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Bioequivalence Evaluation of Two Brands of Metformin 500 mg Tablets (Dialon® & Glucophage®) – in Healthy Human Volunteers

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ABSTRACT: A randomized, two-way, crossover study was conducted in 24 fasting, healthy, male volunteers to compare the bioavailability of two brands of metformin 500 mg tablets; Dialon® (Julphar, UAE) as test and Glucophage® (Lipha Pharmaceutical Industries, France) as reference product. The study was performed at the International Pharmaceutical Research Centre (IPRC), in joint venture with Al-Mowasah Hospital, Amman, Jordan. The drug was administered with 240 ml of water after a 10-h overnight fasting on two treatment days separated by 1-week washout period. After dosing, serial blood samples were collected for a period of 30 h. Plasma harvested from blood was analyzed for metformin by validated HPLC method with UV-visible detector capable to detect metformin in the range of 0.05–5.0 μg/ml with limit of quantitation of 0.05 μg/ml. Various pharmacokinetic parameters including AUC_{0-t}, $AUC_{0-\infty}$, C_{max}, T_{max}, $T_{1/2}$, and λ_Z were determined from plasma concentrations of both formulations and found to be in good agreement with reported values. AUC_{0-t}, $AUC_{0-\infty}$ and C_{max} were tested for bioequivalence after log-transformation of data. No significant difference was found based on ANOVA; 90% confidence interval (97.9–110.8% for AUC_{0-t}, 97.4–110.7% for $AUC_{0-\infty}$; 95.3–110.5% for C_{max}) of test/reference ratio for these parameters were found within bioequivalence acceptance range of 80–125%. Based on these statistical inferences, it was concluded that Dialon® is bioequivalent to Glucophage®. Copyright © 2002 John Wiley & Sons, Ltd.

Key words: metformin; bioequivalence; pharmacokinetics; HPLC; Julphar

Introduction

Bioequivalence of two formulations of the same drug comprises equivalence with respect to the rate and extent of their absorption. The area under concentration time curve (AUC) generally serves as the characteristic of the extent of absorption while the peak concentration (C_{max}) and the time of its occurrence (T_{max}), reflect the rate of absorption, especially in fast releasing drug formulations [1,2]. The present study was conducted to evaluate the bioequivalence of two brands of metformin 500 mg tablets in fasting, healthy human volunteers.

Metformin is an oral antihyperglycaemic agent used in the management of non-insulin-dependent diabetes mellitus (NIDDM) [3,4]. It reduces blood glucose levels, predominantly by improving hepatic and peripheral tissue sensitivity to insulin without affecting the secretion of insulin [3]. It is considered the drug of choice of the biguanide class due to the lesser risk of associated lactic acidosis as compared to phenformin [5]. Chemically, it is 1,1-dimethylbiguanide hy-

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drochloride ($C_4H_{11}N_5$,HCl) with a molecular weight of 165.6 [6].

Defects which cause hyperglycemia and type II diabetes mellitus include alterations in pancreatic insulin secretion, elevations in hepatic glucose production, and peripheral insulin resistance [3]. Metformin acts to reverse two of the above defects via three mechanisms: (1) reduction in hepatic glucose production, (2) reduction in intestinal glucose absorption, and (3) increased insulin sensitivity [7–10].

Metformin differs from other agents used for treating type II diabetes mellitus in several important respects. Unlike suplfonylureas, metformin does not increase insulin secretion and is not associated with hypoglycaemia at therapeutic doses except in special situations [11–13]. It also does not cause weight gain [11].

Gastrointestinal absorption of metformin is incomplete with an absolute bioavailability of 50–60% (under fasting conditions) and 20–30% of an oral dose is recovered in faeces [14,15]. Studies using single oral doses of metformin tablets 500–2550 mg indicate that there is a lack of dose proportionality with increasing doses, which is due to decreased absorption rather than an alteration in elimination [3,7,16–19]. Food decreases the extent and slightly delays absorption; an approximately 40% lower peak concentration was reported following administration of a single 850 mg tablet of metformin with food [7,17]. Metformin is negligibly bound to plasma proteins in contrast to sulfonylureas which are more than 90% protein bound [7,17–19].

Intravenous single-dose studies in normal subjects demonstrate that metformin is excreted unchanged in the urine and does not undergo hepatic metabolism nor biliary excretion [7,17−19]. Following oral administration, approximately 90% of the absorbed drug is eliminated via the renal route within the first 24 h, with a plasma elimination half-life of approximately 1.5–6.2 h [3,7,16–19].

Objectives of the study

The purpose of this study was to determine the bioequivalence of a new tablet formulation of metformin (Dialon®) produced locally in United Arab Emirates by Gulf Pharmaceutical Industries, Julphar, in comparison with Glucophage® from Lipha Pharmaceutical Industries, France.

Material and Methods

Study products

Test Product	Dialon® 500 mg tablets
Batch No.	0009, Expiry 04/2003
Manufacturer	Gulf Pharmaceutical Industries, Julphar, United Arab Emirates
Reference Product	Glucophage® 500 mg tablets
Batch No.	1786, Expiry 07/2005
Manufacturer	Lipha Pharmaceutical Industries, France

Study subjects

Twenty-four (24) healthy adult male volunteers participated in this study at Al-Mowasah Hospital, Amman, Jordan. The mean age was 23 $\pm$ 3.49 years with a range of 18–31 years, mean body weight was 66 $\pm$ 7.84 kg with a range of 52–80 kg and mean height was 174 $\pm$ 5.54 cm with a range of 166–184 cm. On the basis of medical history, clinical examination and laboratory investigation (hematology, blood biochemistry, and urine analysis), no subject had a history or evidence of hepatic, renal, gastrointestinal or hematologic deviations or any acute or chronic diseases or drug allergy to sulfonylureas. Consumption of alcohol or beverages or food containing methylxanthines was not permitted for the volunteers 48 h prior to the study and after drug administration until the last blood sample was collected in the respective study phase. The subjects were instructed to abstain from taking any medication for at least 1 week prior to and during the study period. Informed consent was obtained from the subjects after explaining the nature and purpose of the study. The study protocols were approved by the Institutional Review Board (IRB) of Al-Mowasah Hospital, Amman-Jordan.

Drug administration and blood samples collection

This study was based on a single dose, randomized, two treatment, two periods crossover design. On the morning of phase I, after an overnight fasting (10 h) volunteers were given single dose of either formulation (reference or test) of metformin with 240 ml of water. Following drug administration, 100 ml of glucose 10% solution was administered at approximately 0.5, 1.5, 2, 2.5, 3.0 and 5.0 h. In addition, 20% glucose solution was given to any subject who exhibited symptoms of hypoglycaemia. Lunch and dinner were served at 5 and 12 h, respectively, after drug administration. Volunteers were ambulatory during the study but prohibited from strenuous activity. Approximately, 10 ml of blood samples for metformin assay were drawn through indwelling cannula before (0 h) and at 0.33, 0.66, 1.0, 1.33, 1.66, 2.0, 2.5, 3.0, 3.5, 4.0, 5.0, 6.0, 8.0, 10.0, 12.0, 16.0, 24.0, and 30.0 h after dosing. The blood samples were collected in glass tubes containing heparin, and centrifuged at 3500 rpm for 10 min; plasma was separated and kept frozen at -20°C in properly labeled tubes. After a period of 7 days the study was repeated in the same manner to complete the crossover design.

Sample preparation for HPLC injection

A 50 µl internal standard (chlorpheniramine 50 µg/ml) was added to 200 µl of plasma sample. The sample was vortexed for 30 s, 300 µl of acetonitrile was added and vortexed for 1 min then centrifuged for 3 min at 12800 rpm. The 100 µl of supernatant layer was transferred to another 0.75 ml eppendorf centrifuge tube then diluted by 500 µl of mobile phase, vortexed for 30 s; 50 µl of aliquot sample was then injected to column and peak area was recorded.

Chromatographic conditions

Plasma samples were analyzed for metformin according to reported HPLC methods [20,21] with some modifications and validated before the study. All solvents used were of HPLC grade and were purchased from Merck (LiChrosolv-Darmstadt, Germany); other chemicals and reagents were of analytical grade. Metformin and chlropheniramine were obtained from Julphar, UAE.

The HPLC system was from Shimadzu Kyoto, Japan, and it consisted of a solvent delivery pump (LC-007ADvp), a system controller(SCL-007Avp), an auto-injector (SIL-007Avp), and an UV-visible detector (SPD-007Avp); integration was done using Class VP-5 software version 5.03. Chromatographic separation was performed using Nucleosil 100-5CN (5 µm) ($125 \times 4\,mm^2$) HPLC cartridge column. The mobile phase consisted of 80% acetonitrile and 20% 0.01 M potassium dihydrogen phosphate (pH 3.5 adjusted with glacial acetic acid), and eluted at a flow rate of 0.6 ml/min at an ambient temperature. The effluent was monitored using UV detector at 234 nm. The peak area was measured, and the peak area ratio of drug to internal standard and the concentration were calculated by Class VP-5 software (version 5.03) Shimadzu. Each analysis required less than 9 min. The method was validated by following international guidelines [22].

Pharmacokinetic analysis

Pharmacokinetic analysis was performed by means of model independent method using Kinetica™ 2000 computer program [23]. The elimination rate constant (λ_Z) was obtained as the slope of the linear regression of the log-transformed concentration values versus time data in the terminal phase. Elimination half-life ($T_{1/2}$) was calculated as $0.693/\lambda_Z$. Area under the curve to the last measurable concentration (AUC_{0-t}) was calculated by the linear trapezoidal rule. Area under the curve extrapolated to infinity ($AUC_{0-\infty}$) was calculated as $AUC_{0-t} + C_t/\lambda_Z$, where C_t is the last measurable concentration.

Statistical analysis

For the purpose of bioequivalence analysis AUC_{0-t}, $AUC_{0-\infty}$ and C_{max} were considered as primary variables. Two way analysis of variance (ANOVA GLM procedure; KineticaTM 2000 Computer program [23]) for crossover design was used to assess the effect of formulations, periods, sequences and subjects on these parameters. Difference between two related parameters was considered statistically significant for

p-value equal to or less than 0.05. Parametric 90% confidence intervals [24] based on the ANOVA of the mean test/reference (T/R) ratios of AUCs and C_{max} were computed.

Results and Discussion

Metformin was well tolerated by the volunteers; unexpected incidents that could have influenced the outcome of the study did not occur. There was no drop-out and all volunteers who started the study continued to the end and were discharged in good health.

The described analytical method was proven sensitive and accurate for determination of metformin plasma concentration. Retention times were 5.9 and 7.5 min for metformin and chlorpheniramine (internal standard), respectively. Under the described conditions, the lower limit of quantitation was 0.05 μg/ml using 0.2 ml of plasma. The relationship between concentration and peak area ratio was found to be linear within the range of 0.05–5.00 μg/ml. The intra-day accuracy of the method for metformin ranged from 98.22 to 104.0%, while the intra-day precision ranged from 2.04 to 14.00%. The inter-day accuracy ranged from 96.28 to 106.0%, while the inter-day precision ranged from 3.08 to 16.98%. Stability study showed that metformin was stable in plasma for 6 weeks when stored at −20°C.

Both formulations were readily absorbed from the gastrointestinal tract and metformin was measurable at the first sampling time (0.33 h) in all 24 volunteers. The mean concentration-time profile of the two formulations is shown in the Figure 1 indicating that the mean plasma drug concentration profiles of the two brands were closely similar and superimposable. Peak concentration were attained at 2.8 and 2.95 h after drug administration and then declined rapidly but were still detectable up till 24 h. All calculated pharmacokinetic parameter were in good agreement with reported values [3,7,15,17,19].

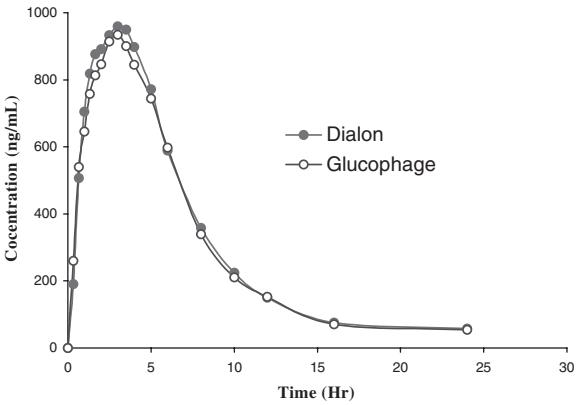

Figure 1. Mean plasma concentration of metformin 500 mg tablets after oral administration of single dose of two brands to 24 healthy human volunteers

Table 1 shows the pharmacokinetic parameters for the two brands of metformin 500 mg tablets. The extent of absorption is a key characteristic of drug formulation, and therefore AUC is an important parameter for comparative bioavailability (bioequivalence) study [25]. However, the other two parameters, C_{max} and T_{max}, are also

Table 1. Pharmacokinetic parameters of metformin tablets (Mean ± Standard deviation; $n = 24$)

Pharmacokinetic parameter	Dialon® (Test)	Glucophage® (Reference)	ANOVA GLM (*p*-value)	90% CI
AUC_{0-t} (ng/ml h)	6888.72 ± 1469.45	6613.88 ± 1453.96	0.2811 (0.4481)[a]	97.9–110.8% (91.3–103.5%)
$AUC_{0-\infty}$ (ng/ml h)	7293.19 ± 1573.26	6996.56 ± 1427.62	0.3266 (0.5049)[a]	97.4–110.7% (91.4–104.0%)
C_{max} (ng/ml)	1036.08 ± 192.98	1016.08 ± 224.44	0.5707 (0.7779)[a]	95.3–110.5% (91.5–106.5%)
T_{max} (*H*)	2.88 ± 0.85	2.95 ± 0.81	0.7165 (0.7690)	—
$T_{1/2}$ (*H*)	3.40 ± 0.83	3.27 ± 0.74	0.4089 (0.4537)	—
λ_Z (*H*)	0.21 ± 0.05	0.22 ± 0.04	0.5253 (0.8899)	—

[a] Statistics was applied on Ln-transformed data. Parenthesis values indicate analysis for periods.
Values are given as Mean ± SD.

important features of the plasma level profile and could affect the therapeutic use of a drug [26] and hence were also considered in the study. The relative bioavailability of Dialon® was 105.74% for AUC_{0-t}, 105.51% for $AUC_{0-\infty}$, and 104.76% for C_{max}.

The most important objective of bioequivalence testing is to assure the safety and efficacy of generic formulations. When two formulations of the same drug are equivalent in the rate and extent to which the active drug becomes available to the site of drug action, they are bioequivalent and thus consider therapeutically equivalent [27]. To demonstrate bioequivalence certain limits should be set depending on the nature of drug, patient population, and clinical end points. It is generally accepted that for basic pharmacokinetic characteristics, such as AUC and C_{max}, the standard equivalence range is 0.8–1.25 [24]. The results of statistical analysis are shown in Table 1.

The mean and standard deviation of AUC_{0-t}, $AUC_{0-\infty}$ and C_{max} of the two products did not differ significantly, suggesting that the blood profiles generated by Dialon® are comparable to those produced by Glucophage®. Analysis of variance (ANOVA) for these parameters, after log-transformation of the data, showed no statistically significant difference between the two formulations either in periods, formulations or sequence, having p value greater than 0.05. Ninety percent confidence intervals also demonstrated that the ratios of AUC_{0-t}, $AUC_{0-\infty}$ or C_{max}, of the two formulations lie within the FDA acceptable range of 80–125%.

For T_{max} the parametric point estimate of difference (test–reference) was -0.07 h, and found to be within the acceptance limits ($\pm$ 20% of reference mean).

Plasma levels may be used as surrogate parameters for clinical activity; therefore results of this study suggest equal clinical efficacy of the two brands of metformin.

Conclusion

Statistical comparison of the AUC_{0-t}, $AUC_{0-\infty}$ and C_{max} clearly indicated no significant difference between Dialon® and Glucophage® tablets in any of the calculated pharmacokinetic parameters. The confidence intervals for the ratios of mean AUC_{0-t}, $AUC_{0-\infty}$ and C_{max} indicated that these values were entirely within the bioequivalence acceptance range of 80–125% (using log-transformed data). Based on the above we can conclude that Dialon®, manufactured by Gulf Pharmaceutical Industries, UAE is bioequivalent to Glucophage®, manufactured by Lipha Pharmaceutical Industries, France, and that both products can be considered equally effective in medical practice

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