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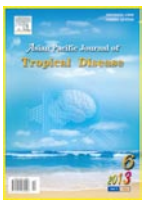

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Comparative *in vitro-in vivo* correlation analysis with pioglitazone tablets

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PEER REVIEW

Peer reviewer

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Comments

This is no doubt a good study in which the authors evaluated the dissolution profile, absorption profile and assess the IVIVC correlation for both test and generic products of PTZ. Aftermath of the study reveals that both formulations are similar and there is a nonlinear IVIVC correlation for PTZ IR tablets.
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ABSTRACT

Objective: To assess the *in vitro-in vivo* correlation of immediate release formulation of pioglitazone 30 mg film coated tablet.
Methods: *In vitro* release data were obtained for test and reference formulation using the USP paddle method (Apparatus 2) at 50 r/min and with the temperature of 37 °C in the dissolution medium of 0.1 mol/L hydrochloric acid of pH 1.2. Twelve healthy volunteers were administered both test and reference pioglitazone 30 mg tablet orally and blood samples were collected over 24 h period. *In vivo* drug concentrations were analyzed by a simple, fast and precise reverse phase binary HPLC method with UV detection to establish a correlation between *in vitro* release and *in vivo* absorption data.
Results: Similarity factor (f_2) and dissimilarity factor (f_1) were determined for the time intervals of 5, 10, 15, 30, 45, 60, 75, 90, 105 and 120 min and the obtained values were 65.17%, 59.37%, 63.62%, 66.61%, 68.89%, 70.73%, 72.27%, 73.59%, 74.65% and 75.67% for f_2 and 9.43%, 9.00%, 5.42%, 3.86%, 3.07%, 2.56%, 2.20%, 1.94%, 1.82% and 1.65% for f_1 at respective time intervals. Mean dissolution time for test and reference products were obtained at 3.06 and 3.40 min respectively. f_2 and f_1 values obtained were within the acceptable range f_2 (50%-100%) and f_1 (<15%).
Conclusions: Comparison of dissolution profiles corroborate that the test and reference formulations are similar and there is no linear *in vitro-in vivo* correlation.

KEYWORDS

In vitro-in vivo correlation, Pioglitazone, Immediate release tablets, Similarity factor (f_2), Dissimilarity factor (f_1)

1. Introduction

In recent years, the concept and application of the *in vitro-in vivo* correlation (IVIVC) for pharmaceutical dosage forms have been a main focus of attention of pharmaceutical industry, academia, and regulatory sectors. Development and optimization of formulation is an integral part of manufacturing and marketing of any therapeutic agent which is indeed a time consuming and costly process. Correlation between *in vitro* and *in vivo* data is often used during pharmaceutical development in order to reduce development time and optimize the formulation. After a proper validation, IVIVC predicts the *in vivo* bioavailability results from *in vitro* dissolution data, and this simulation reflects the *in vivo* behavior of the various formulation[1]. The supposition to assure product quality and performance characteristics of immediate release oral solid dosage formulations for specific post approval changes based on the guidance released by the center for Drug Evaluation and Research (CDER) at the Food and Drug Administration (FDA)[2]. According to Scale-Up and Post Approval Changes (SUPAC) immediate release (IR) guidance, a manufacturer will frequently require to demonstrate that the dissolution profiles of the pre-change product and post change product are similar. In Bangladesh, all local manufacturers are yielding generic products by manufacturing process

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and equipments modification compared to the innovators products after getting approval from drug administration. Hence it is inevitable to have supplements for SUPAC in both local and generic products. For doing so, we made an attempt to find out the similarity of our local product with generic product and to establish IVIVC of our local product. This present communication deals with the IVIVC of pioglitazone (PTZ) 30 mg tablet with its property to release from the dosage form and *in vivo* drug performance. SUPAC IR suggests that dissolution profiles may be compared by determining similarity and dissimilarity factor (f_2 and f_1 metric) that are recently introduced by Moor and Flanner[3]. SUPAC IR also states that an f_2 value between 50%-100% suggests that the two dissolution profiles of local and reference formulations are similar[4-6].

PTZ is a thiazolidinedione (TZD) derivative of novel oral hypoglycemic agent for the management of type 2 diabetes mellitus (T2DM)[7,8]. It is one of the peroxisome proliferator-activated receptor (PPAR-γ) agonists that increases transcription of insulin-responsive genes and thus increases insulin sensitivity. It leads to regulation of carbohydrate and lipid metabolism as well as adipocyte differentiation. PTZ stimulates the uptake of glucose and fatty acids into cells by promoting the synthesis and expression of cellular glucose and fatty acid transporters[9,10]. Many studies of PTZ demonstrated the improvement of glycemic control, Hb1c, fasting glucose levels and significant decrease in triglycerides and an increase in high density lipoprotein (HDL) cholesterol levels, with no overall effect on total cholesterol and low density lipoprotein (LDL) cholesterol[11]. In common with other TZDs, PTZ ameliorates insulin resistance associated with T2DM without stimulating insulin release from pancreatic β cell, thus lowering the risk of hypoglycemia[12]. A single dose of 30 mg of PTZ has no hypoglycemic or hypolipidemic effect or liver toxicity within 24 h of treatment among healthy Bengali males[13].

The structural formulation of PTZ hydrochloride is (±)-5-[p-[2-(5-ethyl-2-pyridyl) ethoxy]-2, 4 thiazolidinedione hydrochloride. The empirical formula is $C_{19}H_{20}N_2O_3S.HCl$. The molecular weight is 392.90. As the case of PTZ, in the fasting state, after oral administration, it was first measurable in serum within 30 min. After absorption from the gastrointestinal tract, peak plasma concentrations were observed within 2 h[14-16]. It was rapidly absorbed within 1 h, achieved peak concentrations at 2-3 h. The absolute bioavailability ranged between 70%-96% with a mean value of 83%. Food slightly delays the time to peak serum concentration 3-4 h, but does not alter the extent of absorption[17,18]. PTZ is highly bound to plasma proteins (>99%) mainly to serum albumin. To a lesser extent, it also binds to other serum proteins[19]. PTZ is metabolized mainly by CYP3A4 and CYP2C8/9[20,21]. PTZ and its metabolites were excreted via urine (15%-30%). The remainders were excreted into bile and feces[9].

2. Materials and methods

2.1. In vitro dissolution test

Six immediate release tablets of each test (Glucozon®, Aristopharma Ltd., Bangladesh) and reference (Actos®, Takeda Pharmaceutical, USA) products of PTZ were taken in the *in vitro* release kinetic study. The dissolution test was carried out according to the procedure described in the USP paddle method (Apparatus 2). The paddle rotation speed was maintained at 50 r/min at 37 °C. The release test was carried out in 900 mL of dissolution medium (0.1 mol/L hydrochloric acid) using a dissolution tester. Samples of 10 mL were withdrawn at time 0, 5, 10, 15, 30, 45, 60, 75, 90, 105, and 120 min and were analyzed by UV spectrophotometer at a detection wave length 269 nm.

2.2. In vivo absorption study

2.2.1 Subjects

Twelve healthy Bangladeshi adult volunteers ranging in age from 21 to 30 years [mean (23.93±2.73) years], in weight from 58 to 76 kg [mean (61.40±7.98) kg] and height from 166 to 185 cm [mean (164.93±4.87) cm] producing a body mass index (BMI) of 22.57±1.47 were enrolled in this study.

2.2.2. Study design

The study was conducted according to the declaration of Helsinki[22]. Each volunteer signed an informed consent document before entering the study. Ethical permission was taken to approve the protocol and consent form of this study from the institutional ethical review committee (Faculty of Pharmacy, University of Dhaka). Twelve healthy male volunteers randomly received each of test and reference products of a single oral dose of PTZ 30 mg tablet following 15 d wash out period in the *in vivo* study. Before enrollment, each subject was determined to good health through routine checkup and laboratory tests of medical history, physical examination, electrocardiogram (ECG). No medications were used for at least two weeks before the study. Tobacco and alcohol were forbidden throughout the study, the physical examination was taken again. Exclusion criteria included any history of a significant gastrointestinal condition that could potentially impair the absorption or disposition of the study medicine, previous history of allergy to any medication, donation of blood within 30 d preceding the first dose of the study or use of investigational agent within 30 d of study entry. They were informed about the risk, benefits, procedures and aims of the study as well as their right as research subjects.

2.2.3. Sample collection

Venous blood samples (6 mL) were collected in heparinized tubes at fasting hour and at 0.5 1.0, 1.5, 2.0, 2.5, 3.0, 5.0, 8.0, 12.0 and 24.0 h after dosing from each subjects. The centrifugations of the collected samples were done at 3000 r/min for 20 min followed by isolation of the plasma. Plasma samples were stored in tubes at -20 °C until analysis.

2.3. Bioanalytical method

A simple, fast and precise reverse phase binary HPLC (Shimadzu prominence module) method was developed for the separation and quantification of PTZ from plasma. This quantification was carried out using Luna C_{18} (250 mm×4.6 mm, 5 μm) column (Phenomenex, Torrence, California, USA) and mobile phase comprised of acetonitrile and ammonium acetate (pH 4.5, 20 mmol/L) in proportion of 60:40 (v/v). The flow rate was 1.0 mL/min and the effluent was monitored at 269 nm. The retention time was (6.1±0.2) min. The method was validated in terms of linearity, precision, accuracy, specificity, limit of detection and limit of quantification. In

the interday and intraday assay, the coefficient of variances (CVs) for PTZ was less than 6.4% for all quality control concentrations.

2.4. Data analysis

The area under the plasma concentration-time curve to 24 h (AUC_{0-24}) was determined by the trapezoidal rule, and the area under the serum concentration-time curve extrapolated to infinity ($AUC_{0-\infty}$) for both test and reference products were calculated according to the following formula:

$$AUC_{0-\infty} = \frac{AUC_{0-t} + C_t}{K_{el}}$$

where C_t is the last quantifiable serum level[23,24].

The percent of drug absorption was calculated by means of model dependent techniques such as Wagner-Nelson procedure. According to Wagner-Nelson equation[25],

$$\frac{A_t}{A_o} = \frac{C_t + K_{el} \times AUC_o^t}{K_{el} \times AUC_0^\infty} \quad \text{(Equation 1)}$$

Here, A_t/A_0 denotes the fraction of drug absorbed at time t, C_t is the plasma drug concentration at time t, K_{el} is elimination rate constant, AUC_0^t and AUC_0^∞ are the area under the plasma concentration-time profile curve at time t and ∞ respectively.

Similarity factor (f_2) and dissimilarity factor (f_1) were also determined by using the equations (Equation 2 and Equation 3) developed by Moore and Flanner[3].

$$f_2 = 50 \log [\{1 + 1/n (R_t - T_t)\ 2\}^{-0.5} \times 100] \quad \text{(Equation 2)}$$

$$f_1 = [\{|R_t - T_t|/R_t\} \times 100] \quad \text{(Equation 3)}$$

Where R_t and T_t are the percent drug dissolved at each time point for the reference and test products, respectively; n is the number of dissolution sample times and t is the time points for collecting dissolution samples.

The mean dissolution time ($MDT_{in\ vitro}$), was also calculated both for local and reference formulations by using equations 4[26,27].

$$MDT_{in\ vitro} = t_{mid}\ \Delta M/\Delta M \quad \text{(Equation 4)}$$

Where t_{mid} is the time at the midpoint between times t_i and t_{i-1}, and ΔM is the amount of drug dissolved between t_i and t_{i-1}.

3. Results

Dissolution pattern for both test and reference tablets is represented in Table 1. From the graphical presentation, it was observed that the dissolution pattern of reference product was almost similar to that of local product (Figure 1).

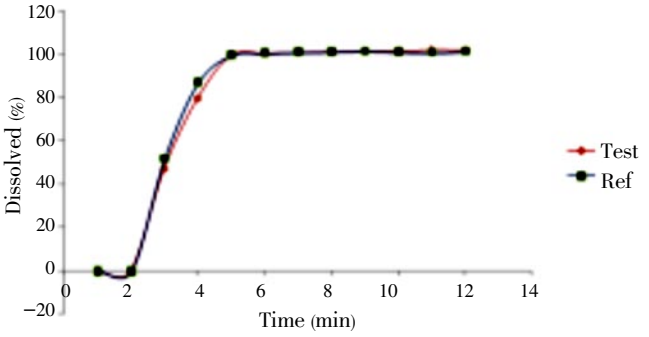

Figure 1. Comparison of mean dissolution rate between test (Glucozon®) and reference (Actos®) products.

f_2 and f_1 for reference and test products are also presented in Table 1. Table 1 shows f_2 for 5, 10, 15, 30, 45, 60, 75, 90, 105 and 120 min, and the obtained values were 65.17%, 59.37%, 63.62%, 66.61%, 68.89%, 70.73%, 72.27%, 73.59%, 74.65% and 75.67% for f_2, and 9.43%, 9.00%, 5.42%, 3.86%, 3.07%, 2.56%, 2.20%, 1.94%, 1.82%, and 1.65% for f_1 at respective time intervals. The maximum and minimum f_2 values were 75.67% and 59.37%, respectively, which were within the acceptable range of 50%-100%. The maximum value for f_1 was 9.43% which was also within the acceptable range (less than 15%). The percent of drug released were calculated from dissolution profiles and percent of drug absorbed were obtained from the Equation 1 and is shown in Table 2 for both reference and test products.

Table 1

Dissolution profile for local and reference product of pioglitazone 30 mg IR tablets.

	Sample	Released (%)									
		5 min	10 min	15 min	30 min	45 min	60 min	75 min	90 min	105 min	120 min
Local (test) Product	Tab1	51.65	78.72	99.20	100.39	100.26	101.23	101.25	102.30	102.50	102.50
	Tab2	46.13	79.23	100.21	102.15	102.25	102.50	102.42	102.30	102.50	102.30
	Tab3	48.56	80.71	100.12	101.60	102.32	102.31	102.15	103.20	103.50	103.40
	Tab4	46.66	80.86	99.23	99.99	100.10	100.20	101.20	101.30	101.50	101.40
	Tab5	45.58	79.86	98.86	99.98	100.28	100.25	101.76	101.56	102.53	102.11
	Tab6	44.23	78.20	99.58	100.23	100.50	100.70	100.62	100.01	100.32	100.48
	Mean±SD	47.13±2.62	79.59±1.07	99.53±0.54	100.72±0.92	100.95±1.04	101.19±1.00	101.56±0.66	101.77±1.09	102.14±1.09	102.03±0.99
Reference Product	Tab1	50.84	84.98	99.89	101.23	102.40	102.89	103.80	103.20	103.40	103.50
	Tab2	52.95	88.51	100.78	101.65	100.90	101.65	101.02	101.30	101.30	101.35
	Tab3	52.36	87.51	99.77	100.14	100.88	100.89	100.96	101.63	99.77	101.84
	Tab4	52.65	88.01	100.83	100.95	101.20	101.35	102.40	101.20	101.30	102.08
	Tab5	52.60	87.92	100.12	101.30	102.23	102.14	102.20	101.10	101.10	101.10
	Tab6	50.87	86.40	98.50	100.10	100.50	100.60	100.60	100.61	100.32	100.69
	Mean±SD	52.04±0.94	87.22±1.307	99.98±0.85	100.89±0.64	101.35±0.78	101.58±0.83	101.830±1.205	101.50±0.89	101.19±1.24	101.76±0.98
Similarity & dissimilarity factor	f_2 (%)	65.17	59.37	63.62	66.61	68.89	70.73	72.27	73.59	74.65	75.67
	f_1 (%)	9.43	9.00	5.42	3.86	3.07	2.56	2.20	1.94	1.82	1.65

SD= Standard Deviation, f_1 = Dissimilarity factor, f_2 = Similarity factor.

Table 2

Mean percentage of drug released and absorbed for test and reference product (%).

Time	Test		Reference	
(min)	Drug released	Drug absorbed	Drug released	Drug absorbed
0	0	0	0	0
30	99.53±0.92	11.42±1.51	99.98±0.85	13.25±1.74
45	100.72±1.04	24.59±2.94	100.89±1.67	27.71±2.99
60	100.95±1.00	32.49±2.98	101.35±1.43	36.56±3.04
75	101.19±0.66	41.11±5.06	101.58±0.96	45.56±5.12
90	101.56±1.09	53.65±4.71	101.83±1.56	60.75±5.71
105	101.77±1.09	64.96±4.96	101.50±1.69	70.97±4.75
120	102.14±0.99	71.64±6.32	101.19±0.67	79.87±6.79

Data are expressed as mean±SE.

Simulation of percent dissolution data with percent absorption data for both test and reference products followed a nonlinear IVIVC correlation analyzed by level A correlation articulated in Figure 2.

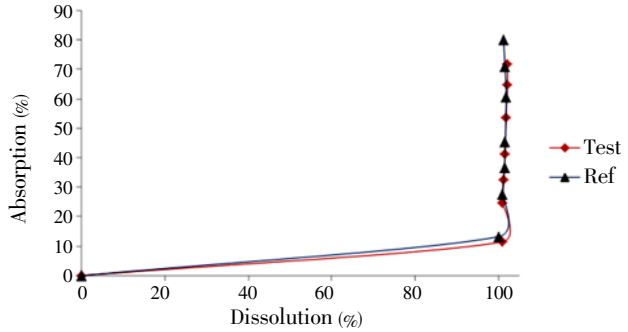

Figure 2. Nonlinear level A correlation (IVIVC) for test (Glucozon®) and reference (Actos®) products.

4. Discussion

An appropriate dissolution conditions could be adapted for routine and in-process quality control of PTZ tablet formulations to ensure *in vivo* performance. It was of interest, therefore, to explore if condition of dissolution of this study, which is very similar to what is proposed by FDA, correlates with serum plasma profiles obtained from bioavailability studies. The dissolution rate for both test and reference products were almost proportionately increased till 30 min. More than 99% dissolution completed as PTZ was IR tablet. The dissolution rate changes were almost stagnant and the dissolution curve followed turning away to the horizontal direction from 30 to 120 min. The simulation of the curve represented that they are similar formulation. On the basis of dissolution efficiencies of the test and reference products, no major differences were found in their dissolution performances. Percent of drug absorption was calculated from the mean serum drug concentrations, using a well-established Wagner-Nelson equation for both reference and local products. The percent of drug release data were plotted against the percent of drug absorption data to press for the IVIVC relationship.

A point-to-point correlation which is the foundation of an acceptable and reliable correlation was achieved but nonlinear. In addition, using the pair-wise procedure based on f_2 which is a model independent approach was followed. Level A correlation represents a point-to-point (1:1) relationship between *in vitro* dissolution rate and *in vivo* input rate of the drug from the dosage form which manifested the similar pattern of nonlinear movement for both reference and test products. Primarily rapid rate of dissolution and steeped-up absorption after 30 min for both reference and test products were the basis of nonlinear relationship. PTZ film coated tablet was an IR formulation. As dissolution is not a rate limiting step and absorption can not keep up with dissolution in IR products, the fraction of drug absorbed against the fraction of drug released profile would be non-linear type which was obtained in our present study.

Aftermath of the present study, it is concluded that test and reference formulations are similar and there is no linear IVIVC correlation between them. Moreover, the findings suggest the bioequivalence which should be carried out as the only reliable and acceptable means to ensure the interchangeability and *in vivo* equivalence of PTZ generic drug products.

Conflict of interest statement

The authors declare that there is no conflict of interest.

Acknowledgements

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Comments

Background

In recent years, the concept and application of the IVIVC for pharmaceutical dosage forms have been a main focus of attention of pharmaceutical industry, academia, and regulatory sectors. Development and optimization of formulation is an integral part of manufacturing and marketing of any therapeutic agent which is indeed a time consuming and costly process. After a proper validation, IVIVC predicts the *in vivo* bioavailability results from *in vitro* dissolution data, and this simulation reflects the *in vivo* behavior of the various formulations. For doing so, this study made an attempt to find out the similarity of the local product with generic product and to establish IVIVC for local product of PTZ.

Research frontiers

Studies are being performed in order to determine the similarities of dissolution profile, *in vivo* drug concentrations at different time interval after administration to 12 healthy volunteers for both test and reference PTZ 30 mg immediate release tablet separately. Eventually simulation of absorption data with dissolution data for both local and generic product is being followed to assess the IVIVC correlation of PTZ.

Related reports

The data regarding peak drug concentration and absorption profile are varied with the Chinese, Japanese, Germany and Tehran population [Souri *et al*. (2008)]. This variation may be due to ethnic and racial variation, the predominant cause of variation of drug response. But the absorption and dissolution profile are similar for both local and reference products indicate that both formulations are similar. There is no linear IVIVC correlation for both test and reference products. These findings are compatible with many other studies carried out in different countries such as India and Pakistan.

Innovations & breakthroughs

Data regarding IVIVC correlation of PTZ is scanty and this sort of study is the first time for PTZ in Bengali population. This findings have uncovered the *in vivo* absorption profile of drug is the key to predict the *in vivo* performance of the drug.

Applications

The findings of the study have significant role in the prediction of *in vivo* drug performance of PTZ. It also suggests that the tested local product is similar to the innovator product in terms of similar pattern of dissolution and absorption behavior. Nonlinear IVIVC correlation may lead to the bioequivalence study to re-ensure the safety profile through evaluation of pharmacokinetic parameters.

Peer review

This is no doubt a good study in which the authors evaluated the dissolution profile, absorption profile and assess the IVIVC correlation for both test and generic products of PTZ. Aftermath of the study reveals that both formulations are similar and there is a nonlinear IVIVC correlation for PTZ IR tablets. Immediate release tablet may lead to this nonlinearity. The results are interesting and suggested that test product may predict the same *in vivo* drug performance to that of reference products.

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