Bioequivalence Study of Two IR Metformin Formulations: an Open-Label, Randomised, Two Treatments, Two-Way Crossover Study in Healthy Volunteers

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Abstract

Pharmacokinetic properties of two immediate-release formulations of Metformin 250mg were studied in 24 healthy male volunteers under fasting condition. Volunteers were given either the test or reference formulation randomly at first study interval, and crossed over at second study interval. Non-compartmental model was used to analyse the pharmacokinetic parametres. Results had shown no significant difference between two formulations in terms of maximum plasma concentration and the time to reach such maximum concentration (C_{max} and T_{max}). The half-life ($T_{1/2}$) and the elimination rate constant (K_e) were found to be comparable. There was no statistical significant difference for the area-under-the-curve (AUC) as well. No adverse event was reported. In conclusion, the test formulation was bioequivalent with the reference formulation.

Keywords: Metformin; Pharmacokinetics; Bioequivalence; Bioavailability

Introduction

The biguanide, Metformin, has anti-glycemic action which lowers basal and postprandial plasma glucose level. It does not stimulate insulin secretion and has little risk of inducing hypoglycaemia [1].

Under fasting condition, the oral bioavailability of metformin 500mg immediate release tablet is approximately 50% to 60% [2]. Studies using higher doses of metformin show a lack of dose proportionality due to reduced absorption of the drug. Following the administration of one tablet of 500 mg extended release metformin, the peak plasma concentration is reached approximately at a median value of 7 hour (range of 4 to 8 hour) [1]. The peak plasma level for immediate release formulation is approximately 20% lower than the extended release tablet, but the extent of absorption is similar for both formulation as measured by the area under the plasma concentration curve (AUC). About 90% of orally administered metformin is eliminated via the renal route with an elimination half-life of approximately 6.2 hour. It is excreted in urine as unchanged drug, and no metabolite has been identified in human [2,3].

Pharmacokinetic studies show co-administration of metformin with food reduces both the extent and rate of absorption of metformin. The therapeutic level differs as well for fasting condition and after meal [3]. Mean C_{max} is reduced by approximately 40%, while AUC is 25% lower, and T_{max} is prolonged by approximately 35 minutes, when metformin tablet is administered after food [1].

Side effects of metformin are commonly reported as taste disturbances, gastrointestinal disorders such as nausea, vomiting, diarrhoea, abdominal pain and loss of appetite. Lactic acidosis is rare, as reported in about one in 33,000 patients taking metformin (Glucophage) over a year [1].

The objective of this study is to evaluate if the generic metformin 250mg immediate release (IR) tablet, manufactured by Hovid Ltd Malaysia, is bioequivalent to an approved metformin 250 mg IR tablet (reference formulation, manufactured by Sunward Pharmaceutical Pte Ltd, Singapore) in healthy volunteers.

Materials and Methods

Study population

Male volunteers aged between 21-55 year-old with a body mass index (BMI) between 18.5 to 29.9 or between 20% of ideal body weight for height and build according to the Metropolitan Life Insurance Company Standards, who were deemed healthy by clinical evaluation were eligible for the study. Clinical assessments performed during screening included detailed medical history, physical examination, 12-lead electrocardiogram and clinical laboratory tests.

Exclusion criteria included any significant clinical deviation from normal, in physical or clinical determination; a history or suspicion of drug dependence and alcohol abuse; hypersensitivity to metformin; had participated in other bioequivalence study or donated blood within the past 8 weeks. The study was approved by the Malaysian Medical Research Ethics Committee (MREC) and informed consent was obtained from all volunteers before any procedures pertaining to the study was performed.

Study design

The study was a randomized, single dose, two periods, two treatments, cross-over study with a washout period of at least 7 days. The volunteers were quarantined in a controlled environment for 10 hours prior to each study day. All volunteers were required to undergo a 10-hour overnight fast prior to drug administration. During the

fasting period, free access of plain water was allowed. The volunteers were randomized into one of two groups, which received a single oral administration of metformin test formulation (Diabetmin 250 mg tablet, Hovid Ltd) or the reference formulation (Sunward Metformin 250 mg tablet, Sunward Pharmaceutical Pte Ltd) on either the first or second study period.

The doses were administered with 240 ml of 20% glucose solution at room temperature. Volunteers were allowed access to plain water 1 hour before and 1 hour after drug administration, and were given 60 ml of 20% glucose solution at hourly basis until 3-hour post dose. Standardized meals were provided to study participants at 4, 7, 10 and 13 hour after dosing. All volunteers were prohibited from consuming any alcoholic or caffeine-containing beverages 24 h before dosing, and no tobacco use was allowed. All study participants were released from the clinical facility 24 h after dosing.

The trial was conducted in accordance to Malaysian Good Clinical Practice guideline.

Blood sampling and analysis

A 5ml blood sample was collected into blood collection tube (Vacutainer®, containing sodium heparin as anticoagulant) one hour before dosing and at 0.5, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 8, 10, 12, 16 and 24 hours after dosing. The blood samples were then centrifuged for 15 minutes at 3500 rpm immediately upon collection and transferred to separate glass tubes to be kept at -40°C for analysis. The plasma levels of metformin were analyzed with a reversed-phase high performance liquid chromatographic (HPLC) method with ultraviolet detection (Waters 600E Multisolvent Delivery System, Waters 2487 Ultraviolet Detector, Waters 717 Plus Autosampler, Maple Street Milford, USA).

Safety assessment

All volunteers were monitored closely by attending clinicians up to 12-hour post dosing. Vital signs were taken for up to 5 times throughout the duration of one study period. A last vital signs monitoring (including blood pressure and pulse rate) was performed prior to the release of volunteers from the clinical facility.

Pharmacokinetics and statistical methods

Only data collected from participants who successfully completed both study periods was included in data and statistical analysis. Data obtained from volunteers who experienced emesis or vomiting in the first 4-hour post dosing will be excluded. If deviation of more than 5% in the blood sampling time had occurred, the actual time will be used to calculate the pharmacokinetic parameters.

Maximum plasma concentration (C_{max}), time to reach maximum plasma concentration (T_{max}), area under the plasma concentration-time curve from time zero to the last measurable concentration (AUC_{0-t}), and total area under the plasma concentration-time curve ($AUC_{0-\infty}$) were estimated from the plasma concentration-time data. C_{max} and T_{max} were obtained directly from the plasma values, while the $AUC_{0-\infty}$ was calculated by adding the area from time zero to last sampling time, t (AUC_{0-t}) and the area from time t to infinity ($AUC_{t-\infty}$). AUC_{0-t} was calculated using the trapezoidal formula; and $AUC_{t-\infty}$ was calculated by dividing the last measurable plasma drug concentration (C_t) with the elimination rate constant (k_e) [4]. In all cases, the $AUC_{t-\infty}$ should be less than 20% of the $AUC_{0-\infty}$. The k_e was estimated from the terminal slope of the individual plasma concentration-time curves after natural logarithmic (ln) transformation of the plasma concentration values and application of linear regression [5]. The values of C_{max}, AUC_{0-t}, AUC_{0-}

$_{\infty}$ and k_e obtained with the two preparations were analysed using the analysis of variance (ANOVA) procedure which distinguished effects due to subjects, periods, and treatments [6]. The effect from product, period, sequence, and subject-within-sequence were included in the ANOVA analysis. The AUC_{0-t}, $AUC_{0-\infty}$ and C_{max} values were subjected to natural logarithmic transformation prior to statistical analysis. On the other hand, the T_{max} values were analyzed using the Wilcoxon Signed Rank Test for paired samples. Statistical analysis was conducted with commercial software, EquivTest PK from Statistical Solution (Cork, Ireland).

Analysis of plasma metformin concentration

Reversed-phase HPLC with UV detection was used to analyse the plasma metformin concentration. Analysis was carried out with Waters 600E Multisolvent Delivery System (Maple Street Milford, USA), Waters 2487 Dual Channel UV-VIS Detector (Maple Street Milford, USA), a Waters 717 Plus Autosampler (Maple Street Milford, USA) and a data acquisition and analysis software, Waters Empower™ 2 Data Sofware (Maple Street Milford, USA). The analytical column, ZORBAX RX-SIL (250 x 4.6mm id, 5μ) (Upchurch Scientific, Oak Harbor, USA), was fitted with a refillable guard column (2mm x 2cm)(Upchurch Scientific, Oak Harbor, USA) and packed with Perisorb RP-18 (30-40 μm, pellicular) was used for chromatographic separation. The mobile phase was a mixture of 10.0% acetonitrile in 0.03 M diammonium hydrogen phosphate buffer, adjusted to pH6.0 with 85% phosphoric acid. Flow rate of the analysis was set at 1.0 ml/min isocratically while the detector operated at the wavelength of 234 nm, sensitivity set at 2.0000 AUFS. Injection volume was adjusted at 50 μl and samples quantified using peak area.

Prior to analysis, all frozen plasma samples were thawed and 250 μl aliquot was measured into a 7-ml glass tube with a Teflon lined screw cap. 250 μl of 2.5 μg/ml cimetidine solution (internal standard solution), 250 μl of 7.5 M sodium hydroxide solution and 5ml of the extraction solvent (butanol:hexane; 1:1 v/v) were then added. The mixture was vortex-mixed for 60 seconds, and then centrifuged for 10 minutes at 3500 rpm. The organic layer was then transferred to another 7-ml glass tube with Teflon lined screw cap, and 250 μl of 1M acetic acid was then added. The mixture was vortexed for 60 seconds and centrifuged for 10 minutes at 3500 rpm. 50 μl of lower aqueous layer was then injected into the column.

Results

A total of 24 eligible volunteers consented to participate in the study. 23 subjects successfully completed both phases and were included in the bioequivalence analysis. One subject withdrew his consent to participate due to personal reason. All subjects recruited were male with median age of 26 years (interquartile range of 10.0 years) and mean BMI of 23.6 ± 4.23 kg/m² (Table 1).

The mean plasma metformin concentration-time profile was similar for both the Diabetmin tablet (test) and Sunward Metformin tablet (Reference) (Figure 1). The peak plasma concentration, C_{max}, for test formulation (727.5 ± 167.9 ng/ml) was similar to that of the reference formulation (772.6 ±171.3 ng/ml; Table 2); Time to reach peak plasma concentration, T_{max}, was shown to be similar between the test and reference product as well (2.98 ± 0.86 hr vs 3.13 ± 0.83 hr; Table 2). The half-life of test formulation (3.70 ± 1.63 hr; Table 2) was comparable to that of the reference formulation (3.65 ± 1.20 hr; Table 2), and the elimination rate constant, K_e, was shown to be

Characteristics	Value
Median age (interquartile range), years	26 (10.0)
Body Mass Index (standard deviation), kg/m²	23.6 (4.23)
Race, *n* (%)	
Malay	18 (78.2)
Chinese	4 (17.4)
Indian	1 (4.4)

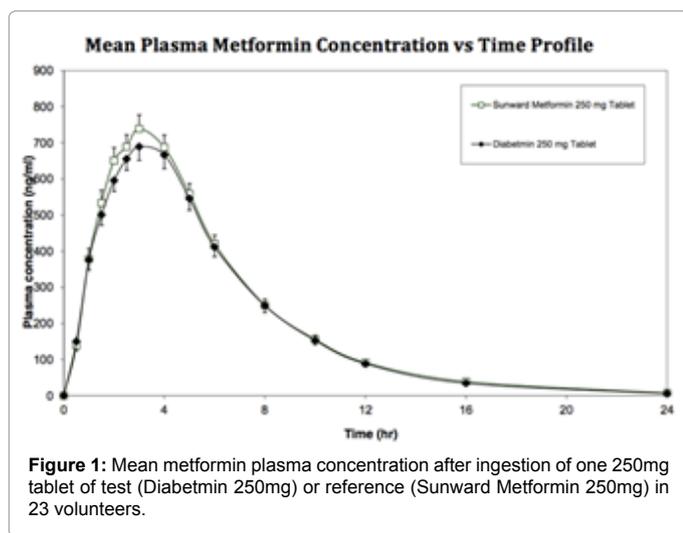**Table 1:** Baseline characteristics of 23 study volunteers.

Figure 1: Mean metformin plasma concentration after ingestion of one 250mg tablet of test (Diabetmin 250mg) or reference (Sunward Metformin 250mg) in 23 volunteers.

PK Parametre	Diabetmin 250mg tablet (Test)	Sunward Metformin 250mg tablet (Reference)
AUC_{0-t} (hr.ng/ml)	4680.7 ± 1163.23	4882.1 ± 1157.44
$AUC_{0-\infty}$ (hr.ng/ml)	4862.5 ± 1153.84	5043.1 ± 1187.02
C_{max} (ng/ml)	727.5 ± 167.89	772.6 ± 171.27
T_{max} (hr)	2.98 ± 0.859	3.13 ± 0.828
$T_{1/2}$ (hr)	3.70 ± 1.629	3.65 ± 1.203
K_e (hr⁻¹)	0.21 ± 0.066	0.21 ± 0.066

Legend: sd, standard deviation; AUC_{0-t}, area under the plasma-concentration curve from dosing to last quantifiable time point; $AUC_{0-\infty}$, area under the plasma-concentration curve from dosing to infinity; C_{max}, peak plasma concentration; T_{max}, time to reach peak plasma concentration; K_e, elimination rate constant

Table 2: Pharmacokinetics parametres of two formulations; the reference drug Sunward Metformin 250mg immediate release tablet and test drug Diabetmin 250mg immediate release tablet, mean ± sd (*n*=23).

similar (0.2110 ± 0.0660 hr⁻¹ vs 0.2091 ± 0.0655 hr⁻¹; Table 2). The area under the plasma concentration curve from time zero (dosing) to the last quantified time point (AUC_{0-t}) were similar for both test and reference formulations (4680.7 ± 1163.3 vs 4882.1 ± 1157.4 hr.ng/ml), and expectedly the $AUC_{0-\infty}$ showed comparable values as well (4862.5 ± 1153.8 vs 5043.1 ± 1187.0 hr.ng/ml).

The arithmetic values of C_{max}, AUC_{0-t}, $AUC_{0-\infty}$ were relatively similar between the test and reference formulations. No significant differences were observed between the logarithmic transformed values of AUC_{0-t} (p=0.0839) and $AUC_{0-\infty}$ (p=0.1166) for both products. The value of T_{max} showed no statistical significant difference using the Wilcoxon Signed Rank Test (p=0.5584). However, analysis of the logarithmic transformed values of C_{max} showed significant difference between the two products (p=0.0330).

A single-dose administration of metformin 250 mg tablet in both formulations was well tolerated. No adverse drug reactions or side effects occurred during the study periods.

Analysis of plasma metformin concentration

Cimetidine was used as the internal standard. Retention time for metformin and cimetidine were approximately 9.0 and 12.3 minutes. The accuracy of the assay was expressed as the percentage of measured concentration over concentration of the spiked value; precision was denoted with coefficient of variation. For within-day validation, the accuracy did not deviate more than ± 13.0% for all concentration determined, with the exception of the limit of quantification where the mean measured concentration deviated by not more than 17.0%; The coefficient of variation values for within-day validation were less than 4.0% for all concentrations. For between-day validation, the mean measured concentration did not deviate by more than ± 7.0% from the actual concentration for all concentrations, while coefficient of variation were less than 9.0% for all concentrations.

Detector response for metformin was found to be linear over the concentration range of 19.5-5000.0 ng/ml with correlation coefficient of at least 0.99. The limit of quantification was set at 19.5 ng/ml with a signal to noise ratio of at least 5:1. The limit of detection was set at 9.8 ng/ml with signal to noise ratio of at least 2:1. The absolute recovery of metformin was all above 87%.

The method was selective. No significant endogenous peak was detected from six different sources of blank plasma at the retention times of metformin and cimetidine.

Metformin was shown to be stable for at least 3 freeze-thaw cycles, and plasma metformin was shown to be stable for 2 months (stored between -15 to -22°C). This was longer than the time period between first sample collection and last sample analysis. Metformin in plasma was shown to be stable for 6 hours in room temperature, while reconstituted solution of metformin was stable for at least 10 hours in the autosampler maintained at ambient room temperature. The drug solution of metformin and cimetidine was shown to be stable for at least 2 months in refrigerated condition, or 9 hours after storage at room temperature.

Discussion

Metformin is typically dosed from 500 mg to 1.5 g daily [1] and it is the first line medication to treat type 2 diabetes mellitus [7]. The 250 mg tablet is to provide a suitable dosage form for patients whose metformin daily dose is either 750 mg or 1250 mg, thus circumventing the need to cut the 500 mg tablet into half.

Bioequivalence can be concluded based on the 90% confidence interval for the ratio of C_{max}, AUC_{0-t} and $AUC_{0-\infty}$ values of test formulation over those of the reference formulation. The two one-sided test procedure as described by Shuirmann, 1987 [8] was used to calculate the 90% confidence interval, at α = 5%. The European Medicines Agency and the Ministry of Health Malaysia (stated in the Malaysian Guideline for the Conduct of Bioavailability and Bioequivalence Studies) specify that the accepted range for AUC_{0-t} and $AUC_{0-\infty}$ is 80.00-125.00% [9,10]. However the range of C_{max} could be wider if it is appropriately justified.

In this study, the 90% confidence intervals for the ratio of AUC_{0-t}, $AUC_{0-\infty}$ and C_{max} of the test formulation over the reference formulation were estimated to be between 0.9225-0.9960, 0.9299-0.9997 and 0.9050-0.9831 respectively, which were all within the acceptable bioequivalence limit of 0.8000-1.2500.

Conclusion

In conclusion, the test formulation Diabetmin 250 mg tablet was

bioequivalent to the reference formulation Sunward Metformin 250 mg tablet.

Acknowledgement

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