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Research Article

Bioavailability and pharmacokinetic study of Metformin 500 mg tablet (SR formulations) in healthy human volunteers

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ABSTRACT

Bioavailability and Bioequivalence studies have become an important part of the clinical research in India. This study was performed to find out the safety and efficacy of two Metformin 500 mg tablet (SR formulations). This study was an open label, balanced, randomized, two treatment, two periods, two sequences, single dose, cross over bioequivalence study under fasting condition. Metformin Hydrochloride (SR) 500 mg tablet was the Test formulation and D-beta SR tablet [containing Metformin Hydrochloride (SR) 500 mg] was the Reference standard. Volunteers were randomly given a single oral dose of the test and the reference formulation under fasting condition, with a washout period of 07 days. Drug concentration in the plasma samples were quantified by using a validated method on LC/MS/MS. Win Nonlin Version 5.2 software was used for statistical calculations. C_{max}, AUC_{0-t} and AUC_{0-∞} values of the test formulation and reference standard were 89.13%, 87.46%, 88.29%, and 112.44%, 123.85% and 123.87%, respectively. C_{max}, AUC_{0-t} and AUC_{0-∞} values of the test formulation and reference standard fall within the acceptable range of 80–125%. So, the present study concludes that the test formulation is bioequivalent to the reference standard.

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INTRODUCTION

Bioavailability era was started as early as 1945, with the first publication of the concept of biological availability. The development of analytical techniques, during the 1960's, made possible the development of methods sensitive enough to allow quantification of drugs or metabolites, initially in the urine and afterwards in the plasma, making possible the evaluation and the comparison of bioavailability of different formulations, as well as demonstration that significant differences among them could occur.

A generic drug will be said to be bioequivalent to the listed drug, if "the rate and extent of the absorption of the drug do not show a significant difference from the rate and extent of absorption of the listed drug when administered at the same molar dose of the therapeutic ingredient under similar experimental conditions in either a single dose or multiple doses".^[1]

Metformin is a biguanide hypoglycemic agent, used in the treatment of type 2 diabetes mellitus patients, who are not responding to dietary changes and exercise. Metformin diminishes hepatic glucose generation (both gluconeogenesis and glycogenolysis), diminishes intestinal absorption of glucose, and improves insulin sensitivity of hepatic and peripheral (muscle) tissues

causing an expanded uptake of glucose into these tissues.^[2,3]

Metformin is the only biguanide available in the market. Metformin has been utilized clinically for a long time, and has been approved following 1995. Metformin has become the first-line therapy for glycemic control when oral agents are indicated in overweight patients. Metformin is beneficial for those conditions, when function of β -cell gets declined; since it does not stimulate insulin secretion from pancreas. As compared to insulin secretagogues (sulfonylureas in particular), Metformin generally not causes hypoglycemia and weight gain. The duration of action of Metformin is short and it has a half-life of 1.3 - 4.5 hours, and it does not bind to plasma proteins. It is not metabolised and is totally renally eliminated.^[4,5]

If there are no contraindications, Metformin can be used in conjunction with diet as first-line therapy in patients not adequately controlled on diet alone. As it has a different mode of action to the sulfonylureas, meglitinide analogues, thiazolidinediones and α -glucosidase inhibitors, it can be valuable when prescribed in combination.^[6]

After the patent expires, several companies may manufacture and market different formulations of a same active substance, with similar qualities and performances, so as the interchangeability among different formulations, when given in equivalent doses, presents the same safety and efficacy.

That's why several pharma companies tried to bring new generic versions of Metformin Hydrochloride (SR) 500 mg tablet into the market. As per the regulation, bioequivalence study should be done before marketing. So, a bioequivalence study was conducted, comparing the Test formulation Metformin Hydrochloride (SR) 500 mg tablet with the Reference standard Dibeta SR tablet [containing Metformin Hydrochloride (SR) 500 mg].

This was a DCGI submission study, so the CDSCO BA/BE Guidelines, 2005 were followed in conjunction with ICMR Guidelines. ICH-GCP Guidelines and Declaration of Helsinki were also referred as and when required. [7,8,9,10]

The objective of the present study was to assess the bioequivalence of the Test formulation Metformin Hydrochloride (SR) 500 mg tablet with the Reference standard Dibeta SR tablet [containing Metformin Hydrochloride (SR) 500 mg] in healthy human volunteers under fasting condition.

MATERIALS AND METHODS

Study Drug:

Metformin Hydrochloride (SR) 500 mg tablet (Test Sample) and Dibeta SR tablet (Reference standard, Torrent Pharmaceuticals Pvt. Ltd., India) were purchased from the local market. Subjects were receiving each treatment according to the randomization schedule. Dosing was done after an overnight fast of not less than 10 hrs and fasting was continued until 4 hrs post dose. Lunch, snacks and dinner were given at 4, 9 & 13 hrs post dose respectively. Administration of the study drug was performed between 09:00-09:10 hrs, on the first day of the study in both the period.

Study Design:

An open label, balanced, randomized, two treatment, two period, two sequence, cross over bioequivalence study of single dose of Metformin Hydrochloride (SR) 500 mg tablet and Dibeta SR tablet [Containing Metformin Hydrochloride (SR) 500 mg] in healthy human volunteers under fasting condition.

After a supervised overnight fast, volunteers were given the assigned formulation (a single oral dose), with 240 ml of 20% solution of glucose, according to the randomization schedule generated and authorized by Biostatistician. To prepare 20% of glucose, 48 gram of commercially available glucose powder was dissolved in 240 mL of water.

This study had got the approval of Hippocrates Independent Ethics Committee (HIEC), New Delhi; to conduct this study and allowed to enroll twelve subjects for this particular study, based on ethical reason.

Volunteers who fulfilled all the study requirements including inclusion and exclusion criteria were allowed to participate in the study.

A balanced block randomization schedule was generated before the start of the study utilizing WinNonlin Version 5.2 by the biostatistician. Subjects received the assigned formulation in each period, according to a randomization schedule with the following possible sequences, TR or RT [where T = Test Product and R = Reference Standard]. During this study, a sum total of 28 blood samples were collected. The pre-dose blood samples (2 x 3 ml) were collected within a period of 1.5 hour before dosing and then blood samples were collected at (1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0, 10.0, 12.0, 18.0, 24.0) hours post-dosing in K₂EDTA vacutainer. Method of collection of pre-dose and post-dose blood samples up to 12 hours, was either indwelling intravenous cannula or scalp vein set put in a forearm vein of the subjects. After 12 hours post-dose samples were taken directly by venipuncture. Sampling till 24 hours was taken into account based on the terminal half life of Metformin which is 6.2 hours. The blood samples were transferred into K₂EDTA sample collection tube.

All the blood samples were centrifuged under refrigeration immediately after its collection to separate plasma at 2 to 10°C, 4000 ± 100 rpm for 10 minutes. The separated plasma were stored in a freezer at a temperature of – (80 ± 10)°C, till the bioanalytical process was over.

Standardized meals prepared by dietician were served to subjects. An overnight fasting of at least 10 hrs was ensured prior to dosing and subjects were asked to continue the fasting till 4 hrs after dosing. After dosing, lunch, snacks and dinner were served at 4 hrs, 9 hrs & 13 hrs respectively. Water was restricted from 1 hr Predose to 2 hrs post dose. Subsequently water was provided ad libitum. 60 mL of 20% glucose solution was provided at the interval of 15 minutes till 4 hrs of post dose, and whenever required after blood glucose monitoring or safety reason.

Pharmacokinetic Parameters & Analysis:

Pharmacokinetic parameters for Metformin were calculated utilizing non-compartmental model of WinNonlin Version 5.2. Primary Parameters were C_{max}, AUC_{0-t}, AUC_{0-∞}; and Secondary Parameters were T_{max}, K_{el}, t_{1/2}, AUC_{0-t} / AUC_{0-∞}. Statistical analyses were performed on Metformin using the WinNonlin Version 5.2.

C_{max}, AUC_{0-t} and AUC_{0-∞} values (90% CI for In-transformed data) of the test product and the reference

standard of the study drug metformin, were considered for evaluating bioequivalence.

RESULTS AND DISCUSSION

Demographic Details:

Age of the enrolled twelve subjects was between 18 and 45 years. Weight of the enrolled twelve subjects was within $\pm 15\%$ of the ideal Height-Weight chart of Life Insurance Corporation (LIC) of India.

Clinical Laboratory Evaluation:

Screening examination was conducted within 21 days prior to study. During screening, medical history of each

volunteer was taken. Laboratory tests (including Hematology, Biochemistry Serology and Urine examination) were also performed for each volunteer. After passing the screening examination, healthy volunteers were enrolled in the study.

Dropout and Withdrawn Subjects:

All the enrolled subjects successfully completed this study, and no dropout/withdrawal was reported.

Graphical Representation:

Mean plasma concentration versus time curve of test product (T) and reference standard (R) has been illustrated graphically in figure 1.

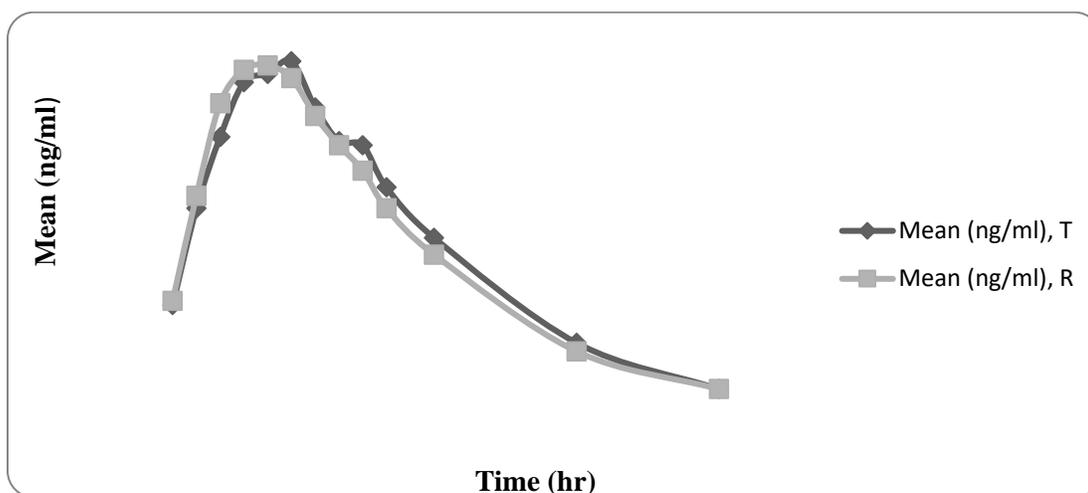


Figure 1: Mean plasma concentration versus time curve of Test product (T) and Reference standard (R)

Estimation of Pharmacokinetic Parameters:

C_{max} and t_{max} were used to find out the rate of absorption of test product and reference standard. $AUC_{0-t} / AUC_{0-\infty}$ (%) was used to find out the adequacy of the sampling time. AUC_{0-t} and $AUC_{0-\infty}$ were used to find out the extent of absorption of test product and reference standard.

AUC_{0-t} , $AUC_{0-\infty}$ and C_{max} values of test product and reference standard were used for bioequivalence estimations.

Mean plasma concentration vs. time curve of Test product (T) and Reference standard (R) were used to find out the pharmacokinetic parameters like C_{max} , t_{max} , $t_{1/2}$, AUC_{0-t} , $AUC_{0-\infty}$ and K_{el} ; using WinNonlin Version 5.2. These data were statistically analyzed and the bioequivalence of test product and reference standard was proved.

Primary and secondary pharmacokinetic parameters of test product (T) and reference standard (R) are depicted in table 1 and table 2, respectively.

Table 1: Primary pharmacokinetic parameters of Test product (T) and Reference standard (R)

Parameters	Test (Mean \pm SD)	Reference (Mean \pm SD)
AUC_{0-t} (ng hr/ml)	5924.45 \pm 1290.10	5768.22 \pm 1425.27
$AUC_{0-\infty}$ (ng hr/ml)	6159.18 \pm 1354.45	5960.31 \pm 1439.23
C_{max} (ng/ml)	621.18 \pm 112.09	621.10 \pm 126.65

SD: Standard deviation, AUC: Area under curve, C_{max} : Maximum plasma concentration

Table 2: Secondary pharmacokinetic parameters of Test product (T) and Reference standard (R)

Parameters	Test (Mean ± SD)	Reference (Mean ± SD)
t _{max} (hrs)	5.25 ±1.65	4.83 ±1.46
AUC _{0-t} /AUC _{0-∞} (%)	96.23 ±1.20	96.71 ±2.00
t _{1/2} (hr)	3.78 ±0.66	3.82 ±0.44
K _{el} (1/hr)	0.19 ±0.03	0.18 ±0.02

SD: Standard deviation, t_{max}: Maximum time point, hr: Hour, AUC: Area under curve, t_{1/2}: Terminal half-life, K_{el}: Elimination rate constant

Confidence Intervals:

Bioequivalence was concluded, since C_{max}, AUC_{0-t} and AUC_{0-∞} values (ln-transformed data) of the test product

(T) and reference standard (R), fall within the acceptable range of 80–125% (to prove bioequivalence between two drugs), as depicted in the table 3.

Table 3: pharmacokinetic bioequivalence parameters of Test product (T) and Reference standard (R)

Pharmacokinetic bioequivalence parameters	Geometric Mean		Point Estimator (%) Test/Reference	90 % Confidence Interval	
	Reference	Test		Lower	Upper
AUC _{0-t} (ng hr/ml)	5574.334	5801.763	104.080	87.460	123.850
AUC _{0-∞} (ng hr/ml)	5765.003	6028.953	104.580	88.290	123.870
C _{max} (ng/ml)	610.745	611.410	100.110	89.130	112.440

AUC: Area under curve, C_{max}: Maximum plasma concentration

Analysis of Variance:

Analysis of variance (ANOVA) was done ($\alpha=0.05$) on C_{max}, AUC_{0-t} and AUC_{0-∞} (untransformed and ln-transformed data). Factors included for Analysis of variance (ANOVA), were randomization sequence, subjects included in that particular sequence, period and treatment effect. These factors were found to be statistically insignificant for C_{max}, AUC_{0-t} and AUC_{0-∞} (untransformed and ln-transformed data).

Safety Assessment:

Both the formulations (Test and Reference) were well tolerated. And during the conduct of study, no Adverse Event (AE)/Serious Adverse Event (SAE)/Death were found out.

C_{max}, AUC_{0-t} and AUC_{0-∞} values (ln-transformed data) of the test formulation and reference standard, fall within the

acceptable range of 80–125% (to prove bioequivalence between two drugs). So, the present study concludes that the test formulation Metformin Hydrochloride (SR) 500 mg is bioequivalent to the reference standard D-beta SR tablet [containing Metformin Hydrochloride (SR) 500 mg].

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