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Bioequivalence Evaluation of a Combine Formulation of Pioglitazone/ Metformin in Healthy Pakistani Volunteers

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Abstract

The purpose of this study was to evaluate bioequivalence of a combine formulation of pioglitazone/metformin 15/850 mg tablet with those of an established branded formulation. An open-label, single-dose, randomized, 2-way crossover study was conducted in fasted healthy Pakistani male volunteers. The concentrations of pioglitazone and metformin in plasma were analyzed by reverse phase High Performance Liquid Chromatography (HPLC). The plasma concentration-time curves were used to obtain pharmacokinetic parameters including AUC_{0-t}, $AUC_{0-\infty}$, and C_{max}. The formulations were considered bioequivalent if the 90% confidence intervals (CIs) for AUC, and C_{max} fell within the interval of 80% to 125%, declared in bioequivalence guidelines. On analysis of variance, no period, formulation or sequence effects were observed for any pharmacokinetic property. The 90% confidence intervals of pioglitazone for the geometric mean ratios of C_{max}, AUC_{0-t} and $AUC_{0-\infty}$ were 93.34% to 103.12%, 86.15% to 106.03% and 85.62% to 107.41%, respectively. Similarly, the 90% CIs of metformin for the geometric mean ratios of C_{max}, AUC_{0-t} and $AUC_{0-\infty}$ were 87.64% to 100.85%, 86.68% to 116.15 and 94.14% to 122.71%, respectively, qualifying the predetermined criteria for bioequivalence based on the rate and extent of absorption.

Keywords: Bioequivalence; pharmacokinetics; Pioglitazone; Metformin; HPLC

Introduction

Numerous oral antidiabetic drugs with different mechanisms of action have been developed to lower glucose and delay the occurrence of serious complications in patients with type 2diabetes [1]. For glycemic control in diabetic patients, monotherapy with an oral antidiabetic agent is not adequate to achieve satisfactory blood glucose control [2]. Thus, combination regimens which include drugs with different and complementary mechanisms of action are recommended for achieving satisfactory blood glucose levels [3-7]. Hypoglycemic agents such as metformin, glipizide, glyburide, repaglinide, rosiglitazone, nateglinide, and pioglitazone are widely prescribed to control blood sugar levels. The majority of combination medicines available to date are based on metformin co-administration.

Therapeutic drug monitoring necessitates the measurement of their plasma concentration for studying the pharmacokinetics of these drugs, assessment of bioequivalence of commercially available tablet formulation and for optimization of dosing in combination therapy [2,8].

The public health care is very expensive in Pakistan due to high cost of prescribed medicine of multinational products. An extensive research to monitor the quality of generic drugs of national pharmaceutical companies may reduce the price significantly. To comply with the regulatory requirements of Pakistan, the relative bioavailability of a pioglitazone/metformin drug product, already marketed in Pakistan, had to be compared with an innovator product. Therefore, the main objective of this study was to assess the bioequivalence of two pioglitazone/metformin 15/850 mg tablets in healthy adult male Pakistani volunteers to achieve the goal of accessibility of medicine to a common man.

Materials and Methods

Chemicals and reagents

Pioglitazone (99.9 %) and metformin (99.4 %) were generously provided by local purveyor Hilton Pharmaceutical (Pvt) Ltd, Karachi.

Blood samples from drug free volunteers were collected, separated plasma and stored at −20°C till further use.

Study design

This was an open-label, single-dose, randomized, 2-way crossover bioequivalence study with 10 days washout period between each administration. Each volunteer received a single dose of either the test or reference formulation of 15/850 mg pioglitazone/metformin, in random order based on computer-generated tables of random numbers.

Calibration standards and quality control

Two samples of (10 mg) pioglitazone and metformin were dissolved in distilled water in two separate 100 ml volumetric flasks to give standard stock solution of 100 µg/ml. Calibration standards were prepared in human plasma by spiking a known amount of pioglitazone and metformin to control plasma (drug-free) samples to produce standards with final concentrations of 0.25, 0.5, 0.6, 0.8, 1.0 and 1.2 µg/ml. Calibration curves were generated by measuring the detector response as peak-area versus concentration of the drug. The accuracy and precision were assessed by measuring the intraday and interday coefficient of variation (CV) at different quality control (QC) concentrations: low, medium and high (0.55, 0.9 and 1.1 µg/ml).

Instrumentation and chromatographic conditions

Pioglitazone and metformin in the plasma samples were analyzed

by HPLC methods with some modifications of already developed methods [9,10]. The analysis was performed on a chromatographic system Shimadzu SP-20, Japan. The analytical columns used to achieve chromatographic separation were Merck C-18, (250 x 4.6) mm; 5 μm, for pioglitazone and MetaSil-Phenyl column (250 x 4.6) mm, 5 μm for metformin. The mobile phase for pioglitazone comprised of acetonitrile: 1.36 % disodium hydrogen phosphate (35:65, v/v), adjusted to pH 7.0 with ortho phosphoric acid. The mobile phase for metformin consist of acetonitrile: 0.02M sodium dihydrogen phosphate (50:50 v/v), adjusted to pH 7.0 with ortho phosphoric acid. Analysis was run at flow-rate of 1 ml/min at 40°C on wavelength of 236 nm.

Sample preparation

For pioglitazone: One ml of plasma and 2 ml of acetonitrile (ACN):trifluoro acetic acid (1%) solution with a ratio of (85:15) was added into a 10 ml glass tube, vortexed for 3 minute and centrifuged (Hettich EBA 21, Germany) at 10,000 rpm for 10 min. Aspirate the upper layer and dry it under water bath. Five hundred (500) μl of mobile phase was added to the tube, vortexed for 3 minutes. The sample filtered by 0.2 micron syringe filter and transferred into an auto-sampler vial (Millipore, MA, USA), and 75 μL was subsequently injected into HPLC system.

For metformin: One ml of plasma sample and 50 μL of 0.05 M Hydrochloric acid (HCl) were added and vortexed for 3 minutes. For protein precipitation 2 ml of ACN was added and the mixture was centrifuged (Hettich EBA 21, Germany) at 10,000 rpm for 10 minutes. The supernatant was dried under nitrogen stream and the extract was reconstituted with 500 μL mobile phase and 75 μL was injected into chromatographic system.

Assay validation

Assay validation was performed according to the FDA guidance for industrial bioanalytical method validation [11]. Linearity was determined using a linear least-squares regression of the six human plasma standards. The sensitivity of the method was expressed as the lower limit of quantification (LLOQ) that could be quantitatively determined with acceptable accuracy and precision. The accuracy and precision were assessed by analyzing six concentrations of QC samples.

Pharmacokinetic and bioequivalence studies in healthy volunteers

A randomized, single-dose, two-period, two-sequence, and crossover design was used for the assessment of the pharmacokinetics and bioequivalence. The study was conducted at the Bioequivalence Study (BeSt) Centre. Drug was randomly given to 24 healthy volunteers under a registered physician. The study was carried out according to principles of good clinical practice (GCP) keeping in view the national legal requirements, the ICH Harmonized Tripartite Guideline for GCP and the ethical principles laid down in the Declaration of Helsinki. Based on this description, they provided written informed consent before participating in the study. The Institutional Review Board of University of Veterinary and Animal Sciences (UVAS) approved the protocol prior to initiate the trial.

Twenty-four volunteers aged between 20–33 years (23.0 ± 3.2 years), with a height between 151–179 cm (170.25 ± 6.98 cm) and with body weights between 50–77 kg (61.50 ±8.04 kg) were assessed as having a healthy status by clinical evaluations. After an overnight fasting of at least 12 hours, subject were randomized to receive test dose

a Tablet Piozer Plus® (Hilton Pharmaceutical (pvt) Ltd., Pakistan) and equivalent oral doses of Competact® (Takeda Institute, Japan) Tablet 15/850 mg with 240 ml of water followed by 60 ml of the glucose solution administered every 15 minutes up to 4 hours after the initial intake dose. Twelve days post administration was considered as a washout period and the subjects were readmitted and received the alternate treatments according to their randomization for treatment sequence. Prior to drugs administration, a control/blank blood sample was collected from each subject. Following drug administration, 8 ml blood samples from each were drawn at 0.25, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 6.0, 8.0, 10, 12, 24, 48 and 72 hours. The blood samples were centrifuged (Hettich EBA 21, Germany) at 10,000 rpm for 10 min at room temperature and plasma was stored at −80°C till further use.

Software

Pharmacokinetic parameters such as Cmax, Tmax, Elim. rate constant, Half_life AUC_{0-t}, $AUC_{0-\infty}$, $AUMC_{0-t}$ and $AUMC_{0-\infty}$, were calculated by EquivTest PK software. After logarithmic transformation of the data, C_{max}, AUC_{0-t} and $AUMC_{0-t}$ values were subjected to ANOVA for a 2 x 2 crossover design to assess effects due to periods, formulation, and sequence using an F test; P <0.05 was considered significant.

Statistical analysis

The 90% confidence interval (CI) and geometric mean ratio between the two products was calculated by using EquiTest PK

Nominal concentration (μg/mL)	Precision (RSD %)*			
	Intraday		Interday	
	P[1]	M[2]	P	M
0.55	1.17	0.91	1.3	1.96
0.9	0.96	0.86	0.93	1.74
1.1	0.68	0.46	0.29	0.4

*RSD = Relative standard deviation, [1]P = Pioglitazone, [2]M = Metformin

Table 1: Intraday and interday Precision for analysis of pioglitazone and metformin in human plasma (n=6).

Amount of Drug added (μg/ml)	Amount of Pioglitazone found (μg/ml)	Accuracy	Amount of Metformin found (μg/ml)	Accuracy
0.55	0.56	102.39	0.58	104.98
	0.56	102.52	0.58	105.09
	0.56	102.30	0.58	105.98
	0.56	102.50	0.58	105.36
	0.56	102.66	0.58	105.57
	0.57	102.77	0.57	104.14
Average		102.52		105.19
0.9	0.88	98.14	0.88	97.40
	0.88	97.81	0.88	98.22
	0.88	97.74	0.88	97.62
	0.89	99.02	0.88	97.57
	0.88	97.92	0.88	97.60
	0.88	97.25	0.87	96.25
Average		97.98		97.45
1.1	1.06	96.10	1.11	100.77
	1.06	96.41	1.10	99.93
	1.06	96.28	1.09	98.95
	1.05	95.72	1.09	99.08
	1.05	95.79	1.08	98.49
	1.06	96.29	1.11	100.56
Average		96.10		99.63

Table 2: Accuracy of pioglitazone and metformin in human plasma (n=6).
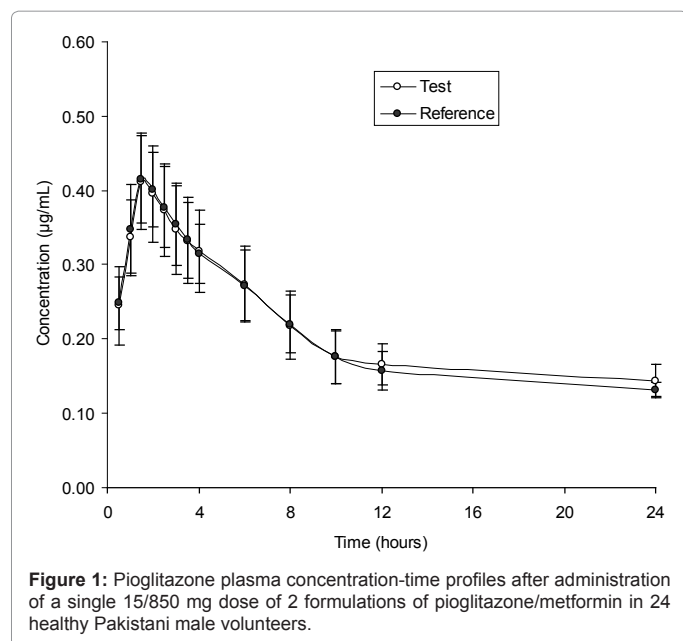
Figure 1: Pioglitazone plasma concentration-time profiles after administration of a single 15/850 mg dose of 2 formulations of pioglitazone/metformin in 24 healthy Pakistani male volunteers.
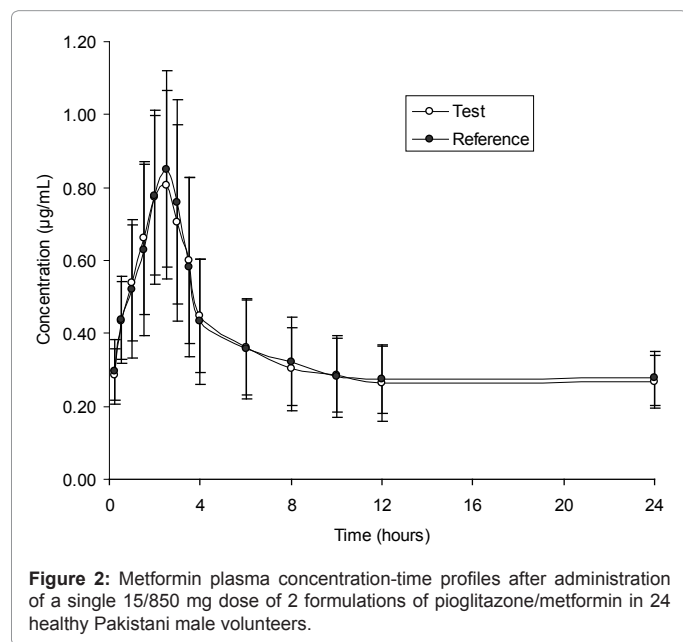
Figure 2: Metformin plasma concentration-time profiles after administration of a single 15/850 mg dose of 2 formulations of pioglitazone/metformin in 24 healthy Pakistani male volunteers.

software. Analysis of variance (ANOVA) was used to check the period, sequence and formulation effect of test and reference.

Results

Separation of drugs

A routine preparation procedure like the extraction, evaporation and reconstitution with the mobile phase were used before reverse phase HPLC separation. No significant endogenous peaks at the retention time of pioglitazone or metformin was recorded using blank sample of human plasma. The retention times of pioglitazone and metformin were 6.4 and 3.8 min, respectively.

Method validation

The results indicated that the validation procedure modified in this

experiment showed good precision and accuracy for both drugs. The standard calibration curves of both drugs showed good linearity within the range of 0.5 to 1.2 µg/ml in human plasma (y = 245886x + 14512, $r^2 \geq 0.9979$) for pioglitazone and (y = 320849x + 21224, $r^2 \geq 0.9971$) for metformin. Intraday and interday precisions and accuracies were determined by analyzing QC samples against a calibration curve, on the same day (n = 6) and on different days (n = 6). This method allowed good precision and accuracy for both drugs and the coefficient of variation values for pioglitazone of both intraday and interday results were below 1.17% and 1.30% (Table 1), respectively and accuracy was 96.10%–102.52% (Table 2). Similarly the coefficient of variation values for metformin of both intraday and interday results were below 0.91% and 1.96% (Table 1), respectively and accuracy was 97.45%–105.19% (Table 2).

Tolerability

Neither major nor minor clinical adverse affects were observed during the entire study period. All participants were available during the study period and three week post study period.

Pharmacokinetic Analysis

The mean plasma concentration–time curves of 2 formulations of pioglitazone is shown in Figure 1. The primary PK parameters for both drugs are listed in Table 3. The mean (SD) C_{max} values of the test and reference formulations of pioglitazone were 0.41 (0.07) and 0.42 (0.06) µg/ml, respectively and the mean (SD) T_{max} values were 0.90 (0.25) and 1.04 (0.41) hours, respectively. Results for the extent of absorption, as determined from mean (SD) AUC_{0-t} and $AUMC_{0-t}$ values, were 3.13 (1.03) and 17.58 (12.15) µg.h/ml respectively, after administration of the test formulation; and 3.28 (1.08) and 19.98 (14.54) µg.h/ml after administration of the reference formulation. The mean (SD) t1/2 was 7.13 (2.00) hours for the test formulation and 8.40 (6.17) hours for the reference formulation. The 90% Cls of the ratios (test vs reference) for the natural log (ln)-transformed AUC_{0-t}, $AUMC_{0-t}$ and C_{max} are shown in Table 3. ANOVA assessment found no significant formulation, sequence, or period effect in the present study. The 90% CIs for the ratios of AUC_{0-t}, $AUMC_{0-t}$ and C_{max} were 86.15% to 106.03%, 89.64-109.17 and 93.34% to 103.12% respectively, correlate the predetermined criteria for bioequivalence. The relative bioavailability of the test formulation was 95.57% (mean AUC_{0-t}), 98.93% (mean $AUMC_{0-t}$) and 98.11% (mean C_{max}), respectively (Table 4).

The mean metformin plasma concentration time profiles after administration of the 2 formulations is shown in figure 2. Similarly, the mean (SD) C_{max} value for metformin with the test formulation was 0.85 (0.28) µg.h/ml, and the T_{max} was 2.27 (0.49) hours. With the reference formulation, the corresponding values were 0.90 (0.28) µg.h/ml and 2.33 (0.38) hours. The t1/2 values with the test and reference formulations were 57.78 (47.04) and 48.74 (36.17) hours, respectively (Table 3). No period or sequence effects were observed for the PK properties in the ANOVA. Statistical analysis showed that the 90% CIs for the ratio of C_{max} (87.64%–100.85%), AUC_{0-t} (86.68%–116.15%) and $AUMC_{0-t}$ (92.07%–108.88%) values for the test and reference products fell within the established regulatory interval of 80% to 125% (Table 4). The mean relative bioavailabilities (test/reference) for C_{max}, AUC_{0-t} and $AUMC_{0-t}$ were 94.02%, 100.34% and 100.12% respectively.

Discussion

Evaluation of bioequivalence of generic product to innovator product is necessary to exclude any clinically important differences in the rate or extent at which the active entity of the drugs become

Parameters	Test		Reference		P value	
	P	M	P	M	P	M
AUC (0-t) ((µg h/mL)) AUC (0-∞) ((µg h/mL))	3.13 ± 1.03 4.70 ± 1.40	7.26 ± 3.72 28.50 ± 19.76	3.28 ± 1.08 5.02 ± 1.99	7.33 ± 4.04 26.40 ± 19.55	0.23 0.20	0.45 0.15
AUMC (0-t) ((µg h/mL))	17.58 ± 12.15	72.49 ± 68.89	19.98 ± 14.54	73.93 ± 72.51	0.20	0.46
Cmax (µg/mL)	0.41 ± 0.07	0.85 ± 0.28	0.42 ± 0.06	0.90 ± 0.28	0.26	0.05
Tmax (h)	0.90 ± 0.25	2.27 ± 0.49	1.04 ± 0.41	2.33 ± 0.38	0.08	0.31
t1/2 (h)	7.13 ± 2.00	57.78 ± 47.04	8.40 ± 6.17	48.74 ± 36.17	0.16	0.11
MRT (h)	10.94 ± 2.79	80.07 ± 65.43	12.54 ± 7.89	67.35 ± 50.59	0.16	0.11

P = Pioglitazone, M = Metformin, AUC = Area under curve, AUMC = Area under moment curve, Cmax = maximal plasma concentration, Tmax = time for the maximal plasma concentration, $t_{1/2}$ = Elimination half life, MRT = Mean residence time

Table 3: Pharmacokinetic parameters (mean ± S.D. of n = 24) of pioglitazone and Metformin after 15/850 mg pioglitazone/metformin tablets based on its plasma concentrations.

Parameter	Pioglitazone		Metformin	
	Ratio	90% CI	Ratio	90% CI
Ln AUC0-t	95.57	86.15-106.03	100.34	86.68-116.15
Ln AUC0-∞	95.90	85.62-107.41	110.07	94.14-122.71
Ln AUMC0-t	98.93	89.64-109.17	100.12	92.07-108.88
Ln Cmax	98.11	93.34-103.12	94.02	87.64-100.85

Table 4: Comparison of 90% CIs of natural log (ln)-transformed parameters for two formulations (test/reference) of pioglitazone/metformin in healthy Pakistani male volunteers.

available at the site of action. Two formulations are considered to be bioequivalent if they are pharmaceutically equivalent and their bioavailability is so similar that they are unlikely to produce clinically relevant differences regarding the safety and efficacy of drug [12]. The purpose of the present study was to compare the bioavailability of 2 formulations of pioglitazone/metformin 15/850 mg tablets, a locally manufactured (test) formulation, Piozer Plus (Hilton Pharm., Karachi), and a reference formulation, Competact (Takeda Inst., Japan).

All pharmacokinetic parameters of pioglitazone derived using the non-compartmental method was found to be similar for both formulations. The test and reference formulations exhibited overlapping plasma profiles indicated that the 2 formulations were similar in both absorption as well as in elimination, (Figure 2). The AUC_{0-t}, $AUMC_{0-t}$ and C_{max} values acquired with the generic and innovator formulations were not significantly different, which reflects the similar PK characteristics of the 2 formulations. However, in this study, the mean AUC_{0-t} value of both test and reference drugs were lower than those found in a previous studies [13-15], following administration of a 30mg pioglitazone tablet and comparable to a study [16], following administration of 15 mg pioglitazone. Moreover, in the present study the C_{max} and T_{max} values of both test and reference formulations were comparable to previous studies [13-15], though their dosage vary as 30 mg pioglitazone tablet and 15 mg pioglitazone tablet [16]. The 90% CIs for the natural log-transformed AUC_{0-t}, $AUMC_{0-t}$ and C_{max} values were well within the bioequivalence range of 0.8 to 1.25, therefore the 2 formulations can be considered bioequivalent according to the bioequivalence guidence with respect to the extent of absorption [12].

The mean plasma concentration–time curves of 2 formulations of metformin after a single oral dose of 15/850 mg of either test or reference formulation of pioglitazone/metformin was recorded almost same during the entire period (Figure 2). Both formulations were apparently readily absorbed, and metformin was measurable at 0.25 hour. The mean AUC_{0-t}, $AUMC_{0-t}$, C_{max} and T_{max} values with the test formulation were numerically lower but not statistically significant from those with the reference formulation, agreed with the previous studies [17-22]. However, the mean half life value with the test formulation was numerically higher but not statistically significant from that with the reference formulation. Pharmacokinetic parameters

when analyzed using ANOVA, statistically non significant differences were observed between the 2 formulations in the logarithmically transformed AUC_{0-t} or C_{max}, also ANOVA found no period or sequence effects for any pharmacokinetic property. The absence of a sequence effect in both parameters suggests that there was no carryover effect. However, further research work is necessary to carry out such studies in female volunteers as well as in patients to make a better conclusion.

Conclusions

It is concluded that both the test and reference formulations (containing two drugs pioglitazone and metformin 15/850 mg tablets) met the acceptance criteria for bioequivalence based on the rate and extent of absorption. Both formulations were well tolerated by young healthy volunteers.

Acknowledgments

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