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Use of Artificial Stomach−Duodenum Model for Investigation of Dosing Fluid Effect on Clinical Trial Variability

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Abstract: Lilly Compound X (LCX) is an oncology drug that was tested in a phase I clinical study using starch blend capsules. The drug was given to a small patient population (4 patients) and showed large inter- and intra-patient variability. In order to evaluate the possible effect of stomach pH on exposure and ways to mitigate the variability issue, artificial stomach−duodenum (ASD) experiments were conducted to investigate the hypothesis that carefully selected dosing fluids would have an impact in minimizing exposure variability caused by the formulation, which could lead to more consistent evaluation of drug absorption in patients. The ASD data corroborates the observed variability, and was a good tool to investigate the effect of stomach pH and potential dosing solutions on duodenal concentrations. Administering capsules co-formulated with Captisol (10% drug load) along with Sprite was shown by the ASD to be an effective way to increase duodenal concentrations as well as to reduce the difference between duodenal concentrations for different gastric pH. The reduction in variability of duodenum AUC (in ASD) is expected to correlate well with a reduction of variability in patient exposure. The dosing regimen of Sprite/Captisol is therefore suggested for future clinical trials involving LCX. Furthermore, for design of early phase clinical trials, ASD technology can be used to assist in choosing the proper dosing solution to mitigate absorption and exposure variability issues.

Keywords: Artificial stomach duodenum; GI simulation; clinical trial variability; variability mitigation; dosing fluid selection; gastric pH effect; oncology

1. Introduction

Drug development is a long, complex and costly process aimed at bringing safe and efficacious drugs to the market. This complexity is due to multiple factors, such as efficacy, clinical safety, toxicity and formulation, causing high rate of attrition in the drug development pipeline.[1] Oncology is a fast growing therapeutic area in drug development, and many pharmaceutical companies are devoting significant resources to develop cancer drugs to satisfy the demands for unmet patient needs. While most oncology drugs in the past were parenteral products, the use of oral cancer agents has increased in recent years[2] despite the fact that developing oral oncology drugs carries its own risks and challenges, such as increased inter- and intra-patient variability in plasma drug exposure due to differences in gender, first pass hepatic metabolism, drug transporters, patient disease state and diet resulting in suboptimal drug exposure (decreased tumor inhibition efficacy) or increased exposure (excess toxicity).[3]

In drug development the selected drug candidate is formulated to be dosed in phase I clinical trial (or first human dose, FHD) to assess the clinical safety of the drug and also

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to carry out studies of drug metabolism and mechanism of action.[4] This phase of clinical testing typically recruits healthy volunteers with the exception of AIDS and cancer drugs where, for mainly ethical reasons, it is performed on patients and in many cases on terminally ill patients. When formulating an FHD oral drug, formulation scientists use different physical properties as well as preclinical animal exposure data to predict factors affecting drug absorption such as solubility, dissolution and the effect of different preclinical formulations on drug plasma levels. However, inter- and intra-patient variability in drug plasma levels or the inability to reach adequate exposure is a problem at this stage of development, particularly in oncology trials. Thus, models were developed to investigate factors affecting drug absorption after oral administration and possible sources of variability in drug absorption. One such approach is an artificial stomach−duodenum (ASD) model. This in vitro model mimics the functions of parts of the human digestive system (particularly stomach and duodenum) thus allowing pharmaceutical and food scientists to study how food and drug formulations are dissolved and transported within the GI tract. Also, it allows for the study of how the physical properties of drugs such as solubility and wettability influence drug absorption, assuming that duodenal drug concentration is proportionally related to absorption.[5] ASD modeling provides an important dynamic tool for investigation of transient effects. This type of model has been successfully used to improve the dynamic in vitro assessment of antacid-induced resistance to stomach acidification and measure their activity on the duodenal milieu as well as in evaluating the effect of nutrients such as proteins and magnesium oxide in neutralizing stomach pH.[6] In another example, Carino et al. successfully utilized a dog ASD model to estimate the relative bioavailability of three different carbamazepine crystalline forms in both fasted and fed states, maintaining agreement with *in vivo* data.[5] Furthermore, Blanquet et al. utilized a computer-controlled multi-compartmental dynamic artificial GI system (TIM-1) to investigate the effect of food and different formulations on the absorption of paracetamole from the gastrointestinal tract and demonstrated the agreement between data obtained from the TIM-1 model and *in vivo* data.[7]

In this article, Lilly Compound X (LCX) is a BCS class II oncology drug that was tested in a phase I clinical study using formulated (starch blend) capsules. The drug was given to a small patient population (4 patients) and showed large inter- and intra-patient variability. In order to evaluate the possible effect of stomach pH on exposure and ways to mitigate the variability issue, human ASD experiments were conducted to investigate the hypothesis that carefully selected dosing fluids would have an impact in minimizing exposure variability caused by the formulation, which could lead to more consistent evaluation of drug absorption in patients.

2. Materials and Methods

2.1. Materials. All chemicals used in the ASD model were of analytical grade. Captisol was obtained from CyDex Pharmaceuticals Inc. (Lenexa, Kansas). Captisol is a poly-anionic β-cyclodextrin derivative with a sodium sulfonate salt separated from the lipophilic cavity by a butyl ether spacer group, or sulfobutylether (SBE). Specifically, Captisol is the hepta-substituted cyclodextrin of this type. Sprite was purchased and used as is after it was degassed for 10 min in an ultrasonic bath at room temperature. Gastric fluids were prepared as dilutions of 0.1 N HCl (Ricca Chemicals). Duodenal fluid (50 mM phosphate buffer, pH 6.8) was made with potassium phosphate monobasic and dibasic (Fisher Chemical), and mimics USP 23 simulated intestinal fluid, excluding enzymes. LCX was developed and manufactured by Eli Lilly and company (Indianapolis, Indiana) for the treatment of cancer. The drug is formulated in capsules in two different ways: a 25 mg dose blended with starch and silicon dioxide for flowability (capsule formulation 1) and a 25 mg dose blended with 225 mg of Captisol (capsule formulation 2).

2.2. Artificial Stomach−Duodenum Model (ASD). The artificial stomach and duodenal human system (Figure 1) was constructed similarly to a previously described method.[5] The system consists of two borosilicate glass chambers, stomach (3 in. diameter, 3.5 in. depth) and duodenum (1.75 in. diameter, 1.9 in. depth). Each chamber has two ports, one a barbed glass tube on a bottom edge for chamber emptying, the other a compression fitting for a 0.25 in. UV−vis fiber-optic probe. All pumps are run with the same model motor (SciLog ACCU CP-8). Stomach and duodenum secretions are controlled with high precision piston pump heads (Fluid Metering Inc. FMI H405), while chamber emptying is carried out with peristaltic pump heads (Tandem 1081). Mixing for both chambers is done by stainless steel paddles controlled by stepper motors (Anaheim Automation 23Y106S-LW8). All equipment is contained within a Forma Scientific reach-in incubator, model 3950, held at a constant 37 °C. All other atmospheric conditions are ambient.

Experimental conditions were set to mimic human physiology. Extensive reviews on this topic have previously been

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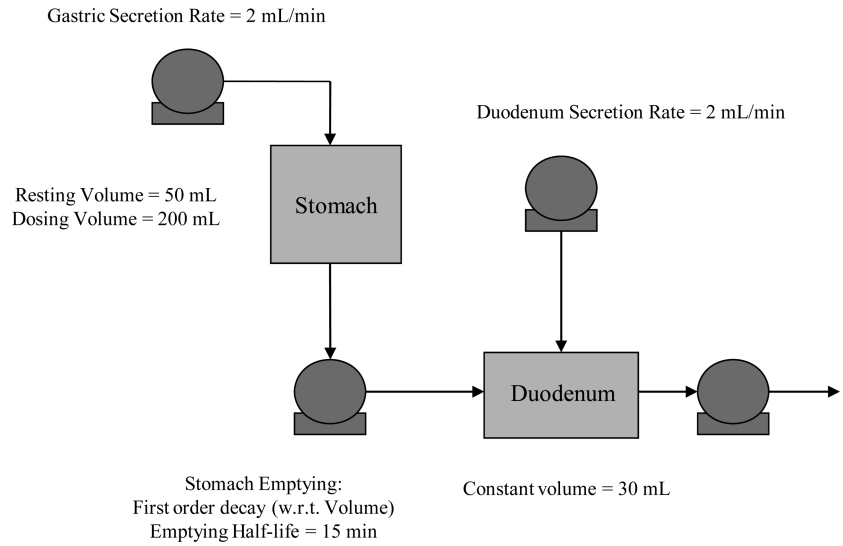

Figure 1. Schematic of ASD model.

conducted by Smeets-Peeters and Dressman.[8] More recent work has been done on physiological simulation, such as the TNO TIM-1 system[9] and further work on simulated GI tract fluids by Dressman et al.[10] Since the largest effect in this study is expected to be pH, and most of the finer adjustments to physiological simulation do not largely impact pH, simpler conditions are chosen that are in the parameter space of previous work for fasted patients. For all experiments, stomach and duodenum secretion rates are held constant at 2 mL/min. The stomach emptying rate is programmatically controlled such that the half-life of returning to the resting volume (50 mL) is 15 min. The duodenum volume is maintained constant at 30 mL by setting the emptying flow rate equal to the sum of the stomach emptying rate and the duodenum secretion rate (residence times vary from 2.3 to 7.5 min). Additionally, a vacuum line is set in the duodenum chamber at a height calibrated to be 30 mL to ensure the volume is maintained at that level.

Fluids used were 0.01 N HCl (pH 2) and 0.000032 N HCl (pH 4.5) as gastric fluids and 50 mM potassium phosphate buffer (pH 6.8) as duodenal fluid. Dosing options investigated for capsule formulation 1 were deionized water, degassed Sprite, and a 1:1 mixture of 20 w/w % Captisol in 0.001 N HCl and deionized water (referred to simply as HCl/Captisol). A dosing option referred to as Sprite/Captisol consists of capsule formulation 2 dosed with degassed Sprite. All doses consist of one capsule (with capsule sinker) and 200 mL of the dosing fluid. Every dosing option was investigated at both stomach pH 2 and 4.5.

Gastric pH was monitored with a process current meter (DP25-S, Omega) utilizing a 4−20 mA transmitter (PHTX-014, Omega) with 12 VDC internal excitation and gel-filled combination pH electrode (PHE-4232). Data was logged on a computer by re-transmission of a 0−5 VDC signal to an analog to digital converter (National Control Devices).

2.3. Quantitative Analysis. Data acquisition in this study was done by fiber-optic UV−visible spectrometry. Dual halogen−tungsten light sources were used (Ocean Optics, DT-MINI-2-GS) with mirrored immersion fiber-optic probes (Hellma, Type 661.622) and Ocean Optics USB2000+ spectrometers. The path length used for this study is 2 mm. Absorbance values were collected as the average of wavelengths across the active drug peak (range of ∼3 nm) and are background corrected by subtraction of average values sufficiently far from the peak (also a range of ∼3 nm). The method was validated by comparison to HPLC measurements of manually pulled samples (HPLC data are not shown in this article).

Table 1. Solubility of LCX in Various Media with and without Captisol

		solubility (mg/mL)	
base media	media pH	w/Captisol	no Captisol
0.01 N HCl	2	>2	1.129
0.001 N HCl	3	0.358	0.025
0.000032 N HCl	4.5	0.014	<0.002
FaSSIF	6.5	0.004	<0.002

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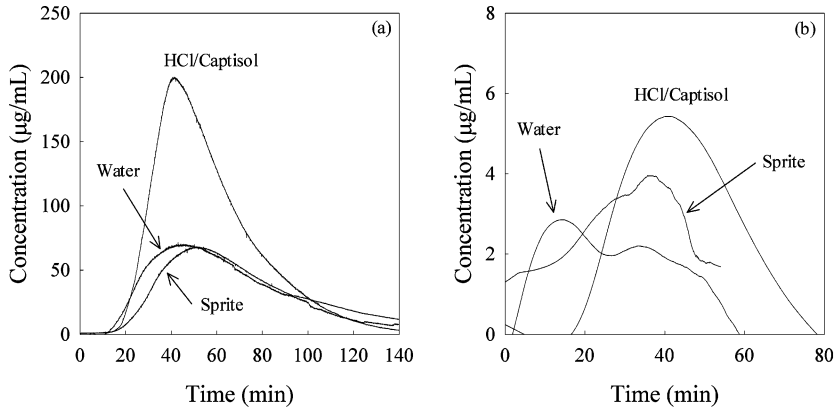

Figure 2. Stomach concentration versus time profiles of capsule formulation 1 administered with water, Sprite or HCl/Captisol at (a) gastric pH 2 and (b) gastric pH 4.5.

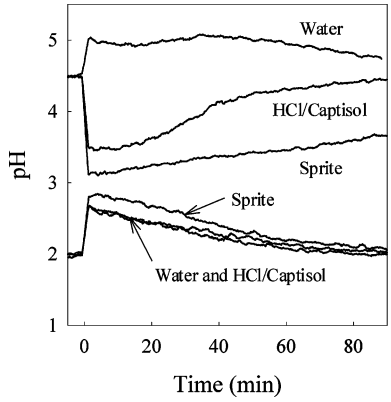

Figure 3. Gastric pH profiles as a function of dosing fluid and native gastric pH.

3. Results and Discussion

LCX has two weakly basic functional groups with pK_a's in the range of 4 and 6. The compound has a high LogP value. Calculated cPassive was highly consistent with the measured permeability (using Caco-2 cells), suggesting that this compound possesses high human intestinal permeability. Table 1 shows a summary of solubility data, including the impact of ~4 w/w% Captisol. LCX has high solubility under acidic conditions (in 0.1N HCl > 2 mg/mL), and its solubility decreases significantly above pH 2. The compound was practically insoluble (solubility <0.002 mg/mL) in simulated fasted and fed intestinal fluids. Captisol enhanced drug solubility at all pH, but seemed to cause more remarkable increases for pH 2 and 3. Despite low solubility in certain pH ranges, early experiments investigating this compound showed that the precipitation of LCX is kinetically slow, and its ability to maintain supersaturated solutions is quite good. No widespread precipitation of drug was noticed in any conditions in this study, even though duodenal concentrations well exceeded equilibrium solubility levels. This emphasizes the importance of dynamic testing, such as ASD modeling.

Capsule formulation 1 was used in clinical trials. In first-in-human (FHD) visit 1, four (4) patients were enrolled and dosed orally once a day without regard to other medications the patients were already taking. The patients were dosed after fasting with a standard glass of water (240 mL). While C_{max} was similar to predicted values in three out of four patients, exposure was much higher than expected and highly variable from patient to patient as well as from day to day. Evaluation of drug−drug interactions (DDI) could not be achieved due to high inter- and intra-subject exposure variability of LCX. However, exposure to the DDI probe drugs (dextromethorphan, midazolam and tolbutamide) was not highly variable, suggesting no problem with data collection at site but rather the formulation used in the trial and the patient physiological conditions which may have played a role in the observed variability. To explain the variability in exposure it is hypothesized that once the drug is dissolved in the stomach at low pH it maintains supersaturation in the intestine, resulting in high exposure. Variability in stomach pH will therefore result in changes in the level of supersaturation in the intestine, thus causing variability in exposure. The fact that three-quarters of the patients were taking a proton pump inhibitor (PPI), which dramatically increases stomach pH, and the exposure in the patients not taking a PPI was the highest, confirms that stomach pH plays a role in drug absorption. However, caution should always be taken in drawing conclusions when the number of patients involved is small.

Nevertheless, variability in the clinical trial is hypothesized to stem from changing stomach solubility resulting in different levels of supersaturation in the intestine. The objective of this study is to assess the effect of stomach pH on drug dissolution and duodenal supersaturation, as well as to investigate the ability of different dosing regimens to potentially reduce this variability. Carino et al. previously correlated ASD solubility with *in vivo* plasma exposure.[5] Thus, the variability in the duodenal concentration results obtained from the ASD model is a reasonable measure of expected variability *in vivo*, and will be used to evaluate all dosing regimens described above on varying gastric pH models.

Figure 2 shows the gastric concentration versus time profiles (for both gastric pH 2 and 4.5) of capsule formulation 1 administered with 200 mL of deionized water, degassed

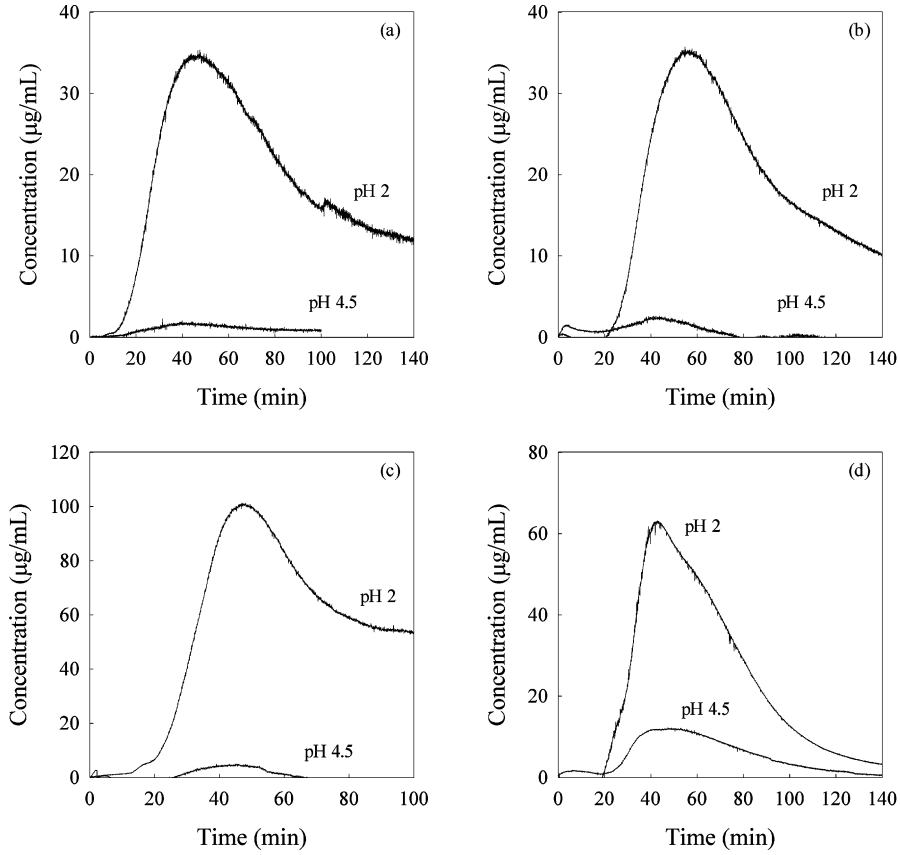

Figure 4. Duodenum concentration versus time profiles starting with gastric pH 2 and 4.5 and using dosing options (a) water, (b) Sprite, (c) HCl/Captisol and (d) Sprite/Captisol.

Sprite or HCl/Captisol solution. It is clear from Figure 2a that HCl/Captisol solution drastically improves drug release at gastric pH 2, while degassed Sprite and water yield roughly equivalent results. However, Figure 2b shows that, at gastric pH 4.5, all dosing options are indistinguishable and yield a small fraction of what was released at gastric pH 2. This is consistent with the clinical observation where the stomach pH difference between patients may be resulting in the large variability.

It is clear that HCl/Captisol enhances drug dissolution at gastric pH 2, but does not seem to have the same effect at gastric pH 4.5. This is possibly due to the ionization state of the drug altering its interaction with Captisol and thus affecting the total solubility of the drug in a Captisol solution.[11] As LCX is a free base with pK_a ~4, the drug is in a positively charged state at lower pH. Since Captisol is an anionic compound, it is likely that electrostatic charges are assisting in complexation, hence causing a greater increase in solubility at lower pH. This pH dependent Captisol−drug interaction and its effect on solubility led to the investigation of the combination of Captisol with Sprite. Sprite is a buffered citric acid solution with a pH of approximately 3.3 (degassed Sprite pH is not significantly different), and will resist a pH change in the ASD system better than an aqueous Captisol solution of approximately the same pH. Figure 3 shows gastric pH levels during experiments with different dosing fluid options. It is evident that, when Sprite is used as a dosing fluid, not only is the pH modified more than in other options but the buffering effect preserves the modification longer. Holding the gastric pH lower with a native gastric pH of 4.5 could potentially maintain the dissolution rate enhancement of Captisol, and reduce the variability of exposure. The pH profile for the Sprite/Captisol dosing option is not shown in Figure 3 because it is nearly identical to the Sprite option.

The investigation of this idea is illustrated in the overlays of duodenum concentration as a function of gastric pH and dosing option (Figure 4). It is evident in all cases, as expected, that lower stomach pH translated into much higher duodenal concentrations. At gastric pH 2, Sprite did not make a significant improvement in duodenal concentration (relative to water); however, both HCl/Captisol and Sprite/Captisol dosing options caused a significant increase in the concentration of the drug in the duodenum. As evidenced by Figure 4d, the Sprite/Captisol combination successfully enhanced drug release at gastric pH 4.5, and consequently increased duodenal concentrations relative to other dosing options. Furthermore, the Sprite/Captisol option reduced the relative duodenal concentration difference between gastric pH 2 and 4.5. Figure 5 quantifies this reduction in relative concentration by comparing the area under the duodenum concentra-

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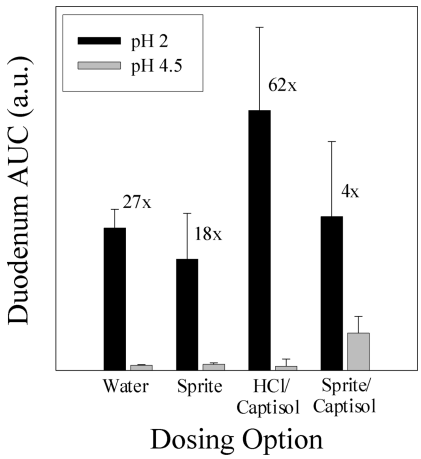

Figure 5. Duodenum AUC of different dosing options for gastric pH 2 and gastric pH 4.5. The numbers above the bars represent the ratio of gastric pH 2 compared to gastric pH 4.5. Error bars indicate standard deviations as determined from two replicates.

tion curve (duodenum AUC) for each gastric pH condition and dosing option. Due to the higher total API solubility in HCl/Captisol at gastric pH 2, and its low solubility at gastric pH 4.5, the expected ratio of gastric AUC to duodenal AUC for the HCl/Captisol dosing option is considerably higher (62 times) than both water (27 times) and Sprite (18 times). However, it is hypothesized that the ability of Sprite to control the pH of the stomach environment allows Captisol to solubilize the drug under both gastric pH levels and therefore reduces the expected variability to a 4-fold difference. Considering that duodenum concentrations can serve as a surrogate for drug absorption, this data suggests that the Sprite/Captisol dosing option (LCX−Captisol coformulation dosed with Sprite) will help to both increase exposure and reduce exposure variability resulting from variable stomach pH environments.

In conclusion, changes in stomach pH are likely contributing to inter- and intra-patient plasma exposure variability seen when capsules of LCX were administered to patients in a phase I clinical trial. The ASD data illustrates this variability and was a good tool to investigate the effect of stomach pH and potential dosing solutions on duodenal concentrations. Administering capsule formulation 2 (coformulated with Captisol) with Sprite was shown by the ASD to be an effective way to increase duodenal concentrations as well as to reduce the difference between duodenal concentrations for different gastric pH. The reduction in variability of duodenum AUC (in ASD) is expected to correlate well with a reduction in patient exposure variability. The dosing regimen of Sprite/Captisol is therefore suggested for future clinical trials involving LCX. Furthermore, for design of early phase clinical trials, ASD technology can be used to assist in choosing the proper dosing solution to mitigate potential absorption and exposure variability issues.

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