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Biorelevant Dissolution Studies of Pioglitazone Hcl Immediate Release Tablets and the Determination of an *In Vitro In Vivo* Correlation

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Abstract

In vitro dissolution testing using biorelevant dissolution media was used to the predict food effects on the absorption of Pioglitazone from immediate release tablets.Dissolution testing was carried out in three dissolution media: hydrochloric acid solution at pH 1.2, in simulated gastric fluid to simulate the gastric fasted state,and in pasteurized milk to simulate the gastric fed state. In order to validate these results, *in vivo* studies were conducted on young Wistar rats under fed and fasted conditions. The *in vivo* studies showed that food delays the onset of action of the drug significantly. The compendial dissolution test in hydrochloride solution at pH 1.2 was not able to predict the food effect. However, the biorelevant dissolution test in simulated gastric fluids predicted correctly that the release of Pioglitazone would be slower in the fed state than in the fasted state. The results demonstrate good correlation between *in vitro* drug release and *in vivo* drug absorption using the biorelevant dissolution test method.

Keywords: Biorelevant dissolution; Pioglitazone; Food effects

Introduction

The use of high throughput screening approaches for discovery of new compounds with pharmacological activity has resulted in the development of drugs with a wide range of physical and chemical properties.To assess whether these compounds possess adequate bioavailability following oral administration, *in vitro* tests that are capable of predicting *in vivo*performance need to bedeveloped.The effectiveness of oral dosage forms relies on the drug dissolving in the gastrointestinal tract fluids prior to absorption into the systemic circulation. Therefore,it is important to be able to predict the rate of dissolution *in vivo*. However, dissolution testing in standard media does not always correlate with *in vivo*results because it does not adequately simulate the physiologic gastrointestinal (GI) environment. Several studies have been carried out to determine the circumstances under which dissolution testing can be predictive for *in vivo* performance in order to design appropriate dissolution testing factors such as composition, volume, flow-rates and mixing pattern of the fluid to simulate the GI environment [1,2]. Due the fact that the majority of drugs currently in development are poorly soluble drugs, it is important thatadequate predictive dissolution testing procedures are available for use in drug development and formulation.

Drug dissolution is animportant rate limiting factor in drug absorption and is directly related to the clinical response for most drugs given orally [3]. In 1995, Amidon and co-workers introduced the biopharmaceutical classification system (BCS) of drugs to reduce the need for *in vivo* bioequivalence studies, by the utilization of *in vitro* dissolution tests as a surrogate for *in vivo* bioequivalence studies [2,4]. When combined with the *in vitro* dissolution characteristics of the drug product, the BCS takes into account three major factors: solubility, intestinal permeability, and dissolution rate, all of which govern the rate and extent of oral drug absorption from immediate release solid oral dosage forms[5].

According to the BCS, drugs can be divided into four classes on the basis of their aqueous solubility and permeability through gastrointestinal mucosa. Class I drugs are defined as those with high intestinal permeability which are able to dissolve readily in aqueous media over the pH range 1 to 8, while Class III are drugs with low permeability but high solubility. The absorption of class III drugs can be successfully predicted by *in vitro* dissolution studies because their solubility is good but permeability is poor.Thus, for Class I and III drugs, dissolution is not the rate limiting step to the oral absorption of these drugs. However, class II and IV drug compounds have limited water solubility,so the choice of dissolution medium is often important in determining the rate of dissolution due to the low solubility of these drugs in aqueous media. Moreover, the solubility of these drugs is insufficient for the whole dose to be dissolved in the gastrointestinal contents under normal conditions. Since dissolution for these substances is the rate limiting step to absorption, the media used in dissolution testing needs to closely represent the existing conditions in the gastrointestinal tract in order to achieve a meaningful *in vitro/in vivo* correlation [1,6]. For these types of drugs biorelevant dissolution testing with two new dissolution media, milk(3.5 % fat), and the USP simulated gastric fluid [7] with or without pepsin (SGF/SGF_{sp}),are designed to reproduce fed and fasted state conditions in the GI tract [8].Several studies indicated that biorelevant dissolution media have strong advantages over compendia media in predicting the *in vivo* performance of certain drug formulations [2] and can serve as an *in vitro* surrogate for *in vivo* bioequivalence studies and for *in vivo* and *in vitro* correlation (IVIVC) studies [9-11].

Pioglitazone HCl (PGZ) is an oral antidiabetic agent that acts by decreasing insulin resistance and is used in the management of type II diabetes mellitus. PGZ improves sensitivity to insulin in muscle and adipose tissues and inhibits hepatic gluconeogenesis. It improves

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glycaemic control while reducing circulating insulin levels [12]. It is reported that oral administration of drug in fasting state shows peak plasma concentration (C_{max}) within 2 hours while food delays the time to reach peak plasma concentration (T_{max}) to 3 to 4 hours but does not alter the extent of absorption [13]. This suggests that food may affect the onset of action, C_{max}, T_{max}, and bioavailability of PGZ.

Due to its low permeability and low solubility, pioglitazone hydrochloride is classified as a class IV drug with limited or no *in vitro/ in vivo* dissolution correlation expected. Note that compounds within this class have poor bioavailability and high inter-individual variability. When attempting to develop a suitable dissolution testing procedure for oral dosage forms of class IV drugs, the selection of an appropriate biorelevant dissolution medium is critical as poor solubility is likely to have an impact on the observedIVIVCs. The aim of this work was to evaluate the performance of pioglitazone hydrochloride oral formulations using USP apparatus II and biorelevant dissolution media and to assess whether biorelevant dissolution studies can be used to successfully predict its'bioavailability *in vivo*.

Material and Methods

Chemicals and solvents

All chemicals and solvents used were of analytical or HPLC grade. PGZwas supplied by the Ranbaxy Laboratory, India. The purity and identityof the drug substance was determined inpre formulation studies by assessing its physical characteristics (physical appearance, melting point and solubility) and using a number of instrumental techniques (FTIR spectral analysis, UV spectral analysis and assay by HPLC). The results obtained complied with the monograph requirements for PGZ in the Physician's Desk Reference [14].

Sodium lauryl sulphate, ammonium hydroxide, alloxane monohydrate, disodium EDTA, hydrochloric acid, and methanol were purchased from SD Fine Chemicals Ltd, India. Citric acid, disodium hydrogen phosphate, 1,2-dichloroethane, acetonitrile, sodium chloride, sodium hydroxide, and potassium chloride were obtained from Merck India Ltd. Potassium dihydrogen phosphate, ortho-phosphoric acid, acetic acid, potassium hydroxide were obtained from Qualigens India Ltd. Generic PGZ immediate release tablets, Pioglar 15 (Pioglitazone,15mg) by Stancare (Ranbaxy Laboratories Ltd, India) were also used.

A hydrochloric acid buffer pH 1.2 (HAB pH 1.2) was prepared by adding 425.0 mL of 0.2 M hydrochloric acid to 250.0 mL of 0.2 M potassiumchloride and diluting to a total volume of 1000 mL with purified water.The pH was then adjusted to 1.2 using a small amount of hydrochloric acid.

Simulated gastric fluid (SGF) (without enzyme) for the fasted state was prepared by dissolving 2.5 g of sodium lauryl sulphate (SLS) and 2.0 g of sodium chloride in HAB pH 1.2 and then making up to a total volume of 1000 mL using HAB pH 1.2. The pH was then adjusted to 1.2 with 2-3 drops of hydrochloric acid (0.01-0.05M).Pasteurized milk was used as the dissolution medium to simulate the gastric fed condition.

Apparatus

A double beam UV Spectrophotometer (UV 1601 Shimadzu, Japan), HPLC (Model SPD-10AVP, Shimadzu, Japan), IR Spectrophotometer (FT/IR-410 Jasco, Japan), USP dissolution apparatus (Hanson Research SR8 plus), and differential scanning calorimeter (Pyris-6 DSC, Perkin Elmer Germany) were used.

HPLC assay conditions

HPLC analysis was carried out using a Symmetry C18 column (5µm, 250 x 4.60mm) at a flow rate of 1.0 mL/minute at room temperature, with an injection volume of 10 µL.The mobile phase was a 10mM potassium dihydrogen phosphate-acetonitrile (50:50v/v) solution adjusted to pH 6.0 using a 0.1 M potassium hydroxide solution, with peak detection was at λ = 225nm. The calibration curve was obtained by plotting the peak areas against standard concentration. Good linearity (R^2 = 0.999) was obtained over the investigated drug concentration range.

Dissolution studies

Dissolution of PGZ in pH 1.2 hydrochloric acid buffer (HAB pH 1.2): A 100µg/mLsolution of PGZ in HAB pH 1.2 was used as thestandard stock solution for the dissolution studies in HAB pH 1.2. A series of standard solutions in the range of 2-20µg/mL were then preparedusing the standardstock solution. A calibration curve was constructed by plotting standard absorbances against their corresponding concentrations and usingthe least square method. The curve obeyed Beer Lambert's law in the investigated concentration range with a high correlation coefficient (R^2= 0.997) indicating good linearity.

Dissolution tests were performed in triplicate using USP Type II dissolution test apparatus at 37 ± 0.5°C and a 50 rpm paddle speed, using 900 mL of HAB pH 1.2 as the dissolution medium. The dissolution medium was pre-warmed to the required temperature before dissolution testing commenced.Aliquots of 5 mL were periodically withdrawn at intervals of 2 min and each time the volume withdrawnwas replaced with an equivalent amount of fresh dissolution medium.The absorbance of each sample wasmeasured at a wavelength of 225 nm and the concentration in µg/ml calculated from the calibration curve.

Dissolution of PGZ in SGF: A100 µg/mL standard working solution was prepared by dissolving PGZ in SGF and then used to make a series of standard solutions in the range of 20-200 µg/mL using SGF. HPLC analysis was carried out using a Shimadzu chromatograph, Model SPD-10AVPon Symmetry C18, 5 µm, 250 x 4.60 mm column, at a flow rate of 1.0 mL/minute and at room temperature. 10mM potassium dihydrogen phosphate-acetonitrile (50:50v/v) adjusted to pH 6.0 with 0.1 M potassium hydroxide was used as mobile phase. Peak detection was performed at λ = 225nm. The calibration curve was obtained by plotting the peak areas against nominal concentration. Good linearity (R^2= 0.999) was obtained over the investigated drug concentration range.

Dissolution studies were performed in triplicate using USP dissolution Type II dissolution test apparatus at 37 ± 0.5°C and 50 rpm paddle speed using 400 mL of SGF. The dissolution medium was pre-warmed to the required temperature before dissolution testing commenced. Aliquots of 5mL were periodically withdrawn at frequent intervals and fresh medium of SGF (pH 1.2) of 5mL was replaced each time. The dissolution test continued for 1 hour 40 minutes.

Dissolution of PGZ in pasteurized milk: A solution of 100 µg/mL of PGZ in anhydrous ethanol was used as a standard stock solution for dissolution in pasteurized milk. From this stock solution serial dilutions in the concentration range of 20-100 µg/mL were made. These dilutions were spiked with 2 mL of milk and the drug was extracted and reconstituted.2 mL of milk sample was centrifuged at 3000rpm for 30minutes and diluted with 4.0 mL of citric acid/disodium

hydrogen phosphate buffer (0.083M/0.033 M, pH 2.8). The mixture was then rotated with 4.0 mL of 1,2-dichloroethane for 3minutes, and centrifuged at 3000rpm for 15minutes. The organic layer was pipetted out and passed through a sodium sulphate drying tube and then evaporated to dryness. The dried residue was then reconstituted in mobile phase [1]. A graph between mean peak area (*y*-axis) versus concentration (*x*-axis) was then plotted. It was found that there was a good linear relationship between mean peak area and concentration with an observedcorrelation coefficient of 0.999.

Dissolution studies were performed in triplicate using USP dissolution Type II dissolution test apparatus at 37 ± 0.5°C and 50 rpm paddle speed using 1000 mL of pasteurized milk. The dissolution medium was pre-warmed to the required temperature before dissolution testing commenced. Aliquots of 2 mL were periodically withdrawn at frequent intervals and fresh medium of pasteurized milk of 2 mL was replaced each time. The dissolution test continued for 1 hour 40 minutes. The concentration of PGZ in the samples was determined using HPLC with% drug release and AUC calculated using equations (1) and (2).

$$\%\ drug\ release = \frac{Concentration\left(\frac{ìg}{mL}\right)\times 900\times 100}{Amount\ of\ drug\ in\ tablet\times 1000} \quad (1)$$

Amount of drug in tablets = 15mg

Volume of dissolution medium = 900 mL

$$AUC = \frac{(C_1 + C_2)(T_2 - T_1)}{2} \quad (2)$$

AUC = area under curve

C_1 = initial concentration

C_2 = final concentration

T_1 = initial time

T_2 = final time

***In vivo* bioavailability of PGZ:** *In vivo* studies were conducted on 18 young Wistar rats, each weighing between 200-250 g. They wererandomly divided into 3 groups, each group containing six rats. Rats in group I acted as a control, rats in group II were given free access to food and water, while rats in group III were fasted overnight before starting the experiment. A solution of PGZwas prepared usingpurified water and administered to rats via the oral route. The dose given to each individual rat was 30mg/kg [4]. After administering PGZ via the oral route, 0.3 mL blood samples were withdrawn at 10, 20, 30, 40, 50, 60, 70, 80, 90 and 100minutes with the concentration of PGZ determined using HPLC [15] after sample extraction [13].

A 100 µg/Ml stock ethanolic solution of PGZ was prepared andused to prepare a number of standard solutions in the concentration range of 1-10 µg/mL. Each of these standard solutions was spiked with 2 mL of plasma.There was a linear response between peak area and concentration with an observed correlation coefficient of 0.996.

2 mL of blood sample taken from the rats was centrifuged at 3000rpm for 30minutes to obtain plasma. Each sample of plasma was then diluted with 4.0 mL with citric acid/disodium hydrogen phosphate buffer (0.083 M/0.033M, pH 2.8). 4.0 mL of 1,2-dichloroethane was added to the mixture with, rotated for 3minutes, and then centrifuged at 3000rpm for 15minutes. The organic layer was pipetted out, passed through a sodium sulphate drying tube, and then evaporated to dryness. The dried residue was then reconstituted in a volume of themobile phase [13].

Results and Discussion

PGZ is a BCS class IV drug with both poor solubility and poor permeability. One of the common methods that are used to increase the aqueous solubility of low soluble drugs is to use the salt forms of drugs in the pharmaceutical formulations. However the aqueous solubility of PGZ hydrochloride salt is still very low (0.7 mM) although it is much higher than the aqueous solubility (0.04 mM) of its corresponding freebase [16]. Pharmacokinetic studies suggest that PGZ release and absorption occurs in the stomach[17], so SGF and pasteurized milk were selected as dissolution media to simulate the gastric fasted and fed conditions. To compare the dissolution profiles in biorelevant dissolution media with that of routine compendial media, the dissolution was also carried out in USP buffer solution HAB pH 1.2.The dissolution profiles for PGZ tablets are shown in Figure 1.

It was observed that there is an appreciable difference between the results obtained in HAB pH 1.2 and simulated gastric fluid (SGF). On comparing the results for*in vitro*fed and fasted conditions (Table 1) it can be seen that food delays the onset of action of PGZ from 16 minutes (T_{max} in fasted state) to 80 min (T_{max} in fed state).

In order to validate results from*in vitro* studies of PGZ, *in vivo* studies were conducted using18 young Wistar rats under fed and fasted conditions. The *in vitro* dissolution data was then comparedwith the *in vivo* bioavailability in order to determine the most biorelevant dissolution medium (Figure 2).

The most common pharmacokinetic parameters, total area under the plasma concentration-time curve ($AUC_{0-\infty}$), peak plasma concentration (C_{max}) and time to reach maximum plasma concentration (T_{max}), were estimated from the plasma concentration-time profiles. The $AUC_{0-\infty}$was calculated by adding the area from time zero to the last sampling time (AUC_{0-t}) to the area from the last sampling time to infinity ($AUCt_{-\infty}$). The former was calculated by applying the trapezoidal rule and the latter by dividing the last measurable plasma

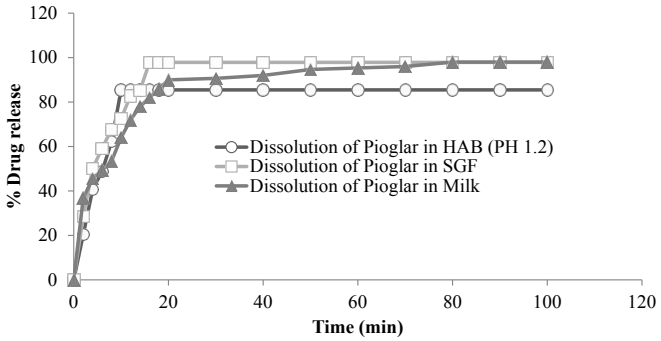

Figure 1: Dissolution profile of PGZ tablets in USP buffer solution hydrochloric acid solution at pH 1.2 (HAB pH 1.2), simulated gastric fluid (SGF) and in pasteurized milk (*n* = 3).

Media	Cmax (µg/mL)*	Tmax (minute)	Maximum % drug release	AUC (µg min/mL)
HAB pH 1.2	14.25 ± 0.04	10.0	85.5	1354.19
SGF	36.72 ± 0.05	16.0	97.920	2943.174
Pasteurized milk	14.7 ± 0.04	80.0	98.0	2737.654

Table 1: Comparison of the results obtained using *in vitro*compendial and biorelevantdissolution media.

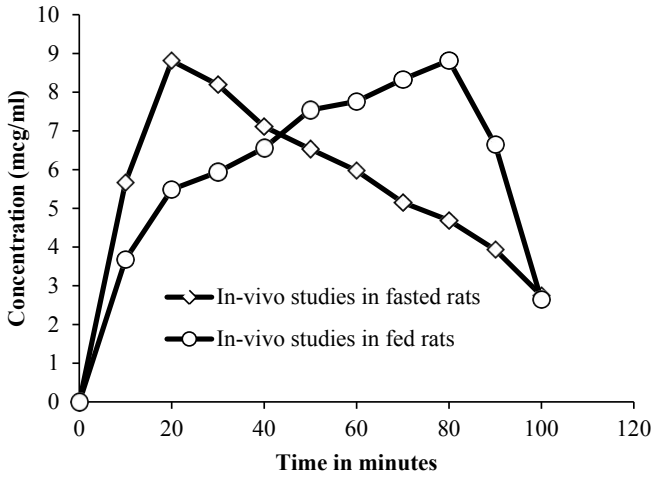

Figure 2: Plasma concentration (Cp) versus time profile of PGZ for rats in the fasted and fed states (*n* = 6).

Parameters	In fed state	In fasted state
Cmax (µg/mL)	8.82	8.81
Tmax (min)	80.0	20.0
AUC (µg min/ml)	620.65	625.25

Table 2: Comparison of the results obtained in *in vivo*fasted and fed states.

drug concentration with the apparent elimination rate constant (ke).

On comparing various biopharmaceutical parameters obtained by *in vitro* dissolution studies (in compendial and biorelevant dissolution media) with *in vivo* results of fed and fasted conditions in rats,it was clear that the values of Cmax, Tmax and AUC for dissolution studies performed in HAB pH 1.2 (compendia dissolution media) were significantly different from the corresponding values obtained by *in vivo* studies (Table 2). The values of C_{max}, T_{max} obtained in biorelevant dissolution media in fed and fasted state were then correlated with the *invivo* data for fed and fasted rats. The value ofT_{max}= 16 min obtained by *in vitro* fasted state (SGF) was similar to the value of 20 minutes obtained for *in vivo* fasted rats and the value of T_{max}(80minutes for *in vitro* simulated fed state (milk)) was identical toT_{max} obtained for *in vivo* fed rats.

Conclusion

Simulation of gastrointestinal conditions for *in vitro* dissolution studies is essential to adequately predict the *in vivo* behavior of PGZ immediate release tablet formulations. To reduce the size and number of human studies required to identify a drug product with appropriate performance in both, the fed and fasted states, it is advantageous to be able to pre-screen formulations *in vitro*. The choice of appropriatebiorelevant dissolution media for such *in vitro* tests is essential in order to correctly forecast the food effect in the pharmacokinetic studies of a drug product. The compendial dissolution test in hydrochloride solution at pH 1.2 was not able to predict the food effect. However, the biorelevant dissolution test in simulated gastric fluids predicted correctly that the release of Pioglitazone would be slower in the fed state than in the fasted state. The results demonstrate good correlation between *in vitro* drug release and *in vivo* drug absorption using the biorelevant dissolution test method.

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