCS class 3 biowaivers require these two excipients to be qualitatively the same and quantitatively very similar to the reference.

Introduction

The Biopharmaceutics Classification System (BCS) is a scientific framework that characterizes drug substances according to their aqueous solubility and intestinal permeability.[1] Solubility, permeability, and drug product dissolution determine the rate and extent of drug absorption from immediate-release (IR) solid oral dosage forms (e.g., tablets and capsules). As BCS class 1 drugs have favorable oral biopharmaceutical properties, the United States Food and Drug Administration (US FDA) and the European Medicines Agency (EMA) have allowed waivers of *in vivo* bioequivalence (BE) studies for such rapidly dissolving IR solid oral dosage forms.[2,3] Rapid dissolution requires >85% of active ingredient be dissolved in 30 min. BCS-based biowaivers have allowed brand and generic products to receive regulatory relief based on *in vitro* data alone, which reduces unnecessary human testing and affords resource savings.[4-6]

The scientific community has suggested that biowaivers be extended to BCS class 3 drugs with a further requirement that dissolution be very rapid (>85% in 15 min).[7-9] IR products of BCS

Abbreviations used: ANDA, Abbreviated New Drug Application; BCS, biopharmaceutics classification system; BE, bioequivalence; HPMC, hydroxypropyl methylcellulose; IR, immediate-release; SLS, sodium lauryl sulfate.

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This article contains supplementary material available from the authors upon request or via the Internet at http://dx.doi.org/10.1002/jps.24643.

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http://dx.doi.org/10.1002/jps.24643
0022-3549/

class 3 drugs can be expected to behave like oral solutions if dissolution is very rapid over a range of pH conditions. If dissolution is very rapid, the rate limiting step for oral absorption would be intestinal membrane permeation or gastric emptying, and not drug dissolution.[6,9,10] BCS class 3 drugs constitute almost 25% of drugs marketed in the United States.[4] Moreover, almost 40% of orally administered drugs on the WHO Model List of Essential Medicines are BCS class 3 drugs.[11] Extending biowaivers to class 3 drugs can reduce development costs and reduce human drug exposure.[4,5,11]

European Medicines Agency allows BCS-based biowaivers for class 3 drugs in very rapidly dissolving IR solid oral dosage forms, and US FDA has recently also proposed the same.[2,3] EMA and US FDA appear to indicate that, for excipients that are not known to affect bioavailability, BCS class 3 biowaivers require that excipients be qualitatively the same and quantitatively very similar. These limitations reflect concerns that excipients have potential to modulate class 3 drug absorption via impacting drug intestinal permeability, motility, or drug stability/metabolism.[6-8] By virtue of class 3 drug absorption being incomplete because of the lower drug intestinal permeability, excipient modulation of drug intestinal permeability and/or drug transit through the gastrointestinal tract are major concerns. Some excipients like sorbitol and mannitol can enhance *in vivo* transit time of low permeability drugs, causing bioinequivalence.[12,13] An additional potential concern is excipient modulation of protein expression with subsequent impact on drug disposition, although we have not seen such evidence in commonly used excipients.[14]

Previously, we employed Caco-2 monolayers to evaluate the effect of nine individual excipients on the Caco-2 permeability of seven low permeable compounds that differ in their physiochemical properties.[15] Generally, most excipients had no influence on drug permeability. Sodium lauryl sulfate (SLS) moderately increased the permeability of almost all the drugs. Hydroxypropyl methylcellulose (HPMC) appeared to increase cimetidine permeability. It was concluded that further work was needed to interpret the *in vivo* consequences of these observations from cell culture.

The objective of the present study was to assess the impact of very large amounts of 14 commonly used excipients on BCS class 3 drug absorption in humans. Study 1 involved two fasted, single-dose, four-way crossover BE studies in healthy human volunteers (i.e., study 1A and 1B). In study 1A, cimetidine was the model BCS class 3 drug.[16] In study 1B, acyclovir was the model BCS class 3 drug.[17] Each study involved 3 test drug capsule formulations, where each formulation contained very large quantities of three excipients. Excipient effect was intended to be assessed via BE of capsule against an oral liquid, although CimTest-2 and AcyTest-2 were the reference formulations employed. Results of study 1A and 1B lead to a subsequent study, denoted as study 2, focusing on HPMC, magnesium stearate, and sorbitol as excipients. Figure 1 illustrates a flowchart of excipient influences across studies 1A and 1B, including the rationale for subsequent study 2.

Materials and Methods

Materials

Cimetidine (study 1A), SLS, and acyclovir were obtained from Spectrum Chemical Manufacturing Corporation (New Brunswick, NJ). Cimetidine (study 2) and sodium hydroxide were obtained from Letco Medical (Decatur, AL). Microcrystalline cellulose (type PH-102) and croscarmellose sodium (type SD-711) were obtained from FMC BioPolymer (Newark, DE). HPMC was obtained from The Dow Chemical Company (Bay City, MI). Corn starch was obtained from Roquette America Inc. (Keokuk, IA). Sodium starch glycolate and lactose were obtained from DMV Fonterra Excipients (Foxhol, the Netherlands). Colloidal silicon dioxide was obtained from Evonik Industries (Aerosil 200 Pharma; Piscataway, NJ). Dibasic calcium phosphate was obtained from JRS Pharma (Patterson, NY). Crospovidone and povidone were obtained from BASF The Chemical Company (Jessup, MD). Stearic acid and magnesium stearate were obtained from Mallinckrodt (St. Louis, MO). Pregelatinized starch was obtained from Colorcon (West Point, PA). Empty hard gelatin capsules were obtained from Capsugel (Morristown, NJ). Propylparaben was obtained from Macron Fine Chemicals (Center Valley, PA). Methylparaben was obtained from Protameen Chemicals, Inc. (Totowa, NJ). Sodium 1-hexanesulfonate, sodium acetate trihydrate, potassium phosphate monobasic, sodium phosphate dibasic heptahydrate, hydrochloric acid, and sodium phosphate monobasic were purchased from Sigma-Aldrich (St. Louis, MO). Cimetidine hydrochloride oral solution 300 mg/5 mL (equivalent to cimetidine) and acyclovir oral suspension 200 mg/5 mL were purchased from Hi-Tech Pharmacal (Amityville, NY). Cimetidine and acyclovir reference standards were purchased from the United States Pharmacopeia (Rockville, MD). All solvents were HPLC grade and were purchased from Fisher Scientific Inc. (Pittsburg, PA).

Study 1: Formulations and In Vitro *Testing*

Cimetidine was used as a model BCS class 3 drug for study 1A. Acyclovir was used as model BCS class 3 drug for Study 1B. Three capsule formulations of each drug were manufactured, collectively involving 14 common excipients, which were: microcrystalline cellulose, HPMC, SLS, corn starch, sodium starch glycolate, colloidal silicon dioxide, dibasic calcium phosphate, crospovidone, lactose, povidone, stearic acid, pregelatinized starch, croscarmellose sodium, and magnesium stearate. These 14 excipients were selected from a list of the 20 most common excipients in oral solid Abbreviated New Drug Application (ANDA) formulations. Not selected from this list were: Opadry, talc, citric acid, sucrose, methyl cellulose, and titanium dioxide. Each capsule formulation contained 100 mg of either cimetidine or acyclovir in study 1A or study 1B, respectively, along with three excipients in quantities higher than those used in typical IR solid oral dosage forms. Capsule compositions are shown in Table 1. Capsules were not intended to exhibit dissolution-limited absorption, although formulation design was limited by the need to use only very large quantities of the 14 excipients. Regarding excipient composition, test capsules were not intended to be qualitatively or quantitatively the same as commercial cimetidine tablets or acyclovir capsules.

A Turbula mixer (Turbula, Type: T2F Nr 070759; Basel/Schweiz) was used to mix the drug and 3 excipients into powder blends, which were hand filled into capsules. Cimetidine and acyclovir capsules were manufactured under current good manufacturing practices (GMP) at the University of Maryland GMP Facility.

Capsules of cimetidine and acyclovir were subjected to a panel of 6 quality control (QC) tests: appearance, identification, assay, impurity, uniformity of dosage units, and dissolution, which were performed as specified in the USP monograph for cimetidine and acyclovir, respectively.[18,19] Uniformity of dosage units was performed by the weight variation approach. Furthermore, for each cimetidine and acyclovir whose tablet or capsule USP monograph employs pH 1.2 dissolution media, *in vitro* dissolution studies were also performed at the two additional pH values of 4.5 and 6.8.[20] All dissolution tests were performed on six units of each product using USP apparatus I at 100 rpm and at 37°C in 900 mL of pH 1.2, 4.5, and 6.8 media. pH 1.2 media was 0.1 N HCl. pH 4.5 media was 0.2 M sodium acetate trihydrate, adjusted with HCl to pH 4.5. pH 6.8 media was 0.2 M monobasic potassium phosphate, adjusted with sodium hydroxide to pH 6.8. The commercial oral liquid solution of

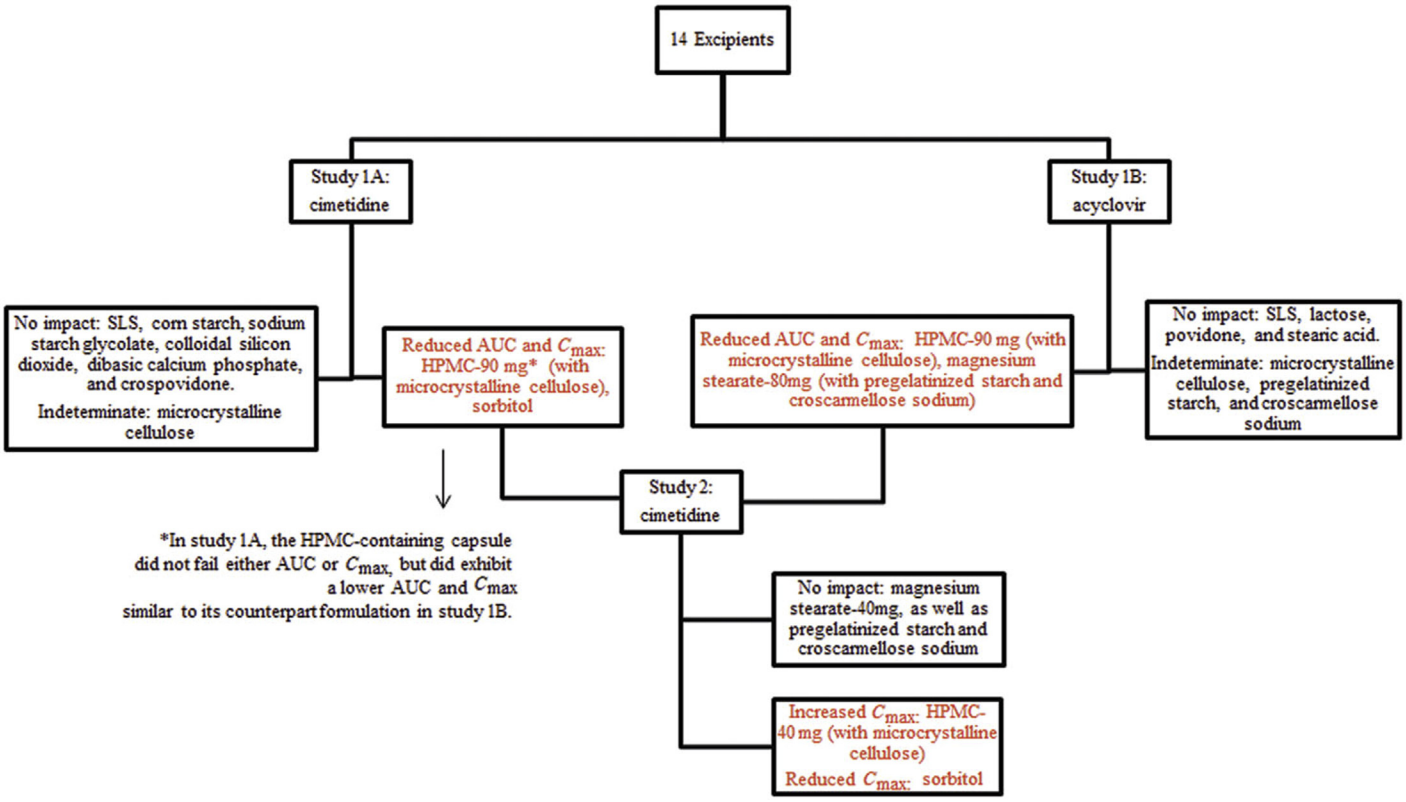

Figure 1. Flowchart of excipient influences across studies 1 and 2.

cimetidine and acyclovir were used as the reference formulation for study 1A and 1B, respectively.[21]

Study 1: In Vivo *Studies*

Separately, for each cimetidine 200 mg and acyclovir 200 mg, a human pharmacokinetic study was conducted in 24 healthy adult volunteers. Each study was an open-label, fasted, single dose, randomized four-way crossover BE study. Study 1A for cimetidine and study 1B for acyclovir were completely separate clinical studies. Both studies were conducted at the General Clinic Research Center (GCRC) at the University of Maryland. All human studies were approved by the Institutional Review Board of University of Maryland Baltimore, as well by the Research Involving Human Subject Committee (RIHSC) of US FDA.

Volunteers fasted overnight for 10 h prior to drug administration. Each subject was given a dose of 200 mg of cimetidine or acyclovir orally (e.g., two cimetidine capsules, 3.33 mL of cimetidine commercial oral solution, two acyclovir capsules, or 5 mL of acyclovir commercial oral suspension) along with 240 mL water. Otherwise, water was not allowed 1 h before and 1 h after drug administration. The subjects were provided lunch and a snack 4 and 6 h after drug administration, respectively.

Blood samples (5 mL, heparinized tubes) for pharmacokinetic analysis were drawn immediately prior to drug administration and at 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 5.0, 6.0, 8.0, and 10.0 h post-dose. The blood samples were centrifuged at 1000g at 4°C for 10 min. Harvested plasma aliquots were stored at −20°C until assayed.

Study 2: Formulations and In Vitro Testing

Study 2 was a follow up study of study 1. Based on study 1 results, it was suspected that HPMC in CimTest-1 and AcyTest-1 formulations (which were compositionally identical, except for drug; Table 2) and magnesium stearate in AcyTest-3 formulation (Table 1) slowed drug dissolution *in vivo*. Additionally, it appeared that

Table 1
Study 1A and 1B Test Formulations: Compositions and *In Vitro* Dissolution

Formulation	Excipient 1	Excipient 2	Excipient 3	% Dissolved in 15 min[a]		
				pH 1.2	pH 4.5	pH 6.8
CimTest-1	Microcrystalline cellulose (300 mg)	Hydroxypropyl methylcellulose (45 mg)	Sodium lauryl sulfate (25 mg)	106 ± 2.0	97.5 ± 1.7	82.6 ± 5.4
CimTest-2	Corn starch (450 mg)	Sodium starch glycolate (100 mg)	Colloidal silicon dioxide (20 mg)	104 ± 1.5	100.1 ± 2.0	100.6 ± 1.4
CimTest-3	Dibasic calcium phosphate (300 mg)	Sodium lauryl sulfate (25 mg)	Crospovidone (50 mg)	95.3 ± 2.8	97.9 ± 1.8	93.9 ± 2.5
AcyTest-1	Microcrystalline cellulose (300 mg)	Hydroxypropyl methylcellulose (45 mg)	Sodium lauryl sulfate (25 mg)	83.9 ± 2.7	70.4 ± 2.8	81.2 ± 3.6
AcyTest-2	Lactose (450 mg)	Povidone (35 mg)	Stearic acid (40 mg)	99.7 ± 0.6	85.1 ± 3.3	67.1 ± 5.1
AcyTest-3	Pregelatinized starch (100 mg)	Croscarmellose sodium (60 mg)	Magnesium stearate (40 mg)	75.6 ± 2.9	73.6 ± 1.7	59.6 ± 3.9

Capsules for study 1A included 100 mg of cimetidine. Capsules for study 1B included 100 mg of acyclovir. All capsules contained three excipients. Study 1A and 1B collectively evaluated 14 excipients across six test capsule formulations. Formulation CimTest-1 and AcyTest-1 employed the same excipients. Sodium lauryl sulfate was included in formulations CimTest-1, CimTest-3, and AcyTest-1. In the *in vivo* study of each formulation, two capsules were administered as a single dose of 200 mg of drug.

[a] Mean ± SEM.

Table 2
Prototype Study 2 Test Formulations

Formulation	Formula	Excipient	% Dissolved in 15 min[a]
CimTest-A-10 mg	Cimetidine (100 mg); microcrystalline cellulose (300 mg); sodium lauryl sulfate (25 mg)	HPMC: 10 mg (2.3%)	92.9 ± 3.3
CimTest-A-20 mg[b]		HPMC: 20 mg (4.5%)	89.5 ± 2.8
CimTest-A-45 mg[c]		HPMC: 45 mg (9.5%)	38.6 ± 8.1
CimTest-A-75 mg		HPMC: 75 mg (15%)	23.5 ± 3.6
CimTest-B-20 mg[b]	Cimetidine (100 mg); pregelatinized starch (100 mg); croscarmellose sodium (60 mg)	Mag st: 20 mg (7.1%)	94.5 ± 2.4
CimTest-B-40 mg		Mag st: 40 mg (13.3%)	60.2 ± 3.2
CimTest-B-40 mg-L		Mag st: 40 mg (8%) + Lactose: 200 mg	60.0 ± 5.0
CimTest-B-40 mg-T[d]		Mag st: 40 mg (13.3%): turbular mixer	29.0 ± 5.1

CimTest-A prototypes differed in HPMC amount, where CimTest-A-20 mg was selected for clinical study 2 as it showed very rapid dissolution. CimTest-B prototypes differed in magnesium stearate amount and processing, where CimTest-B-20 mg was selected for clinical study 2 as it showed very rapid dissolution in pH 6.8 media. The two formulations for clinical evaluation were subsequently denoted, more simply, as CimTest-A and CimTest-B.

[a] Mean ± SEM in pH 6.8 media.
[b] Selected for clinical study 2.
[c] Original formulation CimTest-1 in study 1A.
[d] Original excipient composition in study 1B (i.e., AcyTest-3).

sorbitol impacted cimetidine absorption from commercial oral solution. In study 2, cimetidine was used as the model BCS class 3 drug.

Table 2 lists prototype formulations that were considered for study 2. Capsules containing a range of HPMC (1075 mg HPMC) and magnesium stearate (20-40 mg magnesium stearate) were evaluated *in vitro,* in order to identify compositions that would provide very rapidly dissolving formulations. One selected cimetidine capsule formulation for clinical study 2 was denoted CimTest-A and contained 20 mg of HPMC, a reduced amount compared with the 45 mg of HPMC in CimTest-1 and AcyTest-1 from study 1A and 1B, respectively. The other selected cimetidine capsule formulation for clinical study 2 was denoted CimTest-B and contained 20 mg of magnesium stearate, a reduced amount compared with the 40 mg of magnesium stearate in AcyTest-3 from study 1B. Additionally, CimTest-B differed from AcyTest-3 by using a V-blender (Twin shell drug blender, model LB 331; Stroudsburg, PA) rather than a Turbula mixer, to avoid over-lubrication. Capsules were subjected to *in vitro* dissolution in phosphate buffer of pH 6.8 using USP apparatus I in order to preliminarily identify very rapidly dissolving formulations (Table 2). Cimetidine capsules for clinical study were subjected to the same QC tests that had been performed in study 1A: appearance and identification, assay, impurity, content uniformity, and dissolution.

Given study 1A findings where the sorbitol-containing commercial cimetidine solution provided about 10% lower absorption compared to cimetidine capsules, a sorbitol-free cimetidine oral solution was manufactured and used as the reference for study 2. The commercial cimetidine oral solution was used in study 2 as a test formulation, to assess an impact of sorbitol. The composition of the sorbitol-free solution intended to mimic the commercial product except for sorbitol and was: cimetidine (300 mg/5 mL), methyl paraben (5 mg/5 mL), propyl paraben (1 mg/5 mL), dibasic sodium phosphate heptahydrate (0.568 mg/5 mL), and monobasic sodium phosphate anhydrous (12.62 mg/5 mL). The solution pH was adjusted to 5.1-5.7 with hydrochloric acid (37%) and sodium hydroxide pellets. The sorbitol-free cimetidine oral solution was subjected to appearance and identification, assay, impurity, and microbial testing, and passed all testing. Cimetidine capsules and the sorbitol-free oral solution were manufactured under cGMP at the University of Maryland GMP Facility.

Study 2: In Vivo *Studies*

A human pharmacokinetic study of cimetidine 200 mg was conducted in 24 healthy adult volunteers. The study was an open-label, fasted, single-dose, randomized four-way crossover BE study conducted at the GCRC at the University of Maryland. The study procedure and protocol was the same as study 1.

Quantification of Cimetidine and Acyclovir

Cimetidine was quantified with HPLC using Waters system with a dual wavelength (model 248) absorbance detector (Waters Corporation, Milford, MA). The wavelength was set to 228 nm. The column used was a Phenomenex (Torrance, CA) C18 4.6 × 250 mm^2 column. Cimetidine was analyzed using a previously reported method.[22] Briefly, the quantification process involved combining 250 μL plasmawith 30 μL of 2 M NaOH, 250 μL saturated sodium carbonate solution, and 30 μL of internal standard, famotidine (50 μg/mL) in a 4.5 mL polypropylene tube. The sample was vortexed briefly followed by the addition of 3 mL water saturated ethyl-acetate. The tube was shaken at a low speed for 10 min followed by centrifugation for 10 min at 2000g. The organic layer was transferred to a clean tube. The contents were evaporated to dryness under a stream of liquid nitrogen. The residue was re-constituted in the mobile phase followed by injection onto the HPLC column with an injection volume of 50 μL. The method was linear between 100 and 4000 ng/mL (r^2 = 0.998). The intra and inter-day precision and accuracy were ≤4.2%.

Chromatographic separation of acyclovir was achieved using ultra-HPLC-heated electrospray ionization-tandem mass spectrometry (UHPLC-HESI-MS/MS).[23] The UHPLC system was comprised of an Accela degasser, quaternary pump, and an HTC PAL thermostatted autosampler (Thermo Scientific, San Jose, CA). The column was a Waters BEH C18 50 × 2.1 mm^2 with 1.7 μm particle size. The mobile phase consisted of a linear gradient of 2 mM aqueous ammonium acetate with 0.1% formic acid (A) and 100% methanol with 0.1% formic acid (B). A TSQ Vantage triple quadrupole mass spectrometer (Thermo Scientific) with a HESI source was operated in positive ionization mode using selected reaction monitoring. For acyclovir, transition of *m/z* 226.1 to *m/z* 152.0 was monitored. For ribavirin (internal standard), transition of *m/z* 245.1 to *m/z* 113.0 was monitored. Sample preparation involved combining 200 μL plasma samples (containing 50 μL of 2 μg/mL ribavirin which was used as an internal standard) with 1000 μL of acetonitrile. The resulting mixture was vortexed briefly followed by centrifugation for at 10,000*g* for a minute. Supernatant (1000 μL) was evaporated to dryness under a stream of liquid nitrogen. The residue was re-constituted in 10% methanol containing 0.1% formic acid to yield a final volume of 200 μL. The method was linear between 1 ng/mL and 2000 ng/mL (r^2 = 0.997). The intra and inter-day precision and accuracy were ≤13%.

Quantification of Sorbitol

Sorbitol concentrations in commercial cimetidine solution and acyclovir suspension were quantified via UHPLC–HESI–MS/MS using a UHPLC system comprised of an Accela degasser, quaternary pump, and an HTC PAL thermostatted autosampler (Thermo

Scientific) coupled to a TSQ Vantage triple quadrupole mass spectrometer (Thermo Scientific) with a HESI source was operated in negative ionization mode. The column was a Waters BEH C18 50 × 2.1 mm^2 with 1.7 μm particle size. The isocratic mobile phase was 50% acetonitrile in water with a flow rate of 0.2 mL/min. Sorbitol retention was 0.4 min. Detection of sorbitol was accomplished using selected reaction monitoring where the transition of *m/z* 181 to *m/z* 89.3 was monitored. Sample preparation involved shaking liquid product for 1 min, diluting 100 μL liquid product to 10 mL with water, and briefly vortexing mixture. Then, 200 μL of diluted liquid product was further diluted to 10 mL with water, and briefly vortexed.

Pharmacokinetic and Statistical Analysis

The plasma concentration data were analyzed by noncompartmental analysis using Phoenix WinNonlin version 6.3 (Pharsight, Mountain View, CA). The impact of excipients was determined by evaluating BE of the test formulations. The area under the plasma concentration-time curve ($AUC_{0\text{-}t}$) was calculated using the linear trapezoidal rule. The maximum plasma concentration (C_{max}) and $AUCo_{\text{-}t}$ were subjected to average BE analysis using Phoenix WinNonlin. Each test formulation was compared with the reference formulation, using the 90% confidence interval (CI) approach. Additionally, like C_{max}, time to reach C_{max} (T_{max}) was determined from the observed plasma concentration data. The AUC extrapolated to infinity ($AUC_{0\text{-}\infty}$) was determined by adding the extrapolated area (C_t/K_e) to $AUC_{0\text{-}t}$, where K_e is the terminal elimination rate constant. The half-life ($t_{1/2}$) was calculated as ln 2/K_e.

Results

Quantification of Sorbitol

The sorbitol concentrations in commercial cimetidine solution and acyclovir suspension were measured to be 2355 (±8) mg/5 mL and 1503 (±21) mg/5 mL, respectively. In study 1A and study 2, each 3.33 mL dose of cimetidine commercial oral solution contained an estimated 1568 (±5) mg of sorbitol. In study 1B, each 5 mL dose of acyclovir commercial oral suspension contained an estimated 1503 (±21) mg of sorbitol.

Study 1: Formulations and In Vitro *Testing*

Table SI (see Supporting Information) lists results from *in vitro* QC tests for formulations employed in study 1A for cimetidine and study 1B for acyclovir. All formulations passed all tests. Figure 2 shows the mean dissolution profiles of 3 cimetidine capsule formulations and 3 acyclovir capsule formulations in pH 1.2, 4.5, and 6.8 media. The mean percent dissolved was >85% in 30 min for all cimetidine and acyclovir capsules, indicating capsules were at least rapidly dissolving. All cimetidine capsules were very rapidly dissolving except for CimTest-1. The only acyclovir formulation that was very rapidly dissolving was AcyTest-2 in pH 1.2 and pH 4.5.

Study 1A and 1B: In Vivo *Studies*

The mean plasma concentration-time profiles for study 1A and 1B are shown in Figure 3. Table 3 lists mean pharmacokinetic parameters. Table 4 shows the study 1A cimetidine BE results using the commercial cimetidine solution as the reference. As the commercial cimetidine solution provided about 10% lower absorption compared with cimetidine capsules, Table 5 shows study 1A cimetidine BE results using CimTest-2 as the reference, as CimTest-2 did not appear to reduce or enhance cimetidine drug absorption. CimTest-2 yielded $AUC_{0\text{-}\infty}$ of 2802 h ng/mL, which agrees with 2800 h ng/mL after 200 mg cimetidine oral tablet.[24]

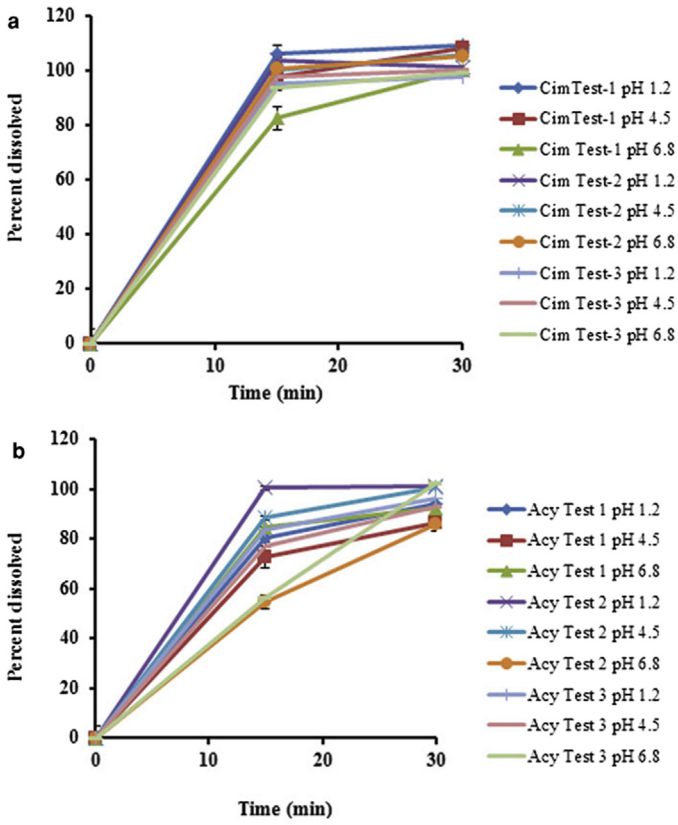

Figure 2. Study 1: mean dissolution profiles of test capsules in 1.2, 4.5, and 6.8 pH media. All formulations were at least rapidly dissolving with the mean % dissolved >85% in 30 min. In panel a, cimetidine test capsule profiles are shown. In panel b, acyclovir test capsule profiles are shown.

CimTest-2 and CimTest-3 provided the highest profiles (Fig. 3), with CimTest-3 being BE to CimTest-2 (Table 5). CimTest-1 was also BE to CimTest-2. The commercial cimetidine solution profile was about 20% lower than the CimTest-2 profile.

Regarding acyclovir from study 1B, AcyTest-2 and commercial acyclovir suspension provided the highest acyclovir absorption and were BE to one another (Fig. 3 and Table 4). Table 4 shows the study 1B acyclovir BE results, using the commercial acyclovir suspension as the reference. Table 5 shows the study 1B acyclovir BE results, using AcyTest-2 as the reference, as AcyTest-2 did not appear to reduce or enhance acyclovir drug absorption. AcyTest-2 yielded $AUC_{0\text{-}\infty}$ of 2145 h ng/mL, which agrees with 4369 h ng/mL after 400 mg acyclovir oral capsule.[25]

C_{max} of AcyTest-1 failed to demonstrate BE compared with AcyTest-2 (Table 5). It was hypothesized that HPMC (45 mg in each CimTest-1 and AcyTest-1 capsule) reduced drug dissolution and hence *in vivo* drug absorption rate. Point estimates from CimTest-1 in study 1A (Table 5) are supportive of this observation for AcyTest-1, as CimTest-1 and AcyTest-1 capsules were qualitatively and quantitatively the same with respect to excipients. A goal of study 2 was to reduce HPMC to yield a very rapidly dissolving formulation, and contain 300 mg microcrystalline cellulose and 25 mg SLS.

$AUC_{0\text{-}t}$ of AcyTest-3 was about 10% less than AcyTest-2 (Table 5). AcyTest-3 contained 40 mg magnesium stearate and employed high shear mixing via a Turbula mixer. Dissolution was rapid but not very rapid. It was hypothesized that magnesium stearate reduced drug dissolution and hence *in vivo* drug absorption. A goal of study 2 was to reduce magnesium stearate to yield a very rapidly dissolving formulation containing reduced magnesium stearate,

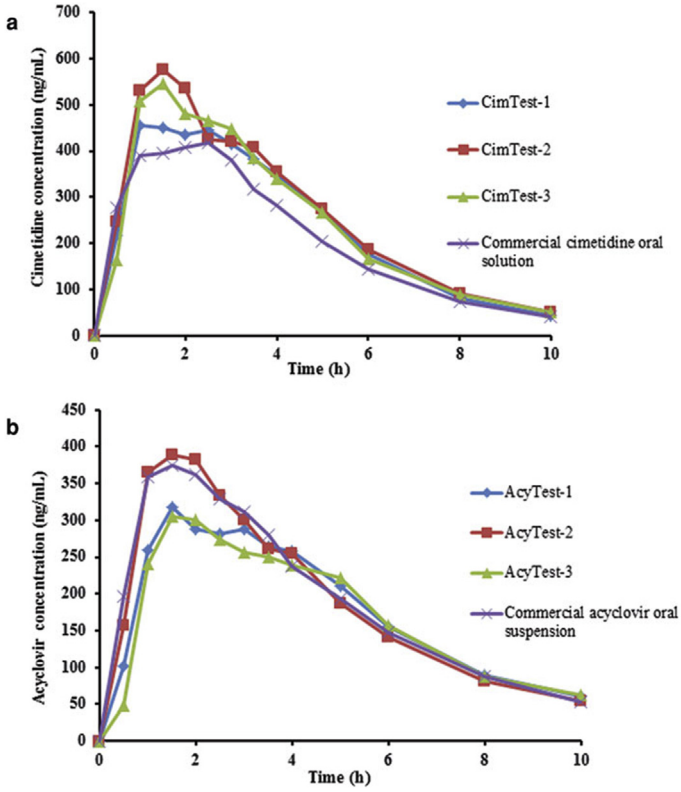

Figure 3. Study 1: mean plasma concentration-time profiles. In panel a, profiles follow a dose of two 100 mg cimetidine capsules or commercial cimetidine oral solution (n = 24 subjects). In panel b, profiles follow a dose of two 100 mg acyclovir capsules or commercial acyclovir oral suspension (n = 24 subjects).

100 mg pregelatinized starch, and 60 mg croscarmellose sodium, using a lower shear mixing approach.

Figure 1 summarizes these findings from study 1 and their impact on study 2 design. From study 1, nine excipients that were concluded to not impact BCS class 3 drug absorption were SLS, corn starch, sodium starch glycolate, colloidal silicon dioxide, dibasic calcium phosphate, crospovidone, lactose, povidone, and stearic acid. Microcrystalline cellulose was indeterminate as it was confounded with HPMC (Table 1). Pregelatinized starch and croscarmellose sodium were indeterminate as they were confounded with magnesium stearate (Table 1).

Study 2: Formulations and In Vitro *Studies*

Table SII (see Supporting Information) lists results from *in vitro* QC tests for cimetidine formulations in study 2. Study 2 followed studies 1A and 1B to examine a formulation with less than 45 mg HPMC (i.e., derivative formulation of CimTest-1 and AycTest-1), a formulation with less than 40 mg magnesium stearate (i.e., derivative formulation of AcyTest-3), and a sorbitol-free solution (i.e., derivative formulation of commercial cimetidine oral solution). All formulations passed all tests. CimTest-A was the HPMC-containing capsule formulation that was placed into the clinic (i.e., identical to prototype formulation CimTest-A-20 mg in Table 2). CimTest-B was the magnesium stearate-containing capsule formulation (i.e., identical to prototype formulation CimTest-B-20 mg in Table 2).

Figure 4 shows the mean dissolution profiles for both clinical cimetidine capsules in pH 1.2, 4.5, and 6.8 media. The mean percent dissolved was >85% in 15 min, indicating very rapid dissolution.

Table 4
Study 1A and 1B: Bioequivalence Results Using Commercial Oral Liquid as the References

Formulation	$AUC_{0\text{-}t}$ Point	$AUC_{0\text{-}t}$	C_{max} Point	C_{max}
	Estimate (%)	90% CI (%)	Estimate (%)	90% CI (%)
CimTest-1	112.0	104.6-119.8	120.4	107.7-134.6
CimTest-2	123.3	115.2-131.9	132.9	118.9-148.6
CimTest-3	117.1	109.3-125.3	134.9	120.7-150.8
AcyTest-1	91.7	80.4-104.7	82.7	72.1-94.9
AcyTest-2	97.4	85.3-111.2	102.9	89.7-118.1
AcyTest-3	87.6	76.7-99.9	87.1	75.9-99.9

Study 2: In Vivo *Studies*

The mean plasma concentration time profiles for study 2 are shown in Figure 5. Table 6 lists mean pharmacokinetic parameters. Table 7 shows the study 2 cimetidine BE results, using the sorbitol-free solution as the reference. CimTest-B was BE to the sorbitol-free reference solution. CimTest-A and commercial cimetidine oral solution were BE to the sorbitol-free reference solution with respect to $AUC_{0\text{-}t}$, but not with respect to C_{max}.

Discussion

Overall Findings

The objective was to assess the impact of very large amounts of 14 commonly used excipients on BCS class 3 drug absorption in humans. Drug absorption was assessed by both $AUC_{0\text{-}t}$ and C_{max}, which are extent of drug absorption and rate of drug absorption metrics. The main concern of excipient effects for BCS class 3 biowaivers is that an excipient may impact drug permeability or motility, and hence extent of drug absorption. Nevertheless, C_{max} was assessed.

United States Food and Drug Administration and EMA allow biowaivers of IR solid oral dosage forms of rapidly dissolving BCS class 1 drugs.[2,3] EMA and US FDA appear to indicate that for excipients that are not known to affect bioavailability, BCS class 3 biowaivers require that excipients in the test and reference products be qualitatively the same and quantitatively very similar.

Table 3
Study 1A and 1B: Mean Pharmacokinetic Parameters After Two Capsules of Each Test Formulation (i.e., 200 mg of Either Cimetidine or Acyclovir)

Parameter	CimTest-1	CimTest-2	CimTest-3	Commercial Cimetidine Solution	AcyTest-1	AcyTest-2	AcyTest-3	Commercial Acyclovir Suspension
C_{max} (ng/mL)	629 ± 50	715 ± 76	706 ± 58	514 ± 42	373 ± 35	469 ± 48	401 ± 48	450 ± 41
T_{max} (h)	1.68 ± 0.22	1.80 ± 0.20	1.97 ± 0.22	2.16 ± 0.22	2.41 ± 0.25	1.47 ± 0.16	2.10 ± 0.26	1.68 ± 0.15
$AUC_{0\text{-}t}$ (h ng/mL)	2397 ± 165	2632 ± 184	2494 ± 164	2107 ± 130	1775 ± 168	1903 ± 194	1712 ± 192	1917 ± 174
$AUC_{0\text{-}\infty}$ (h ng/mL)	2537 ± 176	2802 ± 182	2677 ± 178	2259 ± 134	2048 ± 184	2145 ± 215	2044 ± 237	2158 ± 201
$T_{1/2}$ (h)	2.03 ± 0.11	2.23 ± 0.16	2.13 ± 0.11	2.30 ± 0.11	2.41 ± 0.25	2.99 ± 0.19	3.27 ± 0.28	2.93 ± 0.18
K_e (h^{-1})	0.365 ± 0.020	0.342 ± 0.02	0.344 ± 0.017	0.319 ± 0.01	0.246 ± 0.013	0.249 ± 0.013	0.244 ± 0.018	0.255 ± 0.014

Values are expressed as mean ± SEM.

Table 5
Study 1A and 1B: Bioequivalence Results Using CimTest-2 and AcyTest-2 as the References for Cimetidine Formulations and Acyclovir Formulations, Respectively

Formulation	AUC_{0-t} Point Estimate (%)	AUC_{0-t} 90% CI (%)	C_{max} Point Estimate (%)	C_{max} 90% CI (%)
CimTest-1	90.9	84.9-97.2	90.6	81.0-101.3
CimTest-3	95.0	88.8-101.6	101.5	90.8-113.4
Commercial cimetidine oral solution	81.1	75.8-86.8	75.2	67.3-84.1
AcyTest-1	94.2	82.5-107.5	80.3	70.0-92.1
AcyTest-3	89.9	78.7-102.6	84.6	73.7-97.0
Commercial acyclovir oral suspension	102.7	89.9-117.2	97.1	84.7-111.4

Neither CimTest-2 nor AcyTest-2 contained HPMC or magnesium stearate.

Cimetidine and acyclovir were chosen as model BCS class 3 drugs as they have well established physicochemical and pharmacokinetic properties. BCS-based biowaivers of cimetidine and acyclovir have been previously concluded to be acceptable for IR solid oral dosage forms that contain excipients in amounts typically present in IR solid oral dosage form.[16,17]

Findings here support BCS class 3 biowaivers of IR products that dissolve very rapidly for all 14 studied excipients: microcrystalline cellulose, HPMC, SLS, corn starch, sodium starch glycolate, colloidal silicon dioxide, dibasic calcium phosphate, crospovidone, lactose, povidone, stearic acid, pregelatinized starch, croscarmellose sodium, and magnesium stearate. Moreover, findings here support the maximum allowable amount of each of these excipients to be the quantities shown in Table 8. These amounts are supported by *in vivo* results here from the administration of two capsules of CimTest-2, CimTest-3, AcyTest-2, CimTest-A, and CimTest-B. Because of the failure to demonstrate BE in C_{max} for CimTest-A in study 2, HPMC and microcrystalline cellulose are suggested to follow the qualitative and quantitative limitations in the EMA and draft US FDA guidances.[2,3] However, for the other 12 excipients, results support that test and reference do not have to be either qualitatively the same nor quantitatively very similar. Findings here support that quantities between zero to the maximum amount in Table 8 should be allowable for those 12 excipients (e.g., 900 mg lactose).

Study 1A and 1B

Study 1A and 1B were designed to assess the impact of very large amounts of 14 commonly used excipients on BCS class 3 drug absorption in humans. These 14 excipients were selected from a list of the 20 most common excipients in oral solid ANDA formulations. The quantity of each excipient considered the typical amount in an IR oral solid dosage form, with the intent that results here would "cover" the majority of brand and generic IR tablet and capsule products. The quantity of each excipient was not intended to exceed the maximum amount used in an US FDA-approved product, per US FDA's Inactive Ingredient Search for Approved Products,[27] in part for study safety.

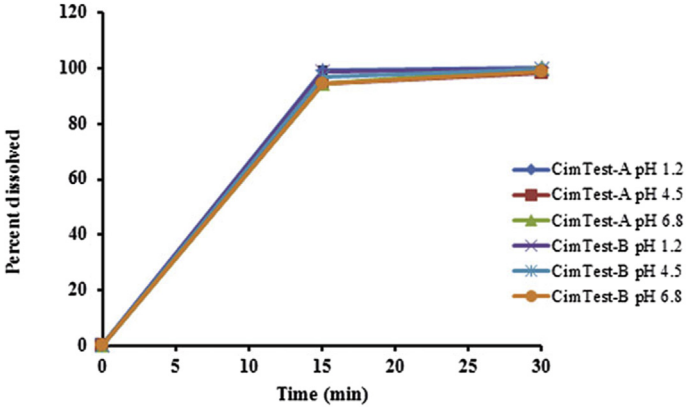

Figure 4. Study 2: mean dissolution profiles of test capsules in 1.2, 4.5, and 6.8 pH media. All formulations were very rapidly dissolving with the mean percent dissolved >85% in 15 min.

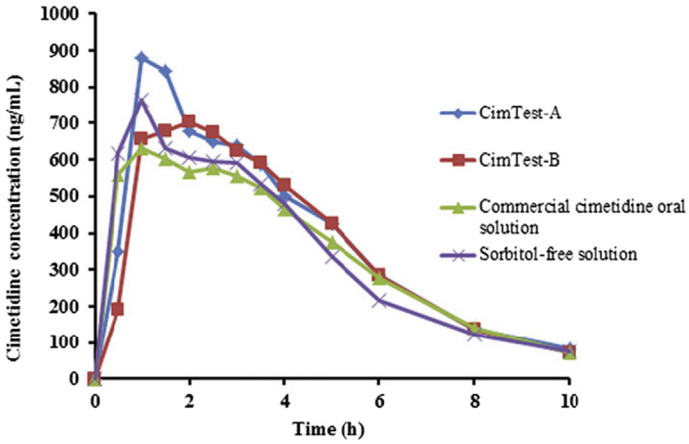

Figure 5. Study 2: mean plasma concentration-time profiles from 200 mg cimetidine. Profiles follow a dose of two 100 mg cimetidine capsules, 3.33 mL of commercial cimetidine oral solution, or 3.33 mL of sorbitol-free oral solution ($n = 24$ subjects).

Table 1 lists the formulations used in study 1A and 1B. Previous cell culture studies indicated that SLS moderately increased the permeability of several low permeability drugs.[15] Also, HPMC appeared to increase cimetidine permeability.[15] Hence, in Table 1, SLS was included in three formulations (CimTest-1, CimTest-3, and AcyTest-1), and HPMC was included in two formulations (CimTest-1 and AcyTest-1). In fact, CimTest-1 and AcyTest-1 employed the identical formulation for each cimetidine and acyclovir. Effort was made to obtain at least rapidly dissolving capsules, which was achieved through the combinations of excipients shown in Table 1. All cimetidine capsules were very rapidly dissolving except for CimTest-1. The only acyclovir formulation that was very rapidly dissolving was AcyTest-2 in pH 1.2 and pH 4.5.

An observation from study 1A and 1B is that the three excipient combination of HPMC, microcrystalline cellulose, and SLS yielded slower drug absorption (i.e., C_{max} reduced by about 10% from CimTest-1 and about 20% from AcyTest-1 in Table 5). SLS was also present in CimTest-3, which did not impact drug absorption. As the major potential concern with SLS was enhanced drug permeability, the lack of increased C_{max} or AUC_{0-t} from SLS-containing capsules found that SLS did not module BCS class 3 drug absorption. Hence,

Table 6
Study 2: Mean Pharmacokinetic Parameters After 200 mg of Cimetidine (i.e., Two Capsules or 3.33 mL of Oral Solution)

Parameter	CimTest-A	CimTest-B	Commercial Cimetidine Solution	Sorbitol-Free Solution
C_{max} (ng/mL)	1098 ± 62	948 ± 60	793 ± 54	927 ± 75
T_{max} (h)	1.43 ± 0.12	2.14 ± 0.22	2.20 ± 0.26	1.83 ± 0.19
AUC_{0-t} (h ng/mL)	3896 ± 181	3661 ± 186	3495 ± 183	3512 ± 193
$AUC_{0-\infty}$ (h ng/mL)	4155 ± 193	3908 ± 187	3746 ± 173	3851 ± 222
$T_{1/2}$ (h)	2.17 ± 0.18	2.18 ± 0.15	2.28 ± 0.17	2.80 ± 0.30
K_e (h^{-1})	0.333 ± 0.014	0.348 ± 0.020	0.338 ± 0.028	0.299 ± 0.025

Values are expressed as mean ± SEM.

Table 7
Study 2: Bioequivalence Results Using Sorbitol-Free Solution as the Reference

Formulation (vs. Reference)	AUC0-t Point Estimate (%)	AUC_{0-t} 90% CI (%)	Cmax Point Estimate (%)	Cmax 90% CI (%)
CimTest-A[a]	112.2	104.4-120.6	122.1	109.4-136.2
CimTest-B[b]	105.2	97.9-113.0	105.0	94.1-117.2
Commercial cimetidine solution	100.2	93.2-107.7	86.9	77.9-97.0

Test capsules and the cimetidine commercial oral solution were bioequivalent to the sorbitol-free solution with respect to AUC.
[a] Two capsules contained a total of 40 mg of HPMC.
[b] Two capsules contained a total of 40 mg of magnesium stearate.

decreased drug absorption from CimTest-1 and AcyTest-1 was hypothesized to be caused by slower dissolution because of the 45 mg HPMC in each of these formulations. Their prototype formulations with more than 45 mg HPMC showed slower dissolution than CimTest-1 and AcyTest-1 (data not shown). HPMC is well known to slow dissolution (e.g., serve as a matrix for extended release).[28,29] HPMC was observed *in vitro* to increase cimetidine permeability,[15] which further suggested an HPMC effect was not via permeability but via reduced dissolution.

A second observation from study 1B is that the 3 excipient combination of pregelatinized starch, croscarmellose sodium, and magnesium stearate yielded slower drug absorption (i.e., about 15% reduced C_{max} from AcyTest-3). Magnesium stearate is known to slow dissolution and possibly reduce drug absorption via over-lubrication. Over-lubrication occurs because of the excessive shearing of magnesium stearate due to excessively long mixing, such that this hydrophobic excipient coats drug particles.[30-33] Over-mixing of magnesium stearate is known to slow the dissolution by forming a coating around drug and other excipients. In study 1B, AcyTest-3 was manufactured at a small scale using a Turbula mixer, which is a high shear process. Hence, the decreased drug exposure from AcyTest-3 was hypothesized to be due to the slower dissolution due to over-mixing of magnesium stearate.

A third observation from study 1A is that the commercial cimetidine oral solution provided the lowest absorption (about 20% less). This solution contained sorbitol which is an osmotically "active" excipient. Sorbitol, in increasingly larger quantities, is capable of reducing the oral absorption of drugs with low intestinal permeability by exerting an osmotic pressure that accelerates the small intestinal transit of drugs. This reduces the time available for drugs to permeate and be absorbed which in turn leads to a decrease in the bioavailability of the drug.[12,13] The sorbitol concentrations in commercial cimetidine solution and acyclovir suspension were measured to be 2355 (±7.6) mg/5 mL and 1503 (±20.9) mg/5 mL, respectively. Hence, the amounts of sorbitol ingested in study 1A (cimetidine) and study 1B (acyclovir) were 1568 and 1503 mg from commercial oral liquid, respectively. Therefore, reduced cimetidine absorption from the commercial cimetidine oral solution would appear to be from sorbitol.

A final observation across studies 1A and 1B is that the following nine excipients did not impact BCS class 3 drug absorption in quantities studied here: SLS, corn starch, sodium starch glycolate, colloidal silicon dioxide, dibasic calcium phosphate, crospovidone, lactose, povidone, and stearic acid. Microcrystalline cellulose was confounded with 45 mg HPMC, such that its assessments from studies 1A and 1B were indeterminate. Pregelatinized starch and croscarmellose sodium were confounded with 40 mg magnesium stearate, such that their assessment from study 1B was indeterminate.

Study 2

One main observation is that magnesium stearate in CimTest-B did not modulate drug absorption. Hence, Table 8 denotes acceptable quantities of magnesium stearate for BCS class 3 biowaivers to be 40 mg (from dosing two capsules here) or less. This result also confirms that pregelatinized starch and croscarmellose sodium

Table 8
Maximum Amount of Excipients That BCS Class 3 Biowaivers Can Accommodate

Excipient	Recommended Maximum Allowable Amount for a Class 3 Biowaiver (mg)	Maximum Excipient Amount Studied Here (mg)[a]	Typical Excipient Amount (when present) in an IR Tablet or Capsule With a Total Weight of 300 mg[26]	Maximum Amount (mg) in Inactive Ingredient Database[27]
Microcrystalline cellulose	Qualitatively the same and quantitatively very similar to reference product	600[b]	100 mg (20%-90%)	1385.3[g]
Hydroxypropyl methylcellulose	Qualitatively the same and quantitatively very similar to reference product	40[b]	10 mg (2%-5%)	444.4[g]
Sodium lauryl sulfate	50	50[b,d]	4.5 mg (0.5%-2.5%)	51.69[g]
Corn starch	900	900[c]	150 mg (25%-75%)	1135[h]
Sodium starch glycolate	200	200[c]	12 mg (4%)	876[g]
Colloidal silicon dioxide	40	40[c]	1.5 mg (0.1%-1%)	100[i]
Dibasic calcium phosphate	600	600[d]	150 mg (25%-75%)	635.5[k]
Crospovidone	100	100[d]	10 mg (2%-5%)	340[j]
Lactose	900	900[e]	240 mg (80%)	1020[g]
Povidone	70	70[e]	7.5 mg (0.5%-5%)	240[k]
Stearic acid	80	80[e]	6 mg (1%-3%)	72[l]
Pregelatinized starch	200	200[f]	150 mg (5%-75%)	435.8[g]
Croscarmellose sodium	120	120[f]	37.5 mg (0.5%-25%)	180[g]
Magnesium stearate	40	40[f]	7.5 mg (0.25%-5%)	400.74[g]

[a] Reflects that two capsules of either cimetidine 100 mg or acyclovir 100 mg were administered in single-dose studies here.
[b] Employed in dosing of capsule formulation CimTest-A in study 2.
[c] Employed in dosing of capsule formulation CimTest-2 in study 1A.
[d] Employed in dosing of capsule formulation CimTest-3 of study 1A.
[e] Employed in dosing of capsule formulation AcyTest-2 of study 1B.
[f] Employed in dosing of capsule formulation CimTest-B of study 2.
[g] Oral tablet.
[h] Oral capsule.
[i] Oral granule.
[j] Oral dispersible tablet.
[k] Oral tablet film coated.
[l] 72 mg from oral table and 180 mg from extended-release table.

were not problematic. Another main observation from CimTest-A is that lowering the amount of HPMC restored drug $AUC_{0\text{-}t}$, as expected. However, as C_{max} confidence limit failed BE by exceeding the 125% upper bound, such HPMC and microcrystalline cellulose must be qualitatively the same and quantitatively very similar to the reference product, for BCS-based class 3 biowaivers. We speculate that microcrystalline cellulose, that is water insoluble, was an unlikely reason for the high C_{max} upper confidence limit. Future work should separately examine possible effects of these larger amounts of HMPC and microcrystalline cellulose.

A secondary observation was that the commercial cimetidine oral solution was BE to the sorbitol-free reference solution with respect to $AUC_{0\text{-}t}$, but not with respect to C_{max}. The amount of sorbitol ingested from the commercial cimetidine oral solution in study 1A and study 2 was 1568 mg, which appears to be an amount that starts to impact drug absorption of poorly permeable drugs. Sorbitol has been shown to exhibit a dose–response effect on the extent of ranitidine absorption, where 1.25, 2.5, and 5 g of sorbitol progressively reduced ranitidine absorption.[12] Ranitidine is a BCS class 3 drug, and its AUC and C_{max} were reduced about 7% with 1.25 g of sorbitol.[12]

Transporter Considerations

A limitation of these studies is that only two drugs were evaluated, cimetidine and acyclovir. It is possible that other BCS class 3 drugs have properties that differ from cimetidine and acyclovir to render those drugs susceptible to other excipient influences that cause modified drug absorption. For example, a drug may be a substrate for an intestinal transporter. In fact, moderate permeability is necessary for a drug to effectively be effluxed,[34,35] such that excipient impact on transporter function is indeed a potential concern for BCS class 3 drugs.[36] Many *in vitro* studies have shown excipient influences on drug permeability.[37,38] However, findings here and observations from the literature implicate that permeability-enhancement *in vitro* is not observed *in* vivo.[39,40] For example, several studies have observed SLS to enhance the permeability of low permeability drugs, including cimetidine.[15,41-43] However, SLS did not enhance drug permeability *in vivo* here. Drug absorption enhancement has been a long-standing goal in drug absorption research, yet no common excipient is known to increase drug permeability in vivo. A recent finding from a phase 2 study implicates the use of sodium N-(8-[2-hydroxybenzoyl] amino) caprylate to enhance the efficacy of oral semaglutide against type 2 diabetes.[40,44] However, these preliminary findings do not mean that common excipients broadly modulate BCS class 3 drug permeability.

Regarding the 14 excipients listed in Table 8, the greatest concern would appear to be a drug that depends on an uptake transporter that an excipient inhibits by virtue of the excipient having molecular structure similarity to the transporter's pharmacophore or recognition site. For example, lactose is a disaccharide from galactose and glucose. Hence, a concern may be that lactose has potential to inhibit a sugar transporter that the drug depends upon for drug absorption. It should be noted that most drugs are absorbed by passive diffusion, rather than by transporter-mediated uptake.[45]

In conclusion, through 3 four-way crossover BE studies, 12 common excipients were found to not impact BCS class 3 drug absorption in humans: SLS, corn starch, sodium starch glycolate, colloidal silicon dioxide, dibasic calcium phosphate, crospovidone, lactose, povidone, stearic acid, pregelatinized starch, croscarmellose sodium, and magnesium stearate. Quantities of these excipients are shown in Table 8. Results further suggest that these excipients need not be qualitatively the same nor quantitatively very similar to reference, but rather simply be not more than the quantities studied here. Meanwhile, as CimTest-A failed C_{max}, BCS class 3 biowaivers require HPMC and microcrystalline cellulose to be qualitatively the same and quantitatively very similar to the reference.

Acknowledgments

This work was supported by US FDA contracts HHSF223200910020C and HHSF223200810041C. This work was supported in part by the University of Maryland Clinical Translational Science Institute, the University of Maryland General Clinical Research Center, and the University of Maryland Mass Spectrometry Center (S0P1841-IQB2014).

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