

Bioequivalence and Pharmacokinetics of Metformin Hydrochloride 1000 mg Tablet Extended Formulation in Healthy Indian Male Volunteers

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Received on 10th January 2014, Accepted on 26th April 2014, Available online from 02nd June 2014

Abstract

This study was to compare the bioavailability and pharmacokinetic properties of test product of Metformin hydrochloride extended release formulation of 1000 mg tablet with reference product of Metformin Hydrochloride (Glucophage®) extended release formulation of 1000 mg in Indian healthy male volunteers. Study design is an open-label, randomized, 2-treatment, single-dose, crossover, bioavailability study to compare test product with reference product in 24 healthy human male volunteers under fed condition. A single oral dose of 1000 mg Metformin (XR) extended release test product, with reference product Metformin Hydrochloride (Glycophage®) extended release was administered as per computer generated randomization schedule during 2 period of the study having 7 days of washout period. A liquid Chromatography mass spectroscopy method was developed and validated as per FDA guideline requirements using Atenolol as an internal standard for the determination of Metformin in human plasma. A non-compartmental pharmacokinetic method was employed to determine the pharmacokinetic parameters (C_{max} , T_{max} , AUC_{0-t} , $AUC_{0-\infty}$ and $t_{1/2}$) of Metformin using Kinetica (version 4.4.1) software. C_{max} , AUC_{0-t} and $AUC_{0-\infty}$ were used to test for bioequivalence after log transformation of Metformin plasma concentration data. The required Bioequivalence acceptance criteria as per regulatory is 90% CI for bioequivalence study and the range is 0.80 to 1.25. The 90% confidence intervals for log transformed data for C_{max} , AUC_{0-t} and $AUC_{0-\infty}$ for test A vs. reference were 83.18-95.64, 88.06-104.99 and 94.76-121.59 respectively.

Keywords: Metformin, Bioequivalence, Pharmacokinetics, LC-MS/MS

INTRODUCTION

Metformin is to improve glycemic control in patients with type 2 diabetes: Metformin is a member of the biguanide class [1]. It improves glucose tolerance in patients with type 2 diabetes, reducing both basal and postprandial plasma glucose levels. Metformin also decreases hepatic glucose production, decreases intestinal absorption of glucose and improves sensitivity by increasing peripheral glucose uptake and utilization. Adults having diabetes type 2 are recommended to take 500 to 2000 mg orally once a day (with the evening

meal). Maximum daily dose is 2500 mg. Safety and effectiveness of Metformin extended-release has not been established in paediatric patients (less than 18 years of age) [2]. Metformin can significantly improve insulin sensitivity in patients that suffer from Diabetes type II (non-insulin dependent). Typically Metformin reduces basal and postprandial hyperglycemia by about 25% in more than 90% of the patients. Metformin is rapidly distributed after absorption, and it is accumulated in the esophagus, stomach, duodenum, salivary glands, and kidneys [3]. It has neither binding to

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plasma proteins nor metabolism, and it undergoes renal excretion. Metformin is a first-choice drug for type 2 DM treatment because of its broad therapeutic advantages. The aim of this study is to evaluate, the bioequivalence of the generic Metformin, with the reference product Glucophage® in healthy male volunteers.

SUBJECTS AND METHODS

Study subjects

The study was conducted according to the Good Clinical Practice and Helsinki Declaration. The protocol, informed consent and case report forms were approved by Ethics committee. 28 subjects were recruited for this study, the 28 subjects admitted had an age (mean \pm SD) of 30.67 ± 8.35 years, BMI range of 22.32 ± 2.43 Kg/m², height of 1.68 ± 0.07 m and weight of 63.03 ± 7.19 kg). Subjects were assessed healthy volunteers, after having been medically examined and clinically tested: complete blood count, urine analysis, and blood biochemistry were normal, and HIV, hepatitis B, were negative. All subjects were briefed on the bioequivalence study details and they all agreed and signed a written informed consent during informed consent process. All volunteers were free to leave the study at any time. Sample size is based on estimates obtained from reported literature. Assuming a formulation ratio (T/R) ranging from 0.95-1.05 and considering intra-subject variability and dropouts a sample size of 28 subjects would be sufficient to show bioequivalence with a power of at least 80%. Hence a sample size of 28 healthy male subjects was included in the study.

Clinical trial design

This study was a randomized, open label, balanced, two-treatment, two-period, two-sequence, single

dose, two-way crossover bioequivalence study under fed conditions with at least 07 days washout period between two consecutive dosing periods. During each period, each subject received Metlong DS 1000 mg tablets (Metformin Hydrochloride) of Panacea Biotec Ltd., India or Glucophage® XR (Metformin Hydrochloride extended release tablets) 1000mg tablets of Bristol-Myers Squibb Company, USA after at least 10 hours fasting period, a high-calorie, high-fat breakfast provided 30 minutes before dosing. Water was not allowed from 1 hour prior to dosing until 2 hours post dosing with the exception of 240 ml of water served during dosing. Blood samples were collected at 0.00, 0.50, 1.00, 1.50, 2.00, 2.50, 2.75, 3.00, 3.25, 3.50, 4.00, 5.00, 6.00, 8.00, 10.00, 12.00, 18.00, 24.00, 36.00 and 48.00 after dosing in 6 ml Serum Vacutainer. The subjects were housed for a period of 12 hours before and 48 hours after the drug administration. Seated blood pressure, pulse rate and oral temperature were measured after check-in, prior to dosing and at 2, 4, 6 and 12 hours (\pm 30 minutes) after drug administration and at check-out. Blood sugar estimation were done at 6 and 12 hours after drug administration. An additional 10 ml blood for screening, 6 ml of blood was withdrawn at the end of Period II for testing blood sugar, serum bilirubin, serum creatinine, blood urea, total white blood cell count, platelet count, hemoglobin, SGOT and SGPT for post study evaluation.

Standard breakfast, lunch, snacks, and dinner were given at 0, 4, 9, and 13 hours after dosing. All meals were the same for both periods. Food with xanthines (such as chocolate), and carbonated drinks were not allowed.

Procedure followed for handling and transfer/shipping of blood samples

A 5ml volume of blood was collected in a pre-labelled Vacutainer tube with Di-Potassium (K₂ EDTA) anticoagulant. The blood tubes were kept in an ice-water bath until the plasma was harvested. The tubes were placed in the centrifuge immediately after blood collection. The plasma was harvested under standard conditions that maximized the yield of plasma and kept it free of extraneous blood components i.e. centrifugation was done at 4000 rpm at 4°C for 10 min. The harvested plasma was transferred into polypropylene tubes. Pipetting of the fuzzy coat formed at the interface was avoided. The plasma tubes were flash-frozen immediately after the plasma was harvested and transferred the tubes to a freezer at -80°C.

While transferring the samples, the samples were packed with sufficient dry ice and it was ensured that the samples remained frozen for more than 5 hours. The samples were collected at times specified under study design and centrifuged under refrigeration. The separated plasma samples were stored in suitably labelled RIA vials at -80°C.

Drug products

Test product (T): Metformin Hydrochloride 1000 mg tablets, manufactured by Panacea Biotec Ltd., India, and reference product (R): Glucophage[®] XR (Metformin Hydrochloride extended release tablets) 1000 mg tablets, manufactured by Bristol – Myers Squibb, USA were used in the current study.

Analytical procedure and method validation

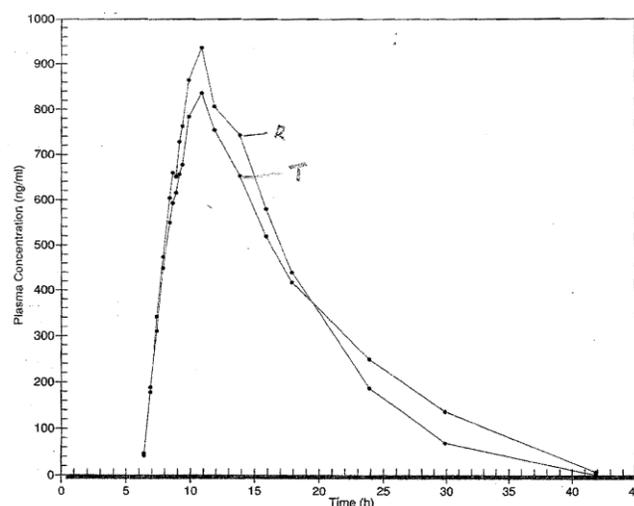
A high performance liquid chromatography Mass spectrophotometry method for the determination of Metformin in human plasma has been validated using Atenolol as the internal standard. Sample preparation consisted of 0.4ml sample in to a clean

RIA vial and 50µl of internal standard (20µl/ml) was added. 1.0 ml Methanol was added and vortexed well. Samples were vibrated at 2500rpm for 10 minutes. The samples were centrifuged at 4500rpm for 10 minutes at 4°C. 1.0mL supernatant was collected and 0.9ml of methanol was added. 5µl of the sample was injected into LC-MS/MS. The mobile phase consisted of acetonitrile: 5mM Ammonium Acetate (80:20 %v/v). Detection of Metformin and the internal standard was achieved by using a TSQ Quantum Ultra LC-MS/MS interfaced with Surveyor LC pump and Surveyor Auto ampler [4-6].

The relationship between concentration and peak area ratio was found to be linear within the range of 46.0939 to 2008.4485ng/ml for Metformin. The limit of quantification was 46.0939ng/ml. The inter-day precision and accuracy of the determination of Metformin were determined before the start of the study sample analysis during method validation.

The used Analytical column is a Varian C18, 5µm (50 × 4.6mm). The mobile phase consisted of Acetonitrile: 5mM Ammonium Acetate 80:20 %v/v). The flow rate was 0.500 ml/min. The method was validated following criteria established by FDA Guidelines [7]. Other validation parameters were also fulfilled by this method. The Intra-day precision was determined by the repeated analysis of plasma samples containing different concentrations Metformin on separate occasions. A single run consists of a calibration curve plus 6 replicates of the LOQC, LQC, MQC and HQC samples. The intraday precision variation ranged between 4.62 to 11.30%.

The inter day precision was determined by the repeated analysis of plasma samples containing different concentrations Metformin on separate occasions. A single run consists of a calibration curve plus 6 replicates of the LOQC, LQC, MQC and

Figure 1. Mean concentration profile of Metformin

All parameters had normality for intra-subject and inter-subject residues. The pharmacokinetic parameters for both formulations are shown in (Table 1).

The mean values and 90% CI for the pharmacokinetic parameters compared are summarized in (Table 2). ANOVA for $AUC_{0-\infty}$ and C_{max} , after logarithmic transformation of the data, revealed that none of the effects examined (formulation, period, within and between-subject variances and carry over) was statistically significant. The 90% CI was within the bioequivalence acceptable range from 80% to 125%, between both formulations proposed that both drug formulations are comparable.

Table 1: Pharmacokinetic Parameters after administration of 1000 mg of test and reference formulation of Metformin in healthy Indian volunteers

Pharmacokinetic Parameters	Mean Values \pm SD (N=24)	
	Reference-R	Test-T
C_{max} (ng/ml)	969.38 \pm 261.37	878.74 \pm 284.61
AUC_{0-t} (hr*ng/mL)	9822.78 \pm 3207.29	9886.32 \pm 4043.78
AUC_{0-inf} (hr*ng/mL)	10602.23 \pm 3408.18	12457.3 \pm 6953.75
T_{max} (h) [†]	5 \pm 1.10	5 \pm 0.94
$T_{1/2}$ (h) [†]	7.37 \pm 1.11	4.74 \pm 9.72
K_{el} (h ⁻¹)	0.15 \pm 0.03	0.12 \pm 0.08

HQC samples for Metformin. The inter day precision variation ranged between 0.93 to 12.26%.

Two concentrations of Metformin quality control (139.046 and 1853.953 ng/ml) (QC) samples in six replicates were used for stability studies, including freeze and thaw, short-term temperature and long-term stability; and the obtained results are as per the acceptance criteria for validation parameters [7] established by FDA guidelines. Standard curves were performed for each analytical batch over a 9 days period, with each volunteer's plasma samples and showed consistent linearity (intercept, slope, and correlation coefficient).

Statistical analysis

In order to assess the effects of treatment, period, sequence of administration, and subjects, in-transformed data for $AUC_{0-\infty}$ and C_{max} , and non-transformed T_{max} were evaluated by means of analysis of variance (ANOVA) (Kinetica[®] Version 4.4.1).

The method suggested by Schuirmann and accepted by the FDA [8] (known as the two one-sided tests) was used to evaluate whether these two formulations of Metformin were bioequivalent. Bioequivalence is accepted if 90% confidence intervals for test/reference ratios of AUCs and C_{max} fell in the range of 0.80-1.25. A p value of less than 0.05 was considered statistically significant [9].

RESULTS

Twenty eight (28) subjects concluded the study without any adverse effects. Pharmacokinetic parameters were calculated for all 24 subjects who were completed both the periods. Blood samples were taken until 48 hours after drug administration; the mean concentration profiles for the 2 formulations were quite similar, as shown in (Figure 1).

Pharmacokinetic Analysis

Pharmacokinetic parameters were calculated by using Kinetica[®] (Version 4.4.1). The non-compartmental analyses of pharmacokinetic parameters and statistical analysis (ANOVA) were done by Kinetica[®] (Version 4.4.1).

The mean, SD, CV (%) and range were calculated for the AUC_{0-t} (ng.h/mL), AUC_{0-inf} (ng.h/mL), C_{max} (ng/mL), T_{max} (h), T1/2 (h), K_{el} (h⁻¹). Individual analysis of variance was performed on the Ln-transformed data of AUC_{0-t}, AUC_{0-inf} and C_{max}. ANOVA was performed. For all analysis, effects were considered statistically significant if the probability associated with 'F' was less than 0.050. AUC_{0-t}, AUC_{0-inf} and C_{max} data, calculated as well as the 90% confidence intervals (based on least-squares means) of geometric means were determined.

Table 2: 90% CI for Ln-transformed parameters (C_{max} and AUC_{0-inf}) of two metformin tablet formulations after a single dose administration to healthy Indian volunteers

Parameter	90%CI (confidence interval) values
C _{max}	83.18-95.64
AUC _{0-t}	88.06-104.99
AUC _{0-inf}	94.76-121.5884

ANOVA was carried out for 24 subjects (C_{max}, AUC_{0-t}, and AUC_{0-inf}). In this study we compared the rate and extent of absorption of Metlong DS (Metformin HCl) 1000 mg tablet (Test) versus Glucophage[®] (Metformin HCl) 1000 mg tablet (Reference) in healthy human subjects. Twenty eight subjects were enrolled into the study and all the 28 subjects were completed the crossover. But the plasma samples of 24 analysed evaluable subjects Metformin concentration data (time vs. concentration) was used for pharmacokinetic analysis. And the same 24 subject's data was used for statistical analysis. ANOVA was carried out for 24 subjects for C_{max}, AUC_{0-t} and AUC_{0-inf}.

The mean C_{max}, AUC_{0-t}, AUC_{0-inf}, T_{max}, T1/2 and K_{el} for the test Metformin hydrochloride 1000 mg formulation (Metlong DS) (Test-T) and reference Metformin hydrochloride 1000mg Tablets formulation (Glucophage[®] XR) (Reference-R) are given in the (Table 1). The actual 90%CI (confidence interval) values of the relative geometric mean for pharmacokinetic parameters (C_{max}, AUC_{0-t} and AUC_{0-inf}) of test to reference formulations are shown in the (Table 2). Based on the statistical results, 90% confidence intervals of the relative geometric mean for C_{max}, AUC_{0-t} and AUC_{0-inf} of test to reference formulation are found to be within the specified range of 80.00 – 125.00% and have shown bioequivalence under fed conditions.

DISCUSSION AND CONCLUSION

In the present study was designed to measure the relevant aspects of the Metformin pharmacokinetics that might use to establish the degree of similarity between the two (test and reference) Metformin formulations indicated above. Plasma concentration- time curves of Metformin Test and reference shown similar graphs overlapped, indicating that Test formulation is similar to the reference formulation. Pharmacokinetic results obtained in the current study shown that conclusive data with respect to therapeutic equivalence since the comparison between the test formulation (Metformin-Hydrochloride) and the reference formulation (Metformin Hydrochloride Glucophage) for C_{max} and AUC 42h showed percentages that fall in the rank of equivalence.

In this study we have compared the test product Metformin hydrochloride 1000 mg tablets to the reference product of Glucophage[®] XR (Metformin Hydrochloride 1000 mg tablets in healthy male volunteers under fed conditions and the result of obtained pharmacokinetic data proved that both products were comparable to each other and falls within the acceptance range of bioequivalence as per the regulatory requirement, the same method

can be used for Metformin clinical trial to quantify the Metformin in human plasma.

Acknowledgement

The authors acknowledged their due thanks to the Management of Quest Life Sciences Pvt Ltd for their support to publish this research work.

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