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Short communication

Quantification of gliclazide by semi-micro high-performance liquid chromatography: application to a bioequivalence study of two formulations in healthy subjects

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Abstract

The objective of the present study was to evaluate the bioequivalence of two formulations of gliclazide in healthy human volunteers. Bioequivalence of the two formulations was determined in 20 healthy subjects with a single-dose, two-period, crossover study. A new high-performance liquid chromatographic method for the pharmacokinetic analysis of gliclazide was developed, using a semi-micro column to quantify gliclazide in plasma samples. Chromatographic separation was achieved with a semi-micro C_{18} column and 40 mM KH_2PO_4 (pH 4.6)–acetonitrile–isopropyl alcohol (5:4:1, v/v/v) as the mobile phase, and with UV detection at 229 nm. The method displayed good precision (coefficients of variation (CV $<$ 8.0%)), was fast (total analysis time 8 min), and required only a small amount of mobile phase (0.22 ml/min), with a reasonable limit of quantification (0.1 μg/ml). The calibration curve was linear in the concentration range 0.1–10 μg/ml. When the pharmacokinetic parameters of gliclazide in the two formulations were calculated and compared statistically using crossover analysis of variance, they were similar, with no statistically significant difference. Ninety percent confidence intervals for AUC_{0-last}, $AUC_{0-\infty}$, and C_{max}, used to evaluate bioequivalence, were in the stipulated range of 0.80–1.25. This result suggests that two formulations are bioequivalent when administered orally at a dose of 80 mg gliclazide.

Keywords: Gliclazide; Bioequivalence; Semi-micro column

1. Introduction

Bioequivalence of two formulations of the same drug involves equivalence with respect to the rate and extent of their absorption. Whereas the area under the concentration–time curve (AUC) generally serves to characterize the extent of absorption, the peak concentration (C_{max}), and the time to C_{max} (t_{max}), reflect the rate of absorption, especially in fast-release drug formulations.

Gliclazide, 1-(3-azabicyclo(3.3.0)oct-3-yl)-3-*p*-tolysulfonylurea, is a second-generation hypoglycemic

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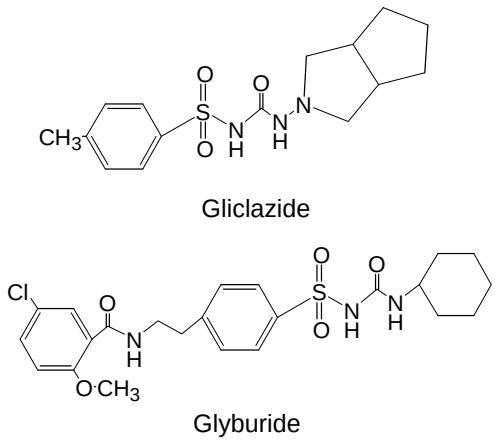

Fig. 1. The structural formulae of gliclazide and glyburide (internal standard).

drug used in the treatment of non-insulin-dependent diabetes mellitus (Fig. 1). It has been suggested that because it acts in the short-term, gliclazide may be suitable for diabetic patients with renal impairment and also for elderly patients in whom reduced renal function may increase the risk of hypoglycemia following the administration of some sulfonylureas [1]. When administered orally, the t_{max} of gliclazide reached within 2–4 h [2–4]. After a single oral dose of 80 mg gliclazide, C_{max} ranged from 3 to 8 μg/ml [2–4]. Its reported bioavailability is 80% [3], whereas the effects of food on the drug are clinically insignificant [5]. Gliclazide shows 85–99% protein binding, and the volume of distribution is 13–24 l [1–3]. It is readily absorbed from the gastrointestinal tract and extensively metabolized in the liver by hydroxylation and by N-oxidation of a number of inactive metabolites [1,2].

Different analytical methods, including gas chromatography [6], high-performance liquid chromatography (LC) with laborious extraction steps [7], a derivatization method [8], analyses with lengthy retention times [9], or expensive solid-phase extraction procedures [10], have been developed to measure gliclazide in biologic samples. Furthermore, most of the methods developed use analytical columns that require large quantities of mobile phase to analyze large numbers of samples in pharmacokinetic studies, including bioequivalence studies. Therefore, a simple and cost-effective LC method using a semi-micro column was developed, suitable for pharmacokinetic studies in terms of specificity and sensitivity, which was applied to a bioequivalence study.

The purpose of the present 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 of the two brands (Samchundang Gliclazide® (Samchundang Pharmaceutical Co., Korea) and Diamicron® (Servier Pharmaceutical Co., France)) using the newly developed LC-based technique.

2. Materials and methods

2.1. Study products

Test product:

- Samchundang (SCD) Gliclazide®—gliclazide 80 mg.
- Batch no.: 2005, expiry: 4/2005.
- Manufacturer: Samchundang Pharmaceutical Co., Korea.

Reference product:

- Diamicron®—gliclazide 80 mg.
- Batch no.: 1SE040, expiry: 8/2004.
- Manufacturer: Servier Pharmaceutical Co., France.

2.2. Study subjects

Twenty healthy adult volunteers participated in this comparative study at Gil Medical Center, Gachon Medical School, Incheon, Korea. Their mean age was 23.5 ± 2.4 years with a range of 21–31 years and mean body weight was 60.1 ± 7.0 kg in a range of 41–71 kg. The volunteers were free from significant cardiac, hepatic, renal, pulmonary, neurologic, gastrointestinal, and hematologic disease, as assessed by physical examination, electrocardiography, and the following laboratory tests including hematology, biochemistry, electrolytes, and urinalysis. No subject had a history or evidence of hepatic, renal, gastrointestinal, or hematologic abnormality or any acute or chronic diseases or allergy to any drugs, including

sulfonylureas. The volunteers were not permitted to consume alcohol, or beverages or food containing methylxanthines, for 72 h before the study or after drug administration until the last blood sample had been collected in each study phase. The subjects were instructed to abstain from taking any medication for at least 1 week before and during the study period. Informed consent was obtained from the subjects after the nature and purpose of the study was explained to them. The study protocols were approved by the Institutional Review Board of Gil Medical Center, Gachon Medical School, Incheon, Korea.

2.3. Drug administration and blood sample collection

This study was based on a single-dose, randomized, two-treatment, two-period crossover design. On the morning of phase 1, after an overnight fasting (over 10 h), volunteers were given a single dose of either formulation (reference or test) of gliclazide with 240 ml of water. Following administration of the drug, a 20% glucose solution was prepared to give to subjects who exhibited symptoms of hypoglycemia. Water intake was permitted 2 h after treatment. No food was permitted for 4 h after treatment. Water, lunch, and dinner were given to all the volunteers according to a schedule. Subjects were not permitted to lie down or sleep for the first 4 h after treatment. Blood samples (approximately 10 ml) for the gliclazide assay were drawn into heparinized tubes through an indwelling cannula before (0 h) and at 0.5, 1, 2, 3, 4, 5, 6, 8, 10, 12, and 24 h after treatment. Blood samples were centrifuged at 2000 *g* for 15 min at 4 °C, and the separated plasma was stored frozen at −20 °C until assayed. After a washout period of 7 days, the study was repeated in the same manner (phase 2) to complete the crossover design.

2.4. Chemicals and reagents

Gliclazide and glyburide (INN: glibenclamide), used as the internal standard (I.S.), were purchased from Sigma–Aldrich (St. Louis, MO, USA) (Fig. 1). LC-grade acetonitrile and isopropyl alcohol, and double-distilled water were used throughout the analysis. All other chemicals and reagents used were of analytical grade.

2.5. Apparatus and LC conditions

The LC system consisted of a Nanospace SI-1 model 2001 pump, a model 2002 UV detector, a model 2023 autoinjector, and a model 2004 column oven (Shiseido Co., Tokyo, Japan). Separation was performed on a Capcell Pak C18 UG120 (5 μm, 250 mm × 1.5 mm i.d.; Shiseido Co.) at column temperature of 26 °C. The mobile phase was composed of 40 mM KH_2PO_4 (pH 4.6 adjusted with phosphoric acid), acetonitrile, and isopropyl alcohol (5:4:1, v/v/v). The flow rate was 0.22 ml/min and the eluate was monitored with UV detection at 229 nm.

2.6. Standards

Stock solutions of gliclazide (1 mg/ml) and the I.S. (glyburide, 1 mg/ml) were prepared in methanol. The working I.S. solution (10 μg/ml) was prepared in methanol everyday.

2.7. Extraction of gliclazide from plasma

The I.S. working solution (100 μl) and 0.5 ml of acetonitrile were added to 0.5 ml plasma samples. After vortex mixing for 10 s, 4 ml of chloroform was added and the mixture was shaken vigorously for 1 min. The mixture was then centrifuged for 15 min at 3000 rpm. The lower organic layer (3 ml) was transferred to a clean glass tube and evaporated to dryness under vacuum at 40 °C. The residue was re-dissolved in 100 μl of mobile phase, and a 30 μl aliquot was injected onto the LC system.

2.8. Pharmacokinetic analysis

Pharmacokinetic analysis was performed using a non-compartmental method. Peak gliclazide concentrations (C_{max}) and the time to C_{max} (t_{max}) were determined by inspection of the individual plasma concentration–time profiles of the drug. The total area under the plasma concentration–time curve (AUC) was calculated using the linear trapezoidal rule. The AUC from 0 to infinity ($AUC_{0-\infty}$) was calculated as $AUC_{0-\infty} = AUC + C_t/k_e$ (where C_t is the last plasma concentration measured). The elimination rate constant (k_e) was determined by linear regression analysis of the log-linear part of the plasma concentration–time

curve. The half-life ($t_{1/2}$) of gliclazide was calculated with as half-life $= \ln 2/k_e$. The clearance (CL/F) of gliclazide was calculated as $CL/F = dose/AUC_{0-\infty}$. The apparent volume of distribution (V_d/F) was calculated as $V_d/F = dose/AUC \cdot k_e$.

2.9. Statistical analysis

Bioequivalence of the two gliclazide products was assessed by calculating individual AUC_{0-last}, $AUC_{0-\infty}$, and C_{max} values. Their ratios (test/reference) using log-transformed data, together with their means and 90% confidence intervals, were analyzed with a parametric method (analysis of variance (ANOVA)) using EquivTest (version 1.0, Statistical Solutions, Cork, Ireland).

The drugs were considered bioequivalent when the difference between two compared parameters was statistically insignificant ($P \geq 0.05$) and the 90% confidence interval for the parameters fell within the range 0.8–1.25.

3. Results and discussion

Fig. 2 shows representative chromatograms of extracted plasma samples. The retention times for gliclazide and glyburide (I.S.) were 4.1 and 6.1 min, respectively (Fig. 2). No endogenous interference was observed with either gliclazide or the I.S. (Fig. 2). The calibration curve was drawn by plotting the peak height ratio versus concentration, which was linear over the range of 0.1–10 μg/ml with the regression equation: $y = 0.3954x - 0.0263$. The intra-day and inter-day precision of the assay was estimated by analyzing four different concentrations of gliclazide

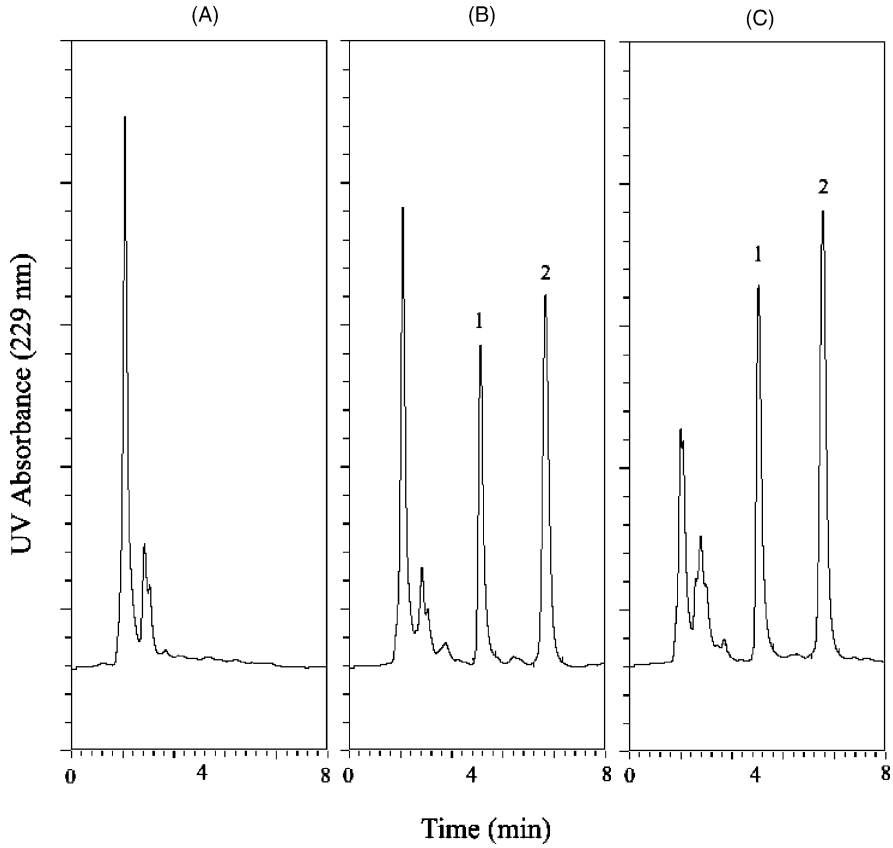

Fig. 2. Representative chromatograms of plasma extracts. (A) Drug-free human plasma; (B) blank plasma spiked with 2 μg/ml gliclazide; and (C) plasma sample from a subject 4 h after an 80 mg oral dose of gliclazide, 1.92 μg/ml. Peak 1: gliclazide and peak 2: internal standard.

Table 1
Intra-day and inter-day coefficient of variation and accuracy in the determination of gliclazide in human plasma

Theoretical concentration (μg/ml)	Intra-day ($n = 6$)			Inter-day ($n = 5$)		
	Concentration found (μg/ml)	CV (%)	Accuracy (%)	Concentration found (μg/ml)	CV (%)	Accuracy (%)
0.1	0.105 ± 0.004	3.9	105.1	0.112 ± 0.009	8.0	111.6
0.2	0.197 ± 0.012	5.9	98.5	0.196 ± 0.015	7.4	95.0
1.0	0.930 ± 0.046	5.0	92.9	0.941 ± 0.054	5.8	91.1
10.0	9.988 ± 0.233	2.3	99.9	10.421 ± 0.429	4.1	100.9

in plasma (Table 1). The results show good reproducibility for the proposed method, with coefficients of variation (CV) for intra-day and inter-day of less than 5.9 and 8.0%, respectively (Table 1). The limit of quantification (LOQ) for gliclazide was 0.1 μg/ml (CV < 8.0%) and the limit of detection (LOD) was 20 ng/ml (signal-to-noise ratio of 3). Recovery was estimated by comparing the peak area of known plasma samples spiked with gliclazide and glyburide with those of the corresponding aqueous solutions, correcting for volume. The recovery of gliclazide, based on peak areas, given as the ratio of extracted normal human plasma/mobile phase when both were previously spiked to a final concentration of 0.1 μg/ml or 1 μg/ml, were 83.5 ± 3.5 and 86.5 ± 4.2% ($n = 4$), respectively. For the I.S., the recovery was 89.1 ± 3.5% ($n = 4$). These results show that the LC analytical method developed here is appropriate for the pharmacokinetic analysis of gliclazide, with good sensitivity and precision. Moreover, despite an analysis of over 500 plasma samples in this study, only a small quantity of the mobile phase (less than 1 l) was used and the analysis was completed within a short period (less than 3 days).

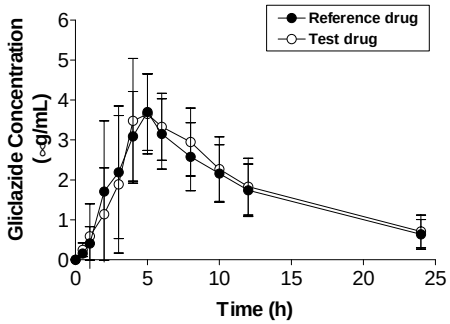

Fig. 3. Mean plasma concentration–time curve for gliclazide after oral administration of 80 mg gliclazide as SCD Gliclazide® (Test drug) or Diamicron® (Reference drug) in 20 healthy subjects.

The mean plasma concentrations of gliclazide after a single oral dose of 80 mg of either formulation of gliclazide in tablet form are shown in Fig. 3. The pharmacokinetic variables for both drugs are shown in Table 2. All calculated pharmacokinetic parameter values were in good agreement with previously reported

Table 2
Pharmacokinetic parameters of gliclazide in two products

Parameters	Reference drug		Test drug	
	Mean ± S.D.	CV (%)	Mean ± S.D.	CV (%)
t_{max} (h)	4.5 ± 1.1	24.7	4.8 ± 1.2	24.9
C_{max} (μg/ml)	4.10 ± 0.96	23.3	4.25 ± 1.08	25.3
k_e (h^{-1})	0.094 ± 0.025	26.1	0.093 ± 0029	31.0
$t_{1/2}$ (h)	7.9 ± 2.3	29.6	8.2 ± 2.8	33.9
AUC_{0-last} (μg/(ml h))	41.3 ± 11.7	28.3	43.1 ± 13.0	30.2
$AUC_{0-\infty}$ (μg/(ml h))	49.7 ± 17.9	36.1	52.9 ± 21.0	39.6
V_d/F (l)	19.4 ± 5.4	28.0	19.2 ± 6.5	34.1
CL/F (l/h)	1.8 ± 0.6	33.4	1.8 ± 0.7	39.3

t_{max}: time to reach peak concentration; C_{max}: peak concentration; k_e: elimination constant; $t_{1/2}$: elimination half-life; AUC: area under concentration–time curve; V_d/F: apparent volume of distribution; and CL/F: oral clearance.

Table 3
Bioequivalence analysis of gliclazide in the test drug and the reference drug

	Reference drug	Test drug	90% CI (test/reference)	Point estimate
C_{max}	4.10 (0.96)	4.25 (1.08)	0.96–1.12	1.05
ln C_{max}			0.97–1.15	
$AUC_{0–last}$	41.29 (11.68)	43.09 (13.03)	1.00–1.09	1.04
ln $AUC_{0–last}$			0.99–1.08	
$AUC_{0–\infty}$	49.67 (17.94)	52.92 (20.97)	1.00–1.10	1.07
ln $AUC_{0–\infty}$			0.99–1.09	

C_{max}: peak concentration; AUC: area under concentration–time curve; and t_{max}: time to C_{max}.

values [1,11] and there was no statistically significant difference between the two formulations. The geometric means and 90% confidence intervals for the SCD Gliclazide® and Diamicron® ratios as log-transformed data are summarized in Table 3. From the mean plasma levels of the 20 Korean subjects who completed the study, the relative bioavailability of SCD Gliclazide® to Diamicron® was 104.4 and 107.0% on the basis of mean $AUC_{0–last}$ and $AUC_{0–\infty}$, respectively (Table 3). For the bioequivalence test, $AUC_{0–last}$, $AUC_{0–\infty}$, and C_{max} were evaluated for the two brands of gliclazide as primary parameters. The means and standard deviations of these parameters for the two brands are very similar, indicating that the pharmacokinetics of gliclazide in the two brands are also similar. ANOVA, after log-transformation of the data, showed no statistically significant difference between the two brands ($P > 0.05$). Furthermore, the 90% confidence intervals for the ratios of test drug to reference drug for $AUC_{0–last}$, $AUC_{0–\infty}$, and C_{max} were also within the range of 80–125%, which is the range accepted by the US and Korean Food and Drug Administration [12,13]. The t_{max} was also analyzed statistically, and the point estimate for individual differences (SCD Gliclazide® versus Diamicron®) was 0.3 h (90% CI of −0.4 to 1.1 h), indicating no significant difference in t_{max} between the two drugs.

The two brands of gliclazide were well-tolerated by the volunteers in both phases of the study, with no hypoglycemic symptoms, and all volunteers completed the study.

4. Conclusion

The developed LC method for the determination of gliclazide using semi-micro column displayed good precision, was fast, and required only a small amount of mobile phase, with a reasonable limit of quantification. When we evaluated the bioequivalence of two 80 mg gliclazide formulation (SCD Gliclazide® and Diamicron®), statistical comparison of $AUC_{0–last}$, $AUC_{0–\infty}$, and C_{max} for two formulations clearly indicated no significant difference. Ninety percent of log-transformed data for the mean ratio (test/reference) of parameters $AUC_{0–last}$, $AUC_{0–\infty}$, and C_{max} for the two formulations had values entirely within the accepted range for bioequivalence of 80–125%. The results of the present study indicate that SCD Gliclazide® 80 mg tablets are bioequivalent to Diamicron® 80 mg tablets, and the two products are clinically interchangeable.

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