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Dissolution Effect of Gastric and Intestinal pH fora BCS class II drug, Pioglitazone: New *in vitro* Dissolution System to Predict *in vivo* Dissolution

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Introduction

The U.S. Food and Drug Administration (FDA) released guidelines based on the Biopharmaceutics Classification System (BCS), which classifies drugs into 4 groups,for the pharmaceutical industry in 2000 [1]. The European Medicines Agency (EMA) and FDA have released guidances for the bioequivalence (BE) and the requirements of the BCS-based biowaiver for immediate release (IR) drug products in BCS class I [1,2]. The expansion ofbiowavers to some BCS class II drugs has been evaluated; particularly weak acidic drugs such as ibuprofen, ketoprofen and naproxen, are potential candidates for biowaivers [3]. These drugs, which are highly permeable, can dissolve quickly at intestinal pH (6.5-7.0) and, therefore, behave like BCS class I drugs in the intestinal tract, even though they exhibit low solubility at acidic pH [4-6]. However, Alvarez and coworkers have been reported that it would be risky to biowaiver BCS class II acidic drugs using just *in vitro* dissolution tests [7]. On the other hand, BCS class II weak basic drugs such as ketoconazole, dipyridamole, and carvedilol, easily dissolve in gastric pH and then may occur the precipitation or reach the supersaturation entering the duodenum due to higher environmental pH [8,9]. Even the limited solubility in intestinal pH, almost complete oral absorption of BCS class II drugslike ketoconazole,carvedilol, and pioglitazone, which dissolve in acidic pH but less dissolve in intestinal pH,has been reported [10-13]. The *in vitro* dissolution study with the USP apparatus II (paddle), which is a golden standard for *in vitro*dissolution study, for thosedrugs would not allow the observation ofprecipitation and supersaturation of those test drugs. Taken together, those reports and concerns raise questions of current*in vitro* dissolution systems and of the discrepancyof *in vitro- in vivo* dissolution. The development of new biopredictive dissolution systems, which emulates *in vivo* condition, would be required to predict the dynamic*in vivo* dissolution effects. Thus, new dissolution systems and techniques such as artificial stomach-duodenal (ASD) model and two-phase testing dissolution systems have been developed and assessed for their feasibilities and reliabilities to predict *in vivo* phenomena[14-19]. Those *in vitro* dissolution systems predict *in vivo* dissolution more accurately for BCS class II drugs than USP dissolution systems, indicating that the current *in vitro* dissolution systems may not be good enough to predict *in vivo* dissolution.

In this article, the new dissolution apparatus called gastro intestinal simulator (GIS) is constructed with three compartments to predict *in vivo* drug dissolution and to monitor the change of drug concentration in each compartment. This system would help to understand the drug dissolution stimulating *in vivo* environment and the transit of drug solution along with GI tract.We examine the dissolution profile of a BCS class II drug, pioglitazone, using the GIS and the feasibility of dissolution result by the GIS to predict *in vivo* dissolution ofpioglitazone.

Experimental

Materials

Pioglitazone tablets were obtained from Takeda Pharmaceutical (Osaka, Japan). High-performanceliquid chromatography (HPLC) grade acetonitrile was obtainedfrom Fisher Scientific (St. Louis, MO). Trifluoroacetic acid (TFA), and allother reagents and solvents were purchased from Aldrich ChemicalCo. (Milwaukee, WI).All chemicals were either analytical or HPLC grade.

GIS apparatus

The *in vitro* GIS model consists of three dissolution chambers representing the stomach, duodenum, and early jejunum with pH monitoring system in each chamber (Figure 1). The stomach and

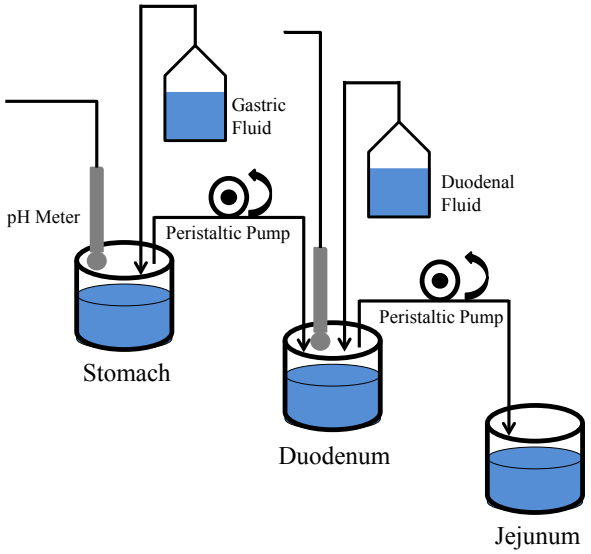

Figure 1: The diagram of the GIS.

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duodenum chambers have capacities of 50-300 mL and 50-125 mL, respectively, and the fluid transit time between those chambers can be adjusted at the range of 1 - 40mL/minute by peristaltic pumps based on the simulation design of the gastric emptying time and GI transit time. The jejunalchamber is only the reservoir from the duodenal chamber and will provide the important information of drug concentration to predict the oral drug absorption of test compound. Those settings can be modified depending on the physiological characteristics of the species.

Preparation of dissolution media

The following dissolution media were used; 0.01N HCl solution without enzymes (pH 2) andUSP simulated intestinal fluid (SIF) without enzymes (pH 6.5).

Dissolution Study of pioglitazone with USP dissolution apparatus II

Dissolution characteristics of a pioglitazone tablet were examined using the USP apparatus II (paddle), Hanson Research (Chatsworth, CA) Model SR6, Serial # 0698-1166 was used for dissolution studies at a rotational speed of 50 rpm at 37.0 ± 0.5°C in 300 mL of the dissolution media; 50 mM phosphate buffer (simulated intestinal fluid: SIF) (pH 6.5). The test tablet was placed in the buffer media to start its dissolution study. Samples were drawn manually at 5, 10, 15, 20, 25, 30, 45, 60, 75, 90, 120, and 180 min and the same sample volume was replaced with the equal amount of blank medium tempered at 37°C.All samples were immediately spun at 2,000 x g for 20 seconds and the supernatant was diluted with the equal volume of methanol for HPLC analysis.

Dissolution study of pioglitazone with GIS

In this experiment, 0.01N HCl solution (pH 2), and 50 mM phosphate buffer (simulated intestinal fluid: SIF) (pH 6.5) were used in each chamber. The initial volumes and pHsof the stomachand duodenum chambers were 300 mL at pH 2, representing 50 mL of stomach fluid plus 250 mL of dose volume, and 50 mL at pH 6.5, respectively. The test tablet was placed in the stomach chamber to start the dissolution study with the GIS. Samples were taken at the specific time points from each chamber over 65 - 120minutes. The fluid was transferred at the range of 1–14 mL/minute to determine the dissolved drug concentration in each chamber. Those chambers were incubated at 37°C and pH changes were monitored by pH probes (Beckman Coulter, Brea, CA). Paddles were provided to mix fluid in each chamber. Fluid volume in duodenum was maintained at the constant volume. All samples were immediately spun at 2,000 x *g* for 20 secondsandthe supernatantwas diluted with the equal volume of methanol. The drug concentration was determined by HPLC analysis.

Solubility studyof a pioglitazone tablet

An excess amount of each drug, a pioglitazone tablet, was placed in 200 mL of each one of the following solutionsto determine the saturated solubility of this pioglitazone product: 3.125 mM phosphate buffer containing 15.4 mMNaCl, 6.25 mM phosphate buffer containing 15.4 mMNaCl,12.5 mM phosphate buffer containing 15.4 mMNaCl,25 mM phosphate buffer containing 15.4 mMNaCl,and 50 mM phosphate buffer containing 15.4 mMNaCl (pH 6.5). The flasks were incubated at 37°C and samples were collected every 45 minutes for 4 hrs. All samples were centrifuged at 12000 x *g* for 3 minutes and the supernatant was diluted with the equal volume of methanol. The drug solubility was determined by HPLC analysis.

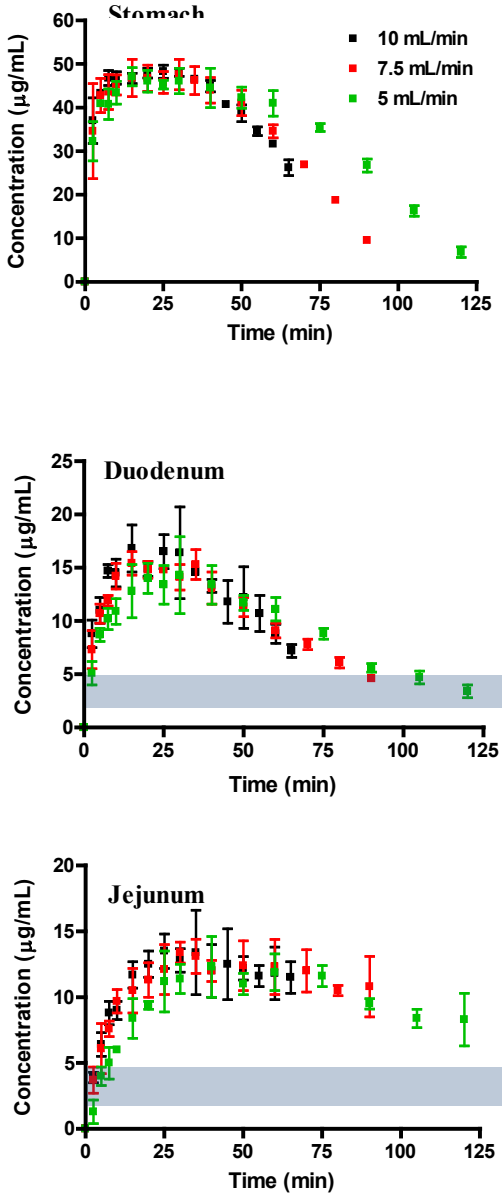


Figure 2: Dissolution profile of pioglitazone tablet in each compartment, stomach, duodenum, and jejunum.

Area ( ) represents the saturated concentration range of pioglitazone from the solubility tests. Values presented are the mean ± S.D., n=3.

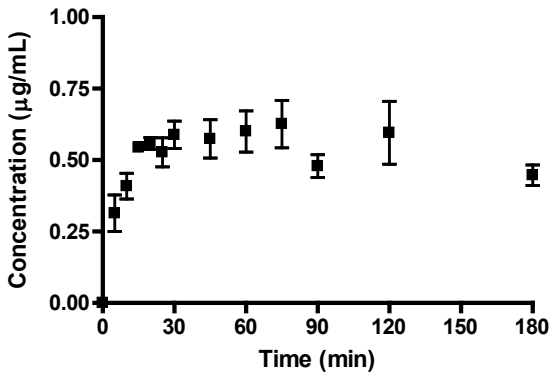
HPLC Analysis

The concentration of pioglitazone was determinedon a Waters HPLC system (Waters, Inc., Milford, MA). The HPLCsystem consisted of two Waters pumps (model 515), a Waters autosampler(WISP model 712), and a Waters UV detector (996 photodiode arraydetector) controlled by Waters Millennium® 32software (version 3.0.1). Samples were resolved in a Cadenza CD-C18 column (3 µm, 3x100 mm) equippedwith a guard column. The mobile phase consisted of 0.1% TFA/water (Solvent A) and 0.1% TFA/acetonitrile (Solvent B) with the solvent B gradient changing from 25 - 45% at a rate of 6.7%/minute during a 10 minute run. Standard curve generated for pioglitazone was

utilized for quantitation of integrated area under peaks. The detection wavelength was 269 nm.

Results

In GIS, the dissolution of pioglitazone tablets with the initial transit time of 10 mL/minuteexhibited the highest pioglitazone concentration (16.8 ± 2.2 µg/mL) in the duodenal chamber (pH 6.5). However, there is no significant difference in all dissolution profileswith the initial transit time of 5 - 10 mL/minute(Figure 2). The dissolution for a pioglitazone tablet in the USP dissolution apparatus II exhibited only 0.6% release (the range of concentration; 0.56 ± 0.04 µg/mL) over 3 hours in 50 mM phosphate buffer (pH 6.5) (Figure 3). A pioglitazonetabletwas quicklydisintegrated and dissolved in the stomach chamber orin the USP vesselwithin 30 minutes regardless of transit timeand dissolution methodology. The solubility studies of apioglitazonetablet showed the solubility range of concentration 1.8 – 4.3 µg/mL in different buffer strength (Table 1). This solubility range of pioglitazone was applied to Figure 2 to indicate the possible pioglitazone concentration in 3.125 - 50 mM phosphate buffer (pH6.5). The highest concentrations of pioglitazone in duodenal and jejunal chambers were 16.8µg/mL and 13.5µg/mL, which were 7.5-fold and 9.25-fold higher than the concentration (1.8 µg/mL) obtained by the solubility tests of pioglitazone in 50 mM phosphate buffer (pH 6.5). On the other hand, the highest concentration of pioglitazone in the USP dissolution apparatus II was 0.6 µg/mL, which was 3.0-folder lower than the concentration obtained by the same tests.



USP dissolution apparatus II with 300 mL of SIF (pH 6.5), paddle rotation set to 50 rpm. Values presented are the mean ± S.D., n=3

Figure 3: Dissolution profile of pioglitazone tablet in SIF (pH6.5) in the USP dissolution apparatus II.

Phospate Conc. (mM)	Tablet
	Pioglitazone Conc. (µg/mL)
3.125	4.25
6.25	3.82
12.5	4.27
25	3.11
50	1.78

The pioglitazone tablet was placed in 200 mL of various concentration of phosphate buffer (pH 6.5).The flasks were incubated at 37°C for 4 hours. The concentration of pioglitazone was determined by HPLC analysis. Values presented are the mean(n=3).

Table 1: The solubility study of pioglitazone in the different concentration of phosphate buffer (pH 6.5).

Discussion

Weak base drugs like ketoconazole and carvedilol might exhibit bioequivalence (BE) using existing *in vitro* dissolution methods like UPS dissolutionapparatus II. However, the progression of *in vivo* dissolution will be far more complicated than the results of *in vitro* dissolution studies with USP dissolutionapparatus due to the pH changes in the GI tract causing the precipitation orthe supersaturation of those BCS class II drugs. Consequently, the *in vitro* dissolution results may not predict*in vivo* dissolution profile. This type of discrepancy between *in vitro*dissolution profilesand *in vivo*dissolution results and, hence, *in vivo* drug absorption, especially for BCS class II and IV drugs, raises the questions regarding the current *in vitro* dissolution tests for bioequivalence.The gastro intestinal simulator (GIS) is the *in vitro* dissolution apparatus to understand the *in vivo* dissolution phenomena of those drugs. The buffer solution in each compartment of the GIS will be transferred from one chamber to the next chamber with various transit rates.

The duodenal and small-intestinal transit timesin the fasted state are approximately 0.5 hour and 3 - 4 hours, respectively, and the gastric residence time is approximately 15 - 60 minutes[20-24].The measured pH of human intestine in the fasted state is reportedly the range of 5.5 to 7.5 in the duodenum and 6.2 to 6.7 in the proximal small intestine[25-27].Therefore, the middle of pH (pH 6.5) was adopted for this set of*in vitro* dissolution studies.ABCS class IIdrug product such as pioglitazone (pKa 5.8 and 6.4) would exhibit much lower solubility in the duodenam and jejunumin pH 6.5 than one in gastric pHdue to its chemical characters.Therefore, the drug which has similar physicochemical characteristicto pioglitazonemay be completely dissolved in the stomach but may occurthe precipitation to inhibit its absorption or reach the supersaturationin small intestine to enhance its absorption.

The average volumes of human intestinal fluid in a fasted state are reportedly 184 mL for duodenum and 63 mL for jejunum [27]. With those volumes at the duodenum and jejunum in a fasted state and the solubility results in 50 mM phosphate buffer, less than 2 % ofpioglitazone(30 mg tablet) would be dissolved at those small intestinal segments at pH 6.5.The dissolution results of pioglitazone tablets in 50 mM phosphate buffer(pH 6.5)with USP dissolution apparatus II displayedeven less drug dissolutionover 3 hours, implying the less absorption of pioglitazone at the small intestine. However, the dissolution results of pioglitazone with the GIS exhibited 3.1-to 9.2-fold higher concentrationthan the obtained saturated concentration in duodenual and jejunal chambers, suggesting the supersaturation of piglitazone in the proximal small intestinal region. As a result, higher *in vivo* absorption of pioglitazone will be anticipated. It has been reported that the orally dosed pioglitazone is well absorbed and its oral bioavailabilityis >80 % [11,12]. Less than 2 % of *in vitro* dissolution profile may not explain well-absorbed oral dose of pioglitazone. This would be the discrepancy between current *in vitro* dissolution profiles and *in vivo* results.

The differences of the dissolution rate and solubility in between acidic pH and pH 6.5 were significant for weak basic drugs and the solubility of pioglitazone would be largely lowered at pH 6.5, which is closer to the one of pKa of pioglitazone. The dissolution rate of pioglitazone would be sensitive to the pH changes in the human intestine. This supersaturation phenomenon will not be observed in current dissolution systems like a USP apparatus II. As a result, it is extremely hard to predict *in vivo* dissolution. However, the GIS has the

capability to observe the supersaturation and, perhaps, precipitation and, hence, to predict better *in vivo* dissolution.

Buffer species and buffer capacity of dissolution medium along with buffer pH clearly have a significant effect on the dissolution rate for test drugs like ketoconazole and pioglitazone. Thus, the selection of dissolution media is crucial. It is likely that the concentration of phosphate buffer (50 mM) is too high for BE studies and may not reflect *in vivo* dissolution media, which is mainly bicarbonate [27,28]. The *in vitro* dissolution rate is clearly dependent on the pH, buffer species and buffer capacity of the medium for drugs products. The USP test does not reflect the human intestinal *in vivo* environment and may not suitable for *in vitro* dissolution study to predict *in vivo* dissolution and, hence, to evaluate bioequivalence. The GIS has the potential to be a standard biopredictive *in vitro* dissolution method for the better prediction of *in vivo* dissolution and, therefore, bioequivalence.

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