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Bioequivalence Evaluation of Two Brands of Gliclazide 80 mg Tablets (Glyzide® & Diamicron®) — in Healthy Human Volunteers

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ABSTRACT: A randomized, two-way, crossover, bioequivalence study in 24 fasting, healthy, male volunteers was conducted to compare two brands of gliclazide 80 mg tablets, Glyzide® (Julphar, UAE) as test and Diamicron® (Servier Industries, France) as reference product. The study was performed at the International Pharmaceutical Research Centre (IPRC), in joint venture with Speciality Hospital, Amman, Jordan. The drug was administered with 240 ml of 20% glucose solution after a 10 h overnight fasting. After dosing, serial blood samples were collected for a period of 48 h. Plasma harvested from blood was analyzed for gliclazide by validated HPLC method. Various pharmacokinetic parameters including AUC_{0-t}, $AUC_{0-\infty}$, C_{max}, T_{max}, $T_{1/2}$, *and* elimination rate constant were determined from plasma concentrations of both formulations. Statistical modules (ANOVA and 90% confidence intervals) were applied to AUC_{0-t}, $AUC_{0-\infty}$, and C_{max} for bioequivalence evaluation of the two brands which revealed no significant difference between them, and 90% CI fell within US FDA accepted bioequivalence range of 80–125%. Based on these statistical inferences, Glyzide® was judged bioequivalent to Diamicron®. Copyright © 2002 John Wiley & Sons, Ltd.

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

Introduction

Bioequivalence of two formulations of the same drug comprises equivalence with respect to the rate and extent of its 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 gliclazide 80 mg tablets in fasting, healthy human volunteers. Although several studies have been published on gliclazide pharmacokinetics, very few of them have focused on the proof of bioequivalence between two brands.

Gliclazide is a second-generation sulfonylurea oral hypoglycaemic agent closely related to glyburide [3–6], effective in controlling blood glucose in type II diabetes mellitus. As for other second-generation sulfonylureas, the potency of gliclazide is greater than that of first-generation agents. It acts mainly by stimulating the islet tissue of the pancreas to secrete insulin and by increasing the sensitivity of peripheral tissues to insulin. Consequently, it is effective only when some residual pancreatic beta-cell activity is present [7]. Thus, hypoglycaemic mechanism of action of gliclazide is mainly related to

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stimulation of insulin secretion from beta cells, but possibly also via direct effects on intracellular calcium transport [3,5,8,9]. Improvement of abnormal first-phase insulin release has been reported in type II diabetic patients [8]. One study provided evidence that gliclazide enhances insulin secretion by increasing pancreatic beta-cell sensitivity to glucose [10]. At the cellular level, sulfonylureas bind to a sulfonylurea receptor in the pancreatic beta-cell inhibiting the adenosine triphosphate-dependent potassium channel (K-ATP). Stabilization of potassium efflux causes depolarization and activation of the L-type calcium channel. Influx of calcium stimulates insulin secretion. The effect of sulfonylureas is similar to that of glucose at the cellular level; however, sulfonylureas only stimulate phase I (initial rapid peak) release of insulin and have no effect on phase II (prolonged insulin release). When sulfonylurea treatment is initiated, insulin levels increase and plasma glucose levels gradually decrease. As the glucose levels decrease, insulin levels also decrease but still remain higher than pre-treatment levels [11].

When administered orally, peak concentrations are achieved within 2–4 h [3,8,12]. After a single 80 mg oral dose, peak concentration ranged from 3 to 8 μg/ml [3,8,12]. Its reported bioavailability is 80% [8] while the effect of food is clinically insignificant [13]. It has 85–99% protein binding and the volume of distribution is 13–24 l [3,8,14]. Liver is the main site of metabolism, and via hydroxylation, oxidation and glucuronidation gliclazide is metabolized to 7 metabolites, majority of those being inactive [8,14,15]. Gliclazide is eliminated primarily as metabolites. The amount of unchanged drug eliminated in the urine varies from <1–20%; 60–70% gliclazide metabolites and conjugates are primarily eliminated via kidneys [3,8,15] and 10–20% via feces [16,17]. The reported elimination half-life is 8–12 h [3,6,8,12,15]; it tends to be longer in elderly patients [14].

Objectives of the study

The purpose of this study was to determine the pharmacokinetic parameters of two brands of gliclazide 80 mg tablets and then to compare these parameters statistically to evaluate the bioequivalence between the two brands. Glyzide® (Gulf Pharmaceutical Industries — Julphar, UAE) was used as test while Diamicron® (Servier Industries, France) was used as reference product.

Material and Methods

Study products

Test product	Glyzide® 80 mg tablets
Batch No.	0006, Expiry 09/2002
Manufacturer	Gulf Pharmaceutical Industries — Julphar, United Arab Emirates
Reference product	Diamicron® 80 mg tablets
Batch No.	9D0802, Expiry 04/2002
Manufacturer	Les Laboratories Servier Industries, Giddy, France

Study subjects

Twenty-four healthy adult male volunteers participated in this study at Speciality Hospital, Amman, Jordan. The mean age was 24.7 ± 6.1 years with a range of 18–40 years and the mean body weight was 74.0 ± 6.3 kg with a range of 54–94 kg. 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 Speciality 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 gliclazide with 240 ml of 20% glucose solution. 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 exhibit symptoms of hypoglycaemia. Lunch and dinner were served after 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 gliclazide assay were drawn into heparinized tubes through indwelling cannula before (0 h) and at 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 5.0, 6.0, 8.0, 10.0, 12.0, 16.0, 24.0, 36.0 and 48.0 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 labelled tubes. After a period of 7 days the study was repeated in the same manner to complete the crossover design.

Chromatographic conditions

An HPLC method was developed and validated at International Pharmaceutical Research Centre (IPRC) Laboratory for gliclazide analysis in plasma samples with UV detection. All solvents used were of HPLC grade and were purchased from Merck (LiChrosolv–Darmstadt, Germany); gliclazide and glyburide (internal standard) reference standards were obtained from Julphar, UAE.

The HPLC system was an isocratic system consisting of a solvent delivery pump (Water, USA; Model 515), Dual λ absorbance detector (Water, USA; Model 2487) and a rheodyne injector (Rheodyne, USA); Millennium Software version 3.0 (Water, USA) was used for data interpretation. Chromatographic separation was performed using Nova-pak Phenyl (Waters, USA) HPLC catridge column (5 µm, 3.9 mm × 150 mm). The mobile phase consisted of 42% acetonitrile and 58% 50 mM potassium dihydrogen phosphate buffer; pH was adjusted to 3.0 using phosphoric acid. The mobile phase was eluted at a flow rate of 2.0 ml/min, and effluent was monitored at a wavelength of 229 nm. Each analysis required not more than 10 min. Quantitation was achieved by measurement of the peak area ratio of the drug to the internal standard. The method was validated by following the international guidelines [18]. The limit of quantitation for gliclazide was 0.20 µg/ml plasma; inter-day CV ranged from 2.19 to 5.96% at three different concentrations; recovery was 92.64%.

Sample preparation for HPLC injection

A 100 µl internal standard (glyburide, 10 µg/ml) was added to 0.5 ml of plasma sample. The sample was vortexed and 5 ml of extraction solvent (6:3:1 acetonitrile:ethyl acetate:dichloromethane) was added and vortexed and then centrifuged. The supernatant layer was transferred to another 10 ml glass tube and evaporated to dryness under nitrogen stream. The residue was reconstituted with 200 µl of mobile phase and transferred to eppendorf tube and centrifuged; 50 µl of the supernatant layer was then injected to column and the peak area was recorded.

Pharmacokinetic analysis

Pharmacokinetic analysis was performed by means of a model independent method. The maximum gliclazide concentrations (C_{max}) and the corresponding peak times (T_{max}) were determined by the inspection of the individual drug plasma concentration–time profiles. The elimination rate constant (λ_Z) was obtained from the least-square fitted terminal log-linear portion of the plasma concentration–time profile. The elimination half-life ($T_{1/2}$) was calculated as $0.693/\lambda_Z$ The area under the curve to the last measurable concentration (AUC_{0-t}) was calculated by the linear trapezoidal rule. The 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. Bioequivalence of the two products was assessed by means of an analysis of variance (ANOVA GLM model) [19] for cross-over design and calculating standard 90% confidence intervals [20] of the ratio test/reference (T/R) using log-transformed data. The products were considered bioequivalent when the difference between two compared parameters was found statistically insignificant ($p \geqslant 0.05$) and 90% confidence intervals for these parameters fell within 80–125% [20].

Results and Discussion

The mean concentration–time profile for the two brands of gliclazide 80 mg tablets is shown in Figure 1. All calculated pharmacokinetic parameter values were in good agreement with the previously reported values [3,8,11,13,14]. The pharmacokinetic parameters for both formulations are given in Table 1. For bioequivalence evaluation various statistical modules were applied to AUC_{0-t}, $AUC_{0-\infty}$ and C_{max} as per current US FDA guidelines [20]. Table 2 shows the probability of $F(\infty)$ values for various source of variation obtained from ANOVA; same table also shows the 90% confidence interval for AUC_{0-t}, $AUC_{0-\infty}$ and C_{max} for log-transformed data.

According to the mean plasma levels of the 24 subjects completing the study, the relative bioavailability was found to be 103.3%, 104.7% and 103.2% on the basis of mean AUC_{0-t}, $AUC_{0-\infty}$ and C_{max}, respectively.

The two brands of gliclazide were well tolerated by the volunteers in both phases of the study; all volunteers who started the study continued to the end and were discharged in good health. Both formulations were readily absorbed from the gastrointestinal tract and gliclazide was measurable at the first sampling time (0.5 h) in majority of the volunteers. Non-compartmental approach was used to determine the pharmacokinetic parameters of gliclazide.

For bioequivalence evaluation, AUC_{0-t}, $AUC_{0-\infty}$ and C_{max} were considered as primary parameters. The mean and standard deviation of these parameters for the two brands were found very close, indicating that the plasma profiles generated by Glyzide® are comparable to those produced by Diamicron®. Analysis of variance (ANOVA), after log-transformation of the data, showed no statistically significant ($p > 0.05$) difference between the brands. Ninety percent

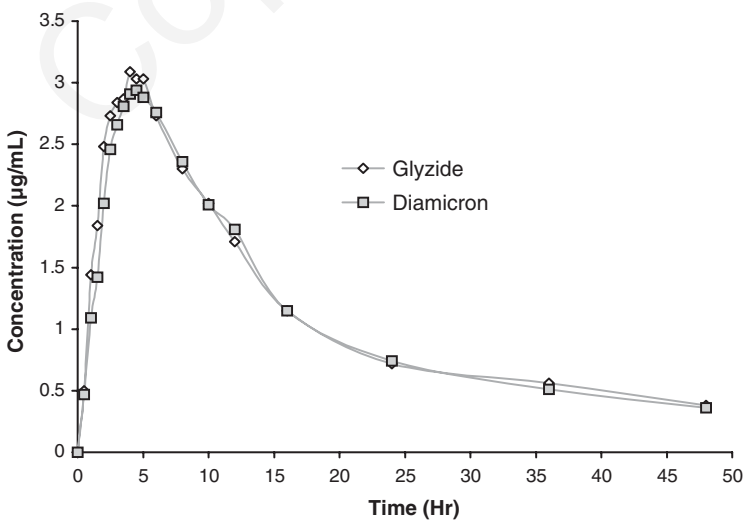

Figure 1. Mean plasma concentration of gliclazide 80 mg tablets after oral administration of single dose of two brands to 24 healthy human volunteers

Table 1. Pharmacokinetic parameters of gliclazide tablets (mean ± standard deviation; $n = 24$)

Pharmacokinetic parameter	Glyzide® (Test)	Diamicron® (Reference)
AUC_{0-t} (μg/ml h)	49.63 ± 17.97	48.81 ± 18.90
$AUC_{0-\infty}$ (μg/ml h)	56.95 ± 23.54	55.27 ± 23.62
C_{max} (μg/ml)	3.65 ± 0.60	3.57 ± 0.68
T_{max} (h)	3.85 ± 1.75	3.96 ± 1.63
$T_{1/2}$ (h)	12.29 ± 4.23	12.40 ± 4.38
λ_Z (h)	0.06 ± 0.02	0.06 ± 0.02

Table 2. Statistical analysis of log-transformed data

Statistical analysis	AUC_{0-t}	$AUC_{0-\infty}$	C_{max}
ANOVA GLM (p-value)	0.1887 (0.2222)	0.0952 (0.3672)	0.3470 (0.9542)
90% CI	99.3–106.5% (99.1–106.3%)	100.1–108.3% (98.2–106.3%)	98.1–107.0% (95.6–104.3%)

Parentheses values indicate analysis for periods.

confidence intervals also demonstrated that the ratio of the AUC_{0-t} or $AUC_{0-\infty}$ or C_{max} of the two brand was within the US FDA accepted range of 80–125%.

For T_{max} the parametric point estimate of difference (test-reference) was −0.11 h, and found within the acceptance limits (± 20% of reference mean).

Conclusion

The statistical comparison of AUC_{0-t}, $AUC_{0-\infty}$ and C_{max} clearly indicated no significant difference in the two brands of gliclazide 80 mg tablets. Ninety percent confidence intervals for the mean ratio (T/R) of AUC_{0-t}, $AUC_{0-\infty}$ and C_{max} indicated that the reported values were entirely within the bioequivalence acceptance range of 80–125% (using log-transformed data). Based on the pharmacokinetic and statistical results of this study, we can conclude that Glyzide® 80 mg tablets (Gulf Pharmaceutical Industries, UAE) is bioequivalent to Diamicron® 80 mg tablets (Servier Industries, France), and that the two products can be considered interchangeable in medical practice.

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