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Using Absorption Simulation and Gastric pH Modulated Dog Model for Formulation Development To Overcome Achlorhydria Effect

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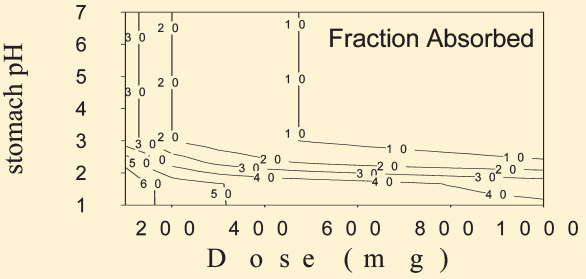
ABSTRACT: Impaired absorption of weakly basic drugs in patients with reduced gastric acidity can lead to loss of efficacy of the therapeutic agent. Hence, a robust formulation which can provide adequate exposure in achlorhydric patients is imperative to achieve the desired efficacy. In this report, formulation development of a weakly basic Merck compound A is described. Compound A shows lower solubility at higher pH and thus is prone to reduced exposure under conditions of achlorhydria, as the compound's solubility increases only in environments of less than pH 2. Several formulations with or without an acidifier were developed and characterized by in vitro dissolution and in gastric pH modified dog model to assess their bioperformance in high gastric pH conditions. To predict the bioperformance of these formulations in humans, a dissolution based absorption model was developed and validated against the observed PPI-interaction data in the clinic and the gastric pH-adjusted dog data. An additional absorption model was developed to allow for incorporation of the dog PK data to provide translation of preclinical to clinical exposure. Based on the in vitro dissolution, in silico absorption modeling and preclinical in vivo data, a citric acid-based formulation (F2) was selected for a human pharmacokinetic study. This study showed that exposures from F2 were not meaningfully different in the presence of proton pump inhibitor (PPI) as compared to non-PPI, thus confirming that the F2 formulation was successful in overcoming the achlorhydria effect. These efforts also highlighted that the complementary use of in vitro/in silico/in vivo (IVISIV) tools may be a helpful strategy in the development of formulations to overcome the achlorhydria effect and achieve adequate exposure in patients with high gastric pH.



KEYWORDS: simulation, modeling, achlorhydria, pharmacokinetics, famotidine, dissolution, dog study

INTRODUCTION

The reduction in bioavailability of weakly basic compounds exhibiting steep pH-solubility profile has often been observed in subjects with reduced gastric acidity (achlorhydria).[1] The changes in gastric pH can be due to disease,[2] demographics,[3] age,[4] and/or concurrent therapy with agents that increase gastric pH, such as antacids, proton pump inhibitors (PPI) or H2 receptor antagonists.[5] A review by Lahner et al. provides a comprehensive summary of the impact of reduced gastric acid secretion on impairment of absorption of several drugs such as ketoconazole, itraconazole, atazanavir, cefpodoxime, enoxacin, dipyridamole, nifedipine and digoxin.[5] In some cases, the reduction in bioavailability of the therapeutic agent can be substantial and consequently lead to subtherapeutic exposure. For example ketoconazole C_{max} can be reduced by 53−93% when coadministered with PPI or H2 receptor antagonists.[6,7] Further ketoconazole malabsorption has been reported in AIDS patients,[8] who are known to have reduced gastric acid secretion thus resulting in suboptimal efficacy.[9] Similarly, the AUC of atazanavir has been reported to be reduced by up to 94% when coadministered with lansoprazole.[10] Since this can lead to significant loss of efficacy, atazanavir coadministration with gastric pH modifiers is contraindicated.[11] Due to the potential for significant malabsorption of weakly basic drugs under reduced gastric acidity conditions, several approaches have been reported to overcome these interactions such as coadministration with acidic beverages, e.g., Coca-Cola[12] and Sprite;[13] codosing with organic acid;[1] and development of solid dosage formulation containing organic acid.[14,15] However, in some cases the above approaches might not solve the achlorhydria issue and hence contraindication with gastric pH modifiers is required, e.g., atazanavir.[11]

Merck compound A (Table 1) is a weakly basic, BCS class II molecule for oncology with a steep pH dependent solubility profile (solubility decreases with increase in pH). In spite of the three pK_a values, the compound does not have any appreciable solubility above pH 2 and hence is prone to reduced absorption in high gastric pH conditions. Phase I single ascending dose studies using a dry filled capsule formulation of the free base form of compound A showed

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significantly reduced exposures, high variability and lack of dose proportionality in patients coadministered with omeprazole. This indicated that a reformulation was required to achieve adequate exposure under high gastric pH conditions. In this paper, we describe the use of biopharmaceutical tools such as in vitro dissolution, absorption simulation, and gastric pH modified dog model to enable development of a solid dosage formulation of a weakly basic molecule to overcome gastric pH related variability in absorption. Although a prior publication has reported development of solid dosage formulation to overcome gastric pH effect,[14] here we attempt to highlight the combined use of in vitro/in silico/in vivo (IVISIV) tools in successful development of a challenging formulation and increase probability-of-success of meeting pharmacokinetic end points in the clinic.

MATERIALS AND METHODS

Materials. Compound A was provided by the Process Research department of Merck Sharp and Dohme. The main excipients used in the formulation development are citric acid (Industria Chimica Valanzana S.P.A.), lactose (Foremost Farm), cellulose microcrystalline, sodium croscarmellose (both from FMC Biopolymer) and magnesium stearate (Avantor Performance Materials). To support the development of solid dispersions the polymer of choice was copovidone (Intl. Specialty Products Technologies); while Hypromellose (Dow Chemicals) and sodium lauryl sulfate (Cognis) were used in the nanosuspension formulation. For the animal studies, pentagastrin and famotidine were purchased from Sigma-Aldrich and Baxter, respectively.

Table 1. Physicochemical and Biopharmaceutical Properties of Compound A (Free Base)

BCS class II
Caco-2 permeability = 39.0×10^{-6} cm/s
human permeability = 3.5×10^{-4} cm/s (projected from Caco-2 data)
molecular weight = 471.5
log P = 2.6
pK_a = 2.4, 3.9, and 10 (corresponds to an acidic group)
mean particle size = 6.5 μm
density = 0.59 g/cm^3
pH−solubility profile
  simulated gastric fluid (SGF, pH 1.2) = 2.75 mg/mL
  pH 2.0 = 0.2 mg/mL
  pH 4.0 = 0.001 mg/mL
  fasted state simulated intestinal fluid (FaSSIF, pH 6.5) = 0.006 mg/mL

Formulations. Several formulations were developed for compound A. Table 2 provides a summary of the formulations developed. Initially to support phase I studies, a dry filled capsule (DFC) formulation (F1) was developed, where the drug substance (free base) was blended with lactose and postlubrication was filled in hard-gelatin capsules. Subsequently to address the achlorhydria effect observed in phase I, reformulation efforts were initiated with the aim of developing a robust formulation, as described in Table 2. First, acidification of the free base formulation (F1-1) was developed by adding citric acid in conjunction with povidone; the latter was added to offer some antinucleation effect to the formulation therefore delaying the precipitation of the free base. Citric acid was chosen among different acidifiers because of its wide use in the food industry and preferred safety profile. A DFC formulation containing the hydrochloride (HCl) salt of compound A was also developed (F2). Although the HCl salt had higher solubility than the free base, its solubility still showed some pH dependency. Hence acidification of the HCl salt formulation was still required. Since both the citric acid and the drug substance were poorly compactable, the filler of choice was microcrystalline cellulose, and its level was chosen to give good granules while maximizing the content of the acidifier.

In parallel, enhanced formulations were developed via hot melt extrusion of the binary mixture of free base and copovidone (HME-1), extrusion of the binary mixture followed by blending with citric acid prior to encapsulation (HME-2), and extrusion of a ternary mixture of free base, copovidone and citric acid (HME-3). Finally, still aiming to increase the supersaturation of the free base, the crystalline drug substance was nanomilled in presence of a suspending aid (HPMC) and surfactant (SLS). The nanosuspension, so designed, resulted to be stable without recrystallization.

Table 2. Compositions of Compound A Formulations

	percentage of total formulation wt						
	dry filled capsule			hot melt extrusion			
	F1[a]	F1-1[b]	F2[c]	HME-1	HME-2	HME-3	nanosuspension
compound A (free base)	62.5	14.2		30.0	15.0	15.0	20.0 mg/mL
compound A (HCl salt)			31.0				
copovidone				70.0	35.0	30.0	
citric acid (comelt)						5.0	
citric acid (extragranular)		42.8	41.9		26.0	20.5	
lactose	16.8				23.0	26.1	
microcrystalline cellulose	16.8	35.0	23.1				
hypromellose							4.0 mg/mL
sodium lauryl sulfate							0.7 mg/mL
povidone		3.6					
sodium croscarmellose	3.0	3.4	3.0			3.4	
magnesium stearate	1.0	1.0	1.0		1.0	1.0	
total	100.0	100.0	100.0	100.0	100.0	100.0	
capsule shell	hard gelatin	hard gelatin	HPMC	hard gelatin	hard gelatin	hard gelatin	

[a] Original formulation. [b] Acidified dry filled capsule. [c] Advanced dry filled capsule.

Dissolution Studies. The dissolution studies were conducted in a USP apparatus II (paddles) using 250 mL of a pH 3.0 medium (2 g/L sodium chloride solution with pH adjusted using concentrated HCl) at 37 °C and paddle speed of 100 rpm. Samples were collected at 5, 10, 20, and 30 min. The samples were filtered using a 1 μm glass-fiber Acrodisc filter and diluted immediately with 0.02% TFA in water:acetonitrile (50:50 v/v) to prevent any further precipitation of compound A from the dissolution medium. These treated samples were analyzed by HPLC using a mobile phase of 0.02% TFA in water:methanol (55:45 v/v) at a flow rate of 2 mL/min and a Phenomenex Onyx monolith column.

Absorption Simulation. The clinical performance of the formulations in patients under PPI treatment was forecast based on the known performance of the F1 formulation in non-PPI treated patients in conjunction with an oral absorption model built in GastroPlus (v6.0; Simulations Plus). Steady state predictions were subsequently obtained via nonparametric superposition in WinNonLin v5.2. For the oral absorption model, the physicochemical properties as shown in Table 1 were used. The default physiological Opt-logD model was used for clinical simulations. For predictions of patients with normal gastric condition, the stomach pH was set to 1.3, whereas for prediction of PPI treatment, the stomach pH was set to 6. Human PK parameters (V/F = 131.7 L, CL/F = 15.6 L/h) were obtained by fitting the oral data in non-PPI treated patients across 100−550 mg BID doses. Given the dose-linear response across a wide dose range, for modeling purposes it was assumed that this observed exposure represented maximum oral bioavailability.

The model employed to predict exposures in humans for new formulations assumes that dissolution in the stomach is the main driver for formulation performance in vivo. While this assumption was initially based on the apparent dose-proportional exposure response in non-PPI treated patients coupled with the fact that such exposures could not be attributed to intestinal solubilization (simulations of F1 formulation at stomach pH of 6 resulted in minimal predicted exposures), the total expected plasma concentration profile was obtained by addition of the amount of drug coming from solubilization in the stomach and the exposure predicted via the PPI-treatment oral absorption model (as an estimate of the amount of drug coming from solubilization in the intestine in cases where incomplete dissolution in the stomach was anticipated). While this approach is expected to provide some overestimation of the exposures, since the two processes—absorption from dissolved drug coming from the stomach and dissolution of undissolved drug in the intestine—are not completely independent in vivo, it is not expected to result in large deviations compared to a more complex model that would separate the two processes. The projected exposure ratios relative to the F1 formulation were compared to the observed dog data to assess accuracy of the predictions. It is worth noting that while one could hypothesize that precipitation would occur upon gastric emptying of dissolved drug to the small intestine, the available data did not point to significant precipitation in vivo with linear exposures across a wide dose range and good agreement of models based on stomach solubilization to observed data. Thus precipitation was not taken into account for formulation projections. Following prediction of single administration PK profiles, steady state exposures for a BID regimen were predicted through nonparametric superposition in WinNonLin v5.2.

Following the completion of dog studies, further simulations were conducted to allow for a translation of observed dog pharmacokinetics to the clinic, especially for the hot melt extrusion (HME)

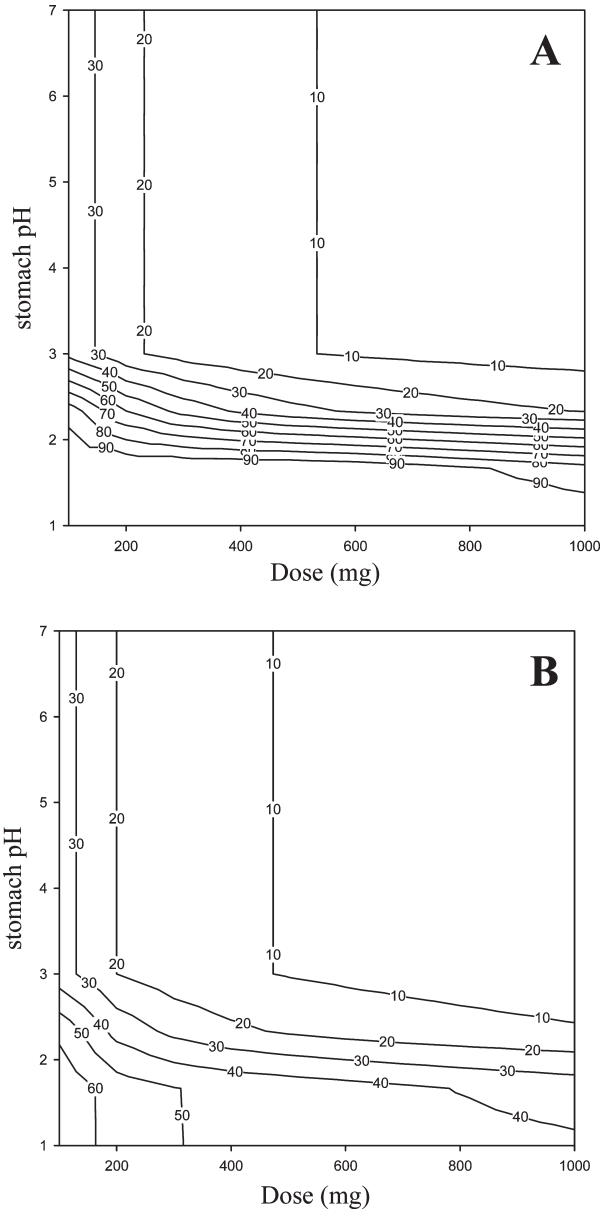

Figure 1. Sensitivity analysis (displayed as contour plot) of fraction absorbed as a function of dose and stomach pH assuming no in vivo precipitation during gastric emptying (A) or 15 min precipitation time (B).

formulations for which traditional dissolution methods may not be fully predictive of in vivo performance. Setup of simulations for the dog data paralleled the methodology described above for the clinical simulations. Dog PK parameters for the models were obtained from the pentagastrin-pretreatment data in analogy to the non-PPI treated patient data used. Composite PK profiles were constructed in a similar fashion to the one described above; however, instead of using the dissolution data, the stomach dissolution factor was back-calculated to allow for the predictions to match the observed dog PK data. Subsequently the adjusted stomach dissolution factor was used for clinical simulations in a manner similar to what was described above. Similarly as above, steady state exposures were predicted through nonparametric superposition in WinNonLin v5.2.

Dog Studies and Pharmacokinetic Analysis. The formulations were tested in male beagle dogs, where the gastric pH was modified

to assess pH dependent absorption as described before.[14] Briefly, after overnight fast, the dogs either were pretreated with a single intravenous bolus injection of famotidine (0.5 mg/kg) approximately 90 min prior to dosing or were pretreated intramuscularly with pentagastrin (0.006 mg/kg) approximately 30 min prior to dosing. Following pretreatment, the dogs were orally administered with the formulations at a dose of 10 mg/kg followed by water rinse (3.5 mL/kg). The 10 mg/kg dose was selected for the animal studies based on a projected human efficacious dose of 770 mg BID. Water was restricted for 1 h following dosing while food was returned at 4 h after dosing. Blood was drawn from a catheter placed into the cephalic vein at predose and at predetermined time intervals postdose. The plasma was separated by centrifugation and analyzed using liquid chromatography/electrospray ionization tandem mass spectrometry. All studies were conducted under a protocol approved by the Merck IACUC.

Area under the curve (AUC_{0-24h}), observed maximum plasma concentration (C_{max}), and time of C_{max} (T_{max}) were calculated using the linear trapezoidal, noncompartmental model in WinNonLin v5.2. Plasma concentration values below LOQ were set at zero for PK calculation purposes.

RESULTS

Impact of Solubility on Formulation Performance under Achlorhydric Condition. The pH−solubility profile of compound A (free base) is shown in Table 1. In the un-ionized state, i.e., above pH 4, the solubility is approximately 1 μg/mL. Due to this extremely poor solubility at the higher pH, it was predicted that the original dry filled capsule (DFC) formulation (F1) of the free base would show reduced absorption under conditions of higher stomach pH as compared to normal gastric pH conditions.

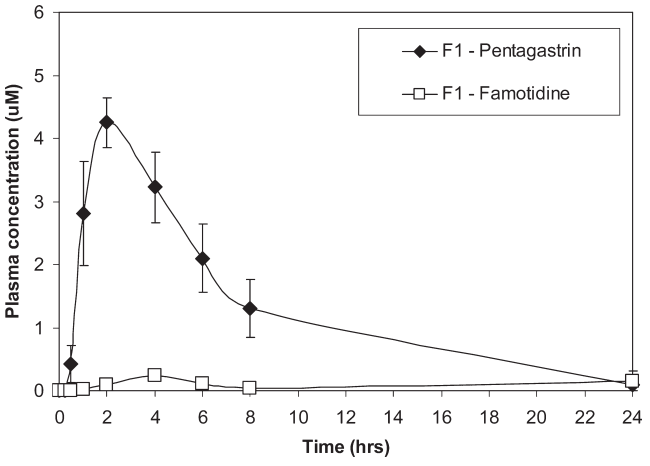

Figure 2. Plasma profiles (n = 6, mean ± SE) of compound A for F1 formulation in famotidine and pentagastrin pretreated dogs at 10 mg/kg.

Absorption simulations showed that increase in stomach pH would lead to a significant decrease in fraction absorbed. Nevertheless, F1 was chosen to kick off first-in-human (FIH) study in patients and any reformulation, if deemed necessary, would be deferred. As expected from modeling outcome (Figure 1), the FIH pharmacokinetic data clearly indicated that the effect of variable gastric pH on exposure was significant. Given that potential for in vivo precipitation is an unknown factor, simulations were conducted both assuming no precipitation (Figure 1A), which could be the case for a highly permeable molecule, and assuming the default mean precipitation time in the software (15 min, Figure 1B). These simulations suggested that the F1 formulation might not perform adequately under high gastric pH conditions and there was a risk of not achieving dose proportional AUC and C_{max} in the phase I single ascending dose studies in oncology patients suffering from achlorhydria or being treated with gastric acid secretion inhibitors. Simulations also suggested that the magnitude of the effect will be dependent on precipitation time as the difference between achlorhydric and normal gastric condition would be lower if precipitation was assumed following initial solubilization. However despite the effect of precipitation, the pH difference would cause a clear effect on exposure across doses in PPI vs non-PPI patients.

Evaluation of F1 Formulation. To further investigate the risk of reduced absoprtion under achlorhydric conditions, the F1 formulation was dosed in beagle dogs under normal (pentagastrin pretreated) and high (famotidine pretreated) gastric pH conditions. This data was in agreement with the simulation predictions and showed that the F1 formulation had a 15-fold lower AUC under high gastric pH conditions as compared to normal stomach pH at a dose of 10 mg/kg (Figure 2 and Table 3). These preclinical results were further validated in the phase I studies in gastric cancer patients, where up to 20-fold reduction in exposure was observed in patients coadministered with PPI as compared to the non-PPI patients (data not shown). Comparable pharmacokinetics of compound A in humans and gastric pH modified dogs suggested that this dog model could serve as a useful model for evaluating the effect of stomach pH on modified formulations. High intersubject variability (% CV = 66−135%) was also observed across doses in AUC_{0-12h}, C_{max} and trough plasma concentration (C_{trough}) for the cohorts codosed with PPI. Due to the high variability, dose proportionality could not be accurately assessed from these studies.

Table 3. Pharmacokinetic Parameters (n = 3 or 6, mean ± SE) of Compound A for Several Formulations in Pentagastrin and Famotidine Pretreated Dogs at 10 mg/kg[a]

formulation	pretreatment	$AUC_{0-24\ h}$ (μM h)	C_{max} (μM)	T_{max} (h)
F1	pentagastrin	30.2 ± 6.46	4.25 ± 0.49	1.5 (1.0−4.0)
	famotidine	2.04 ± 1.19	0.24 ± 0.14	4.0 (4.0−6.0)
F2	pentagastrin	29.0 ± 3.81	5.79 ± 0.80	2.0 (1.0−2.0)
	famotidine	18.4 ± 4.18	3.54 ± 0.62	2.0 (1.0−4.0)
F1-1	famotidine	15.4 ± 6.39	2.53 ± 2.27	1.0 (1.0−2.0)
HME-1	famotidine	9.47 ± 2.87	1.40 ± 0.41	2.0 (1.0−4.0)
HME-2	famotidine	14.5 ± 4.74	2.27 ± 1.02	2.0 (0.5−2.0)
HME-3	famotidine	20.5 ± 4.19	3.19 ± 0.65	2.0 (1.0−2.0)
nanosuspension	famotidine	4.32 ± 1.03	0.41 ± 0.12	2.0 (0.5−4.0)

[a] T_{max} is shown as median value and range.

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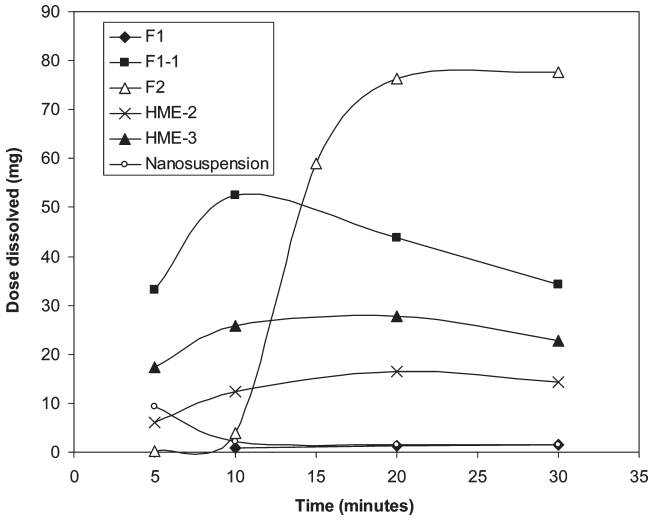

Figure 3. Dissolution profile of compound A in pH 3.0 media for the different formulations.

Even in non-PPI patients fairly large variability was observed (% CV = 55−120%) across doses in AUC_{0-12h}, C_{max} and C_{trough}, from the F1 formulation.

Reformulation To Overcome Gastric pH Effect. Several modified formulations were developed with the aim of enhancing bioperformance in a high gastric pH environment (Table 2). In this work, citric acid (CA) was added as an acidifier in the modified formulations to reduce the microenvironment pH around the dissolving formulation. Citric acid was also chosen due to its acceptable safety profile after oral administration and high aqueous solubility so that there is no dissolution rate limitation of the acidifier. A salt screen resulted in the identification of a stable hydrochloride (HCl) salt of the drug substance with higher solubility than the free base. This HCl salt was also used in formulation development. However, the salt also showed pH-dependent solubility profile, although to a lesser degree than the free base. Hence a decision was made to acidify the HCl salt formulation in order to minimize any gastric pH impact on the bioperformance of the modified formulations. Hot melt extrusion (HME) and nanosuspension formulations using the free base form of compound A were also developed to enhance solubility and maintain adequate supersaturation in a high gastric pH environment. The HME formulations were also acidified to further assist in the solubilization of the drug substance as discussed above.

The dissolution studies of the modified formulations were conducted in the pH 3.0 medium at 100 rpm paddle speed (Figure 3). When biorelevant dissolution media such as FaSSIF (pH 6.5) were used, the formulations quickly reached the solubility limit of the free base and showed minimal solubilization due to very low solubility above pH 2. Similarly, in the case of 2-stage dissolution (i.e., dissolution in pH 3 medium followed by addition of FaSSIF) rapid precipitation of the compound was observed postsolubilization in the pH 3.0 medium. Therefore the dissolution studies reported here were limited to the pH 3.0 medium with a relatively low buffer capacity. Dissolution studies showed that all the modified formulations (except nanosuspension) had greater amount dissolved up to 30 min (expected mean gastric transit time in humans under fasted state) than F1. F1 showed the least amount dissolved under these dissolution conditions, which was consistent with the poor bioperformance of F1 in phase I studies. Hence it was

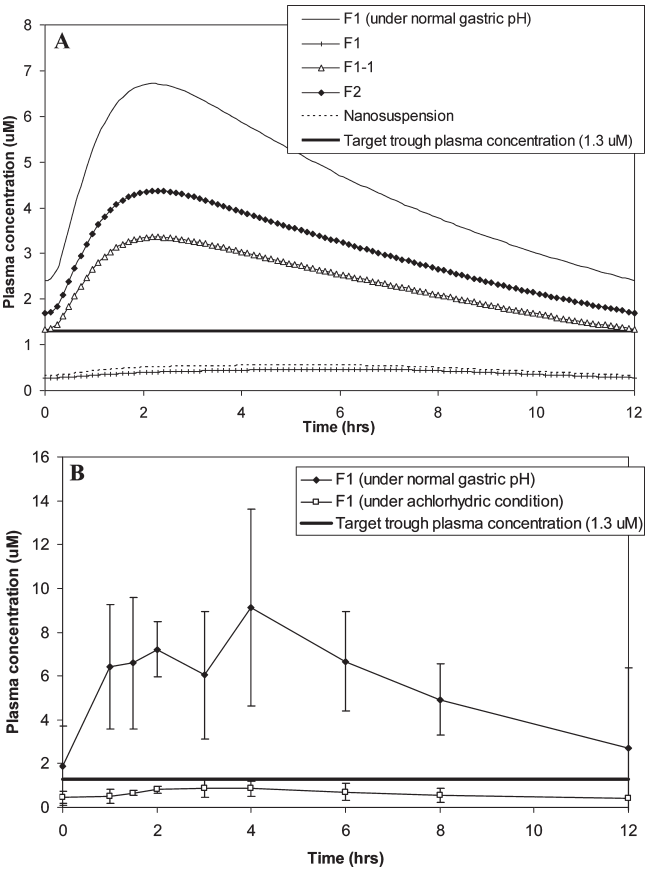

Figure 4. (A) Steady state prediction of bioperformance of the modified formulations in humans based on the dissolution data at a dose of 400 mg BID. The performance of F1 formulation under normal gastric pH condition is shown to highlight the best achievable exposure possible from this formulation in humans. The target trough plasma concentration (1.3 μM) required for efficacy in humans is shown. (B) Observed steady state plasma profiles (n = 2, mean ± SD) for F1 formulation in humans under normal and achlorhydric conditions at a dose of 400 mg BID.

concluded that this dissolution condition was appropriate to assess the bioperformance of the modified formulations. The appropriateness of this dissolution method for future formulation selection was further confirmed since absorption simulations conducted using this dissolution data for F1 had predicted poor bioperformance in humans under high gastric pH conditions (Figure 4A), which was subsequently proven in phase I studies. The observed steady state plasma profiles of the F1 formulation in patients with and without PPI are shown in Figure 4B. At the 400 mg BID dose, data were available for two patients each, in with and without PPI groups. While data from a small number of patients was available at this dose, as previously discussed exposures across non-PPI treated patients were proportional across doses and a larger number of patients. Similarly, F1 formulation showed significant reduction in bioperformance when coadministered with PPI across the dose range in phase I studies. The significantly lower exposure from the F1 formulation in achlorhydric condition as compared to under normal stomach conditions is in agreement with the simulated profiles for F1. This agreement between the simulated and observed clinical data also points to significant contribution of stomach solubilization toward bioperformance. The F2 formulation showed the greatest amount dissolved in the pH 3 medium (~78% dissolved at 30 min) as compared to all other modified formulations,

Table 4. Comparison of Predicted Formulation Bioperformance in Humans from Dissolution Based Absorption Model to the Observed Bioperformance in Dogs

	simulated data based on dissolution (human projection)					obsd data in dog	
formulations	AUC $_{0-24h}$ (μM h)	C_{max} (μM)	C_{trough} (μM)	AUC ratio[a]	C_{max} ratio[a]	AUC ratio[a]	C_{max} ratio[a]
F1[b]	53.90	6.72	2.41	1	1	1	1
			Achlorhydric Condition				
F1	4.74	0.47	0.28	0.08	0.07	0.07	0.06
F1-1	28.45	3.36	1.34	0.53	0.50	0.51	0.59
F2	33.37	4.36	1.70	0.62	0.65	0.61	0.83
nanosuspension	5.55	0.54	0.31	0.10	0.08	0.14	0.09

[a] Relative to F1 in normal gastric pH. [b] In normal gastric pH.

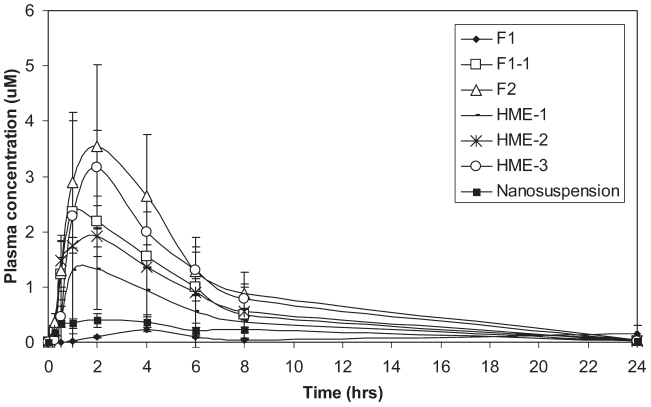

Figure 5. Plasma profiles (n = 3 or 6, mean ± SE) of compound A for various formulations in famotidine pretreated dogs at 10 mg/kg.

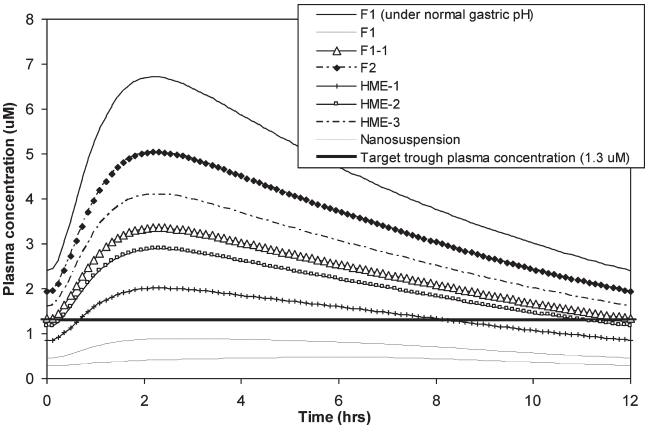

Figure 6. Steady state prediction of bioperformance of the modified formulations in humans based on famotidine pretreated dog data. The performance of F1 formulation under normal gastric pH condition is shown to highlight the best achievable exposure possible from this formulation in humans. The target trough plasma concentration (1.3 μM) required for efficacy in humans is shown.

indicating that this formulation might show better bioperformance than the other formulations under achlorhydric conditions in humans. The lag time (~10 min) observed for the F2 formulation was most likely due to the use of HPMC capsule shell whereas hard gelatin shells were used for the other formulations, which did not show a lag in drug release. Slower release from HPMC capsules as compared to gelatin capsules has been reported before, particularly at higher pH, due to slower disintegration of HPMC capsules.[16]

Absorption simulations based on these dissolution data predicted that the acidified DFC formulations (F1-1 and F2) would meet the C_{trough} target (1.3 μM) in humans under achlorhydric conditions, while the F1 and the nanosuspension would not meet the target (Table 4 and Figure 4). These simulations also predicted that the F2 formulation would show the best exposures of all the other formulations simulated in PPI patients (Table 4) and would be closest to the exposure seen with F1 formulation in non-PPI patients, which in this case was the benchmark for the modified formulations. According to these simulations the F1-1 formulation would barely meet the C_{trough} target in humans but shows much lower overall AUC and C_{max} as compared to F2 (Table 4). Simulations were not conducted for the HME formulations since it is known that traditional dissolution methods may not be fully predictive of in vivo performance of these formulations. Simulations solely based on observed concentrations in dissolution studies in biorelevant media such as FaSSIF resulted in low predicted exposures, similar to the predicted values for F1 simulation of PPI-treatment. Thus stomach solubilization would be a critical factor to drive bioavailability of these new formulations.

Studies in famotidine pretreated dogs showed that the acidified formulations (F1-1, F2, HME-2 and HME-3) had better bioperformance than the nonacidified formulations (Table 3 and Figure 5), as predicted by the dissolution based simulations for formulations F1, F1-1 and F2. Among the conventional acidified formulations, as seen in Table 3 the F2 and HME-3 formulations had the best exposures in famotidine pretreated dogs as compared to F1 in pentagastrin dogs whereas the nanosuspension showed the worst bioperformance as predicted by simulation. Assessment of the F2 formulation in pentagastrin pretreated dogs also showed that there was minimal likelihood of overexposure with this acidified formulation in normal gastric pH condition as indicated by similar bioperformance to F1 in pentagastrin pretreated dogs (AUC ratio = 0.96). To further assess the accuracy of the dissolution based simulation projections, the projected exposure ratios relative to the F1 formulation in humans were compared to the observed dog data (Table 4). The overall good agreement between the predicted human performance of the formulations and the relative exposures in dogs suggested that the dissolution based simulation model was robust and could be used for formulation screening. Subsequently the famotidine dog data were used to simulate bioperformance of the modified formulations in humans. Simulation of

the HME formulations were of particular interest here, since these simulations were not attempted based on the dissolution data. Figure 6 summarizes the outcome of these simulations and shows that most of the acidified formulations would meet the C_{trough} target in humans as predicted by the dissolution based simulations. These projections also forecast that, of all the formulations developed, F2 would have the best bioperformance in achlorhydric conditions and had the highest probability of meeting the target C_{trough} needed for efficacy in humans, which was again in agreement with the dissolution based simulation. Further the formulation rank-ordering (F2 > F1-1 > F1) was also consistent from both models. Based on the simulation, dissolution and animal data, the F2 formulation was selected for pharmacokinetic studies in humans.

DISCUSSION

The negative impact of high gastric pH on bioavailability of weakly basic drug molecules is well-known,[1] and several approaches have been published to overcome gastric pH interactions.[1,12−15] In this paper, the development of a solid dosage formulation of a weakly basic Merck drug (compound A) and complementary use of in vitro/in silico/in vivo (IVISIV) tools to guide formulation development is reported. Absorption modeling (Figure 1) based on the pH-solubility profile of compound A had predicted that a significant impact of higher gastric pH could be expected on the bioperformance of F1. These predictions were subsequently corroborated in dog and human studies. As noted in Results the F1 formulation showed significantly reduced exposures, high variability and lack of dose proportionality when coadministered with PPI in cancer patients. Although high variability was also observed in the non-PPI cohorts across the dose range, this was likely due to high variability in the stomach pH in gastric cancer patients. Hence it was hypothesized that a more robust formulation whose bioperformance is less susceptible to variance in gastric pH would improve the observed variability, achieve adequate exposure under high gastric pH conditions and show appropriate bioperformance under a wide range of administration conditions. For the reformulation, the team focused on development of acidified conventional formulations containing an organic acid as well as enabled formulations such as HME and nanosuspension. The hypothesis for using an acidifier was that the organic acid would increase the solubility of the drug substance in the local environment of the dissolving formulation and thus result in a lower degree of supersaturation in that microenvironment. Due to this unique process, once dissolved the drug substance would be able to remain in the solubilized form, thus providing an opportunity for absorption to take place. The use of organic acid in solid dosage formulations to overcome gastric pH interaction has been shown before. Badawy et al. had shown that tartaric acid based formulation was able to overcome the pH-dependent absorption of BMS-561389 in the famotidine pretreated dogs.[14] Similarly, Derendorf et al. showed that a tartaric acid based extended release formulation of dipyridamole had a 2-fold higher bioavailability than a nonacidified formulation in subjects with higher gastric pH.[15] In the current study, citric acid was chosen as the acidifier primarily due to its good safety profile and low expected chemical interaction with the drug substance in the formulation. The HME and nanosuspension formulations were developed with the aim to increase in vivo dissolution and thus increase the absorption rate and bioavailability of compound A. This rationale was based on published reports where bioavailability of poorly soluble drugs has been significantly increased by using these technologies as compared to other conventional formulations.[17,18] It was hypothesized that, even in the high gastric pH environment, the increased solubility and rapid dissolution afforded by these enabled formulations would result in increased absorption as compared to the F1 formulation and hence a reduced achlorhydria effect will be observed. However, the famotidine dog studies showed that the nonacidified HME and nanosuspension were not as effective in increasing absorption of compound A as compared to the acidified formulations. This was most likely because the addition of a polymer (in the case of HME) or size reduction of the drug substance (nanosuspension) was not as effective as the addition of an acidifier in enhancing solubilization of compound A in the high pH environment. This hypothesis was supported by the higher exposures observed with the acidified HME formulations (HME-2 and HME-3) as compared to the nonacidified HME-1 (Table 3). However, it should be noted that these observations cannot be generalized at this time and more data has to be generated in order to conclude whether these enabled technologies would be able to minimize the achlorhydria effect without the addition of an acidifier. HME-3, which contained intragranular citric acid, showed the highest exposure in famotidine dogs (Table 3). This superior performance can be attributed to intimate contact of the acidifier with drug substance in the formulation, thus enabling better solubilization of the dose. Among the acidified conventional formulations, F2 showed better overall bioperformance in famotidine pretreated dogs (Table 3) and was also predicted to achieve higher C_{trough} in humans as compared to F1-1 (Table 4). The better bioperformance of F2 was most likely due to enhanced solubilization of compound A in high gastric pH conditions due to the use of a combination of the HCl salt of the drug substance and the acidifier (citric acid), whereas F1-1 used the free base form which had much lower solubility in higher pH. F2 formulation also showed similar exposures to HME-3 in famotidine dogs. In the higher gastric pH dogs the F2 formulation showed approximately 9-fold increase in AUC and 14-fold increase in C_{max} as compared to F1. However in pentagastrin dogs (normal gastric pH condition), the exposure from F2 was similar to F1 (AUC ratio = 0.96 and C_{max} ratio = 1.36). These data suggested that while the modified formulation (i.e., F2) was able to significantly improve the exposure in high gastric pH condition as compared to F1, there was minimal likelihood of overexposure in normal gastric pH condition. Both dissolution and dog data based absorption models (Figures 4 and 6) also predicted that the F2 formulation would likely meet the human C_{trough} target under achlorhydric condition.

A valid question when using preclinical animal models for formulation screening prior to human studies is whether metabolic differences between species can lead to different formulation performance. However this will appear unlikely for compound A. Preclinical studies using liver microsome suggested similar primary metabolic route (CYP3A4) in both dogs and humans, and the compound exhibited low in vivo clearance in dogs (plasma clearance = 3.1 mL/min/kg). While no intravenous data is available in the clinic to allow for calculation of clearance, the observed oral exposures are suggestive of low clearance in humans as well (CL/F < 5 mL/min/kg). Thus it would appear most likely that the differences in exposure for different formulations is due to differences in absorption and not metabolism, which is supported by the fact that initial dissolution differences between the formulations agrees with the PK differences. It should be also noted that, given the high permeability (BCS II) of the compound, differences in intestinal permeability between species is also unlikely to contribute to differential formulation behavior in the preclinical model and in the clinic.

Due to a projected stability concern with HME-3, the F2 formulation was selected as the lead formulation for subsequent clinical evaluation. The bioperformance of F2 formulation was evaluated in oncology patients with and without concomitant administration of PPI (omeprazole) at a dose of 770 mg BID (data not shown). This study showed that pharmacokinetics of compound A when incorporated into F2 formulation did not meaningfully change in the presence of PPI as compared to non-PPI (AUC ratio = 0.95 and C_{max} ratio = 0.93). Also, in patients without PPI the exposure achieved from the F2 formulation was similar to F1 (AUC ratio = 1.2), suggesting that the reformulation efforts did not impact pharmacokinetics of compound A under normal gastric pH conditions, as indicated by the dog studies. Further the C_{trough} target (1.3 μM) was achieved in all patients with or without PPI coadministration with the F2 formulation. These human data confirmed that the F2 formulation was successful in overcoming the achlorhydria effect

In this study, several biopharmaceutical tools were used in a complementary fashion to predict clinical outcome. A combination of predictive dissolution assay, absorption modeling and gastric pH modified dog model was used in this project to facilitate formulation development with goals of overcoming the achlorhydria effect and achieving the clinical target (i.e., C_{trough}). Absorption modeling in particular was instrumental in selection of an appropriate formulation for the human PK study, which was key in avoiding multiple clinical studies and hence led to significant cost and time savings. Thus, the IVISIV approach was proven to be an effective process for development of formulations to overcome the achlorhydria effect and achieve adequate exposure in patients with high gastric pH.

CONCLUSION

This work describes the complementary use of biopharmaceutical tools such as dissolution, absorption simulation and dog model to assist in the development of a formulation with adequate bioperformance in achlorhydric conditions. An appropriate dissolution method was developed, which was predictive of formulation performance in dogs and humans. The formulations screened using this dissolution method was tested in a gastric pH modulated dog model to assess performance in normal and high human gastric pH environment. Further, dissolution based and dog data based absorption models were developed and successfully used in formulation screening. This work also highlights the importance of proactive bioperformance risk assessment during early clinical formulation development for compounds with steep pH-dependent solubilities, especially for oncology programs where bridging formulations with very different in vivo performance in patients are not desirable. The effective use of absorption simulation to inform bioperformance risk of formulations in humans can enable better decision making on formulation selection along with dissolution and animal data.

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REFERENCES

(1) Russell, T. L.; Berardi, R. R.; Barnett, J. L.; O'Sullivan, T. L.; Wagner, J. G.; Dressman, J. B. pH-related changes in the absorption of dipyridamole in the elderly. *Pharm. Res.* **1994**, *11* (1), 136–143.

(2) McColl, K. E. L.; El-Omar, E.; Gillen, D. Interactions between H. pylori infection, gastric acid secretion and anti-secretory therapy. *Br. Med. Bull.* **1998**, *54* (1), 121–138.

(3) Morihara, M.; Aoyagi, N.; Kaniwa, N.; Kojima, S.; Ogata, H. Assessment of gastric acidity of Japanese subjects over last 15 years. *Biol. Pharm. Bull.* **2001**, *24* (3), 313–315.

(4) Russell, T. L.; Berardi, R. R.; Barnett, J. L.; Dermentzoglou, L. C.; Jarvenpaa, K. M.; Schmaltz, S. P.; Dressman, J. B. Upper gastrolintestinal pH in seventy nine healthy, elderly, north American men and women. *Pharm. Res.* **1993**, *10* (2), 187–196.

(5) Lahner, E.; Annibale, B.; Delle-Fave, G. Systematic review: impaired drug absorption related to the co-administration of antisecretory therapy. *Aliment. Pharmacol. Ther.* **2009**, *29*, 1219–1229.

(6) Lelawongs, P.; Barone, J. A.; Colaizzi, J. L.; Hsuan, A. T.; Mechlinski, W.; Legendre, R.; Guarnieri, J. Effect of food and gastric acidity on absorption of orally administered ketoconazole. *Clin. Pharm.* **1988**, *7*, 228–235.

(7) Piscitelli, S. C.; Goss, T. F.; Wilton, J. H.; D'Andrea, D. T.; Goldstein, H.; Schentag, J. J. Effects of ranitidine and sucralfate on ketoconazole bioavailability. *Antimicrob. Agents Chemother.* **1991**, *35*, 1765–1771.

(8) Lake-Bakaar, G.; Tom, W.; Lake-Bakaar, D.; Gupta, N.; Beidas, S.; Elsakr, M.; Straus, E. Gastropathy and ketoconazole malabsorption in the acquired immunodeficiency syndrome (AIDS). *Ann. Intern. Med.* **1988**, *109*, 471–473.

(9) Lake-Bakaar, G.; Quadros, E.; Beidas, S.; Elsakr, M.; Tom, W.; Wilson, D. E.; Dincsoy, H. P.; Cohen, P.; Straus, E. Gastric secretory failure in patients with the acquired immunodeficiency syndrome (AIDS). *Ann. Intern. Med.* **1988**, *109*, 502–504.

(10) Tomilo, D. L.; Smith, P. F.; Ogundele, A. B.; Difrancesco, R.; Berenson, C. S.; Eberhardt, E.; Bednarczyk, E.; Morse, G. D. Inhibition of atazanavir oral absorption by lansoprazole gastric acid suppression in healthy volunteers. *Pharmacotherapy* **2006**, *26*, 341–346.

(11) Reyataz prescribing information. http://packageinserts.bms.com/pi/pi_reyataz.pdf.

(12) Chin, T. W. F.; Loeb, M.; Fong, I. W. Effects of an acidic beverage (Coca-Cola) on absorption of ketoconazole. *Antimicrob. Agents Chemother.* **1995**, *39* (8), 1671–1675.

(13) Polster, C. S.; Atassi, F.; Wu, S. J.; Sperry, D. C. Use of artificial stomach-duodenum model for investigation of dosing fluid effect on clinical trial variability. *Mol. Pharmaceutics* **2010**, *7* (5), 1533–1538.

(14) Badawy, S. I. F.; Gray, D. B.; Zhao, F.; Sun, D.; Schuster, A. E.; Hussain, M. A. Formulation of solid dosage forms to overcome gastric pH interaction of the factor Xa inhibitor, BMS-561389. *Pharm. Res.* **2006**, *23* (5), 989–996.

(15) Derendorf, H.; VanderMaelen, C. P.; Brickl, R. S.; MacGregor, T. R.; Eisert, W. Dipyridamole bioavailability in subjects with reduced gastric acidity. *J. Clin. Pharmacol.* **2005**, *45* (7), 845–850.

(16) Al-Tabakha, M. M. HPMC capsules: current status and future prospects. *J. Pharm. Pharm. Sci.* **2010**, *13* (3), 428–442.

(17) Repka, M. A.; Majumdar, S.; Kumar, S.; Srirangam, R.; Upadhye, S. B. Applications of hot-melt extrusion for drug delivery. *Expert Opin. Drug Delivery* **2008**, *5* (12), 1357–1376.

(18) Kesisoglou, F.; Panmai, S.; Wu, Y. Nanosizing - oral formulation development and biopharmaceutical evaluation. *Adv. Drug Delivery Rev.* **2007**, *59* (7), 631–644.