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Pharmacokinetic and Pharmacodynamic Characterization of Gliclazide in Healthy Volunteers

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Pharmacokinetic and pharmacodynamic properties of gliclazide were studied after an oral administration of gliclazide tablets in healthy volunteers. After an overnight fasting, gliclazide tablet was orally administered to 11 volunteers; Additional 10 volunteers were used as a control group (i.e., no gliclazide administration). Blood samples were collected, and the concentration determined for gliclazide and glucose up to 24 after the administration. Standard pharmacokinetic analysis was carried out for gliclazide. Pharmacodynamic activity of the drug was expressed by increase of glucose concentration (ΔPG), by area under the increase of glucose concentration-time curve ($AUC_{\Delta PG}$) or by the difference in increase of glucose concentration ($D_{\Delta PG}$) at each time between groups with and without gliclazide administration. Pharmacokinetic analysis revealed that C_{max}, T_{max}, CL/F (apparent clearance), V/F (apparent volume of distribution) and half-life of gliclazide were 4.69±1.38 mg/L, 3.45±1.11 h, 1.26±0.35 L/h, 17.78±5.27 L, and 9.99±2.15 h, respectively. When compared with the no drug administration group, gliclazide decreased significantly the $AUC_{\Delta PG}$ s at 1, 1.5, 2, 2.5, 3 and 4 h ($p<0.05$). The ΔPGs were positively correlated with $AUC_{gliclazide}$ at 1 and 1.5 h ($p<0.05$), and the correlation coefficient was maximum at 1 h ($r = 0.642$) and gradually decreased at 4 h after the administration. The $AUC_{\Delta PG}$s were positively correlated with $AUC_{gliclazide}$ at 1, 2, 3 and 4 h ($p<0.05$), and the maximum correlation coefficient was obtained at 2 h ($r=0.642$) after the administration. The $D_{\Delta PG}$ reached the maximum at 1 h, remained constant from 1 h to 3 h, and decreased afterwards. Therefore, these observations indicated that maximum hypoglycemic effect of gliclazide was reached at approximately at 1.5 h after the administration and the effect decreased, probably because of the homeostasis mechanism, in health volunteers.

Key words: Gliclazide, Pharmacokinetics, Pharmacodynamics, Human

INTRODUCTION

Gliclazide is a commonly used sulphonylurea hypoglycemic agent in non-insulin dependent diabetes mellitus (NIDDM). There have been several studies of the pharmacokinetics and pharmacodynamics of gliclazide in diabetic patients (Kobayashi *et al.*, 1981; Shiba *et al.*, 1986; Davis *et al.*, 2000; Palmer and Brogden, 1993). For example, Glowka, *et al.* (1998) reported the pharmacokinetic and pharmacodynamic properties of gliclazide in healthy European volunteers. Although there appears no appreciable difference in pharmacokinetics of gliclazide between Japanese and Caucasian subjects (Palmer and Brogden, 1993), racial differences in the pharmacokinetics and pharmacodynamics of a variety of drugs have been well documented. In addition, pharmacokinetic and pharmacodynamic studies has not been carried out for gliclazide in healthy Korean volunteers. Therefore, we have studied pharmacokinetic properties of gliclazide and assessed the glucose-lowering effect for the drug in healthy volunteers.

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METHODS

Subjects

Healthy Korean adult volunteers participated in the study in two groups. Control group consisted of six male and four female volunteers ranged in age between 23 and 40 years of age (in mean±S.D. 28.9±6.2 years) with the body weight of 63.4±8.8 kg. Test group consisted of 11 male volunteers ranged in age between 23 and 30 years (in mean±S.D. 24.5±2.1 years) with the body weight of

Table I. Demographic data of volunteers.

	Control group	Test group	p value
Number (female/male)	10(4/6)	11(0/11)	Not applicable
Age (year)	28.9 ± 6.2	24.5 ± 2.2	0.04
Body weight (kg)	63.4 ± 8.8	69.6 ± 5.7	0.07
Height (cm)	168.9 ± 6.3	176.2 ± 4.0	0.005
Baseline plasma glucose (mg/dL)	91.4 ± 8.3	85.5 ± 5.2	0.06

69.6±5.7 kg. The demographic data of volunteers were showed at Table I. There were no significant differences in body weight and basal glucose concentration between two groups. All subjects were selected after completing a through history and physical examination, and after a normal laboratory examinations which were consisted of hematology, serum chemistry and urinalysis. All subjects were presented with full details of the investigation prior to the written consent. In addition, each subject gave written informed consent to study procedures that were previously approved by the institutional review board of the Institute of Drug Development, Chungnam National University (Daejeon, Korea).

Study design

This study was carried out according to non-blinded, open-label, and parallel study design. All subject were fasted for at least 10 h prior to the timing of the dose. At time zero, an intravenous cannula was inserted into a forearm vein and blank blood samples were collected for the assay of plasma glucose level. After baseline blood sampling, gliclazide tablet (Diamicron®, 80 mg) was orally given to the test group with 240 mL water. The control group received only 240 mL of water without gliclazide administration. In this study, control group was used to calculate the difference of the plasma glucose level with or without the gliclazide administration. All volunteers were consumed with 12 g of white sugar after drug or water administration. All subjects, including the control group, abstained from food intake until the 4 h after the administration Blood samples were collected for the determination of the plasma gliclazide concentration at 0.5, 1, 1.5, 2, 2.5, 3, 4, 5, 7, 9, 12 and 24 h after the oral administration. In addition, plasma glucose concentration was measured at 0.5, 0.75, 1, 1.25, 1.5, 2, 2.5, 3 and 4 h after the administration. Immediately following blood withdrawal, the sample were kept in ice bath and centrifuged within 30 min of the sampling. The plasma samples were, then, stored in 70°C until the analysis.

Determination of gliclazide and glucose in the plasma

In the present work, gliclazide concentration of plasma was determined by HPLC. The procedure was essentially identical to the published method (Davis *et al.*, 2000; Kimura *et al.*, 1980) with a slight modification. Briefly, 400 μL of serum and 40 μL of internal standard (glibenclamide, 20 μg/mL in 50% methanol) were mixed with 4.5 mL of chloroform. The mixture was shaken vigorously for 30 min and centrifuged for 15 min at 1,400 g. The chloroform layer was transferred to a fresh tube and evaporated to dryness under a stream of nitrogen at 50°C. The residue was re-dissolved with 100 μL of acetonitrile and 200 μL of 50% methanol, and 20 μL aliquot of the mixture was injected on to the HPLC column. The separation was carried out on a C18 column (Polaris 5 m C18-A column, MetaChem Co, 250×4.6 mm) at 30°C. The mobile phase was composed with a mixture of 0.04 M KH_2PO_4 (pH 5.2), acetonitrile, and isopropyl alcohol (45:46:9 v/v/v), and was delivered at a flow rate of 1.0 mL/min. The eluting peaks were monitored at a wavelength of 227 nm. Gliclazide peak was clearly separated from peaks of plasma component and internal standard (Fig. 1). The gliclazide concentration in the sample was determined using a calibration curve. Assay parameter of this study was the peak area ratio of gliclazide to internal standard. The calibration curve was linear (correlation coefficient, r=0.999) over the range of 0.2-10 μg/mL. The intra-day coefficients of variation were less than 9.0% and the inter-day coefficients of variation were less than 11% for plasma assays at all concentrations of the quality control samples which include limit of quantification.

The plasma glucose concentration was determined by a hexosekinase/UV method (Stanbio Laboratory, Texas, USA). The calibration curve was linear (correlation coefficient, r= 0.995) over the range of 0-500 mg/dL. The intra-day coefficients of variation were 1.6% and the inter-day coefficients of variation were less than 3.0% for plasma assays.

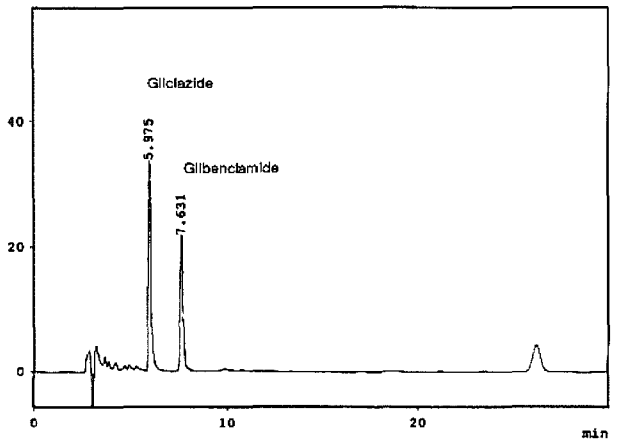

Fig. 1. HPLC chromatogram of a representative plasma sample following oral administration of gliclazide 80 mg.

Pharmacokinetic, Pharmacodynamic, and statistical analysis

The pharmacokinetic parameters of gliclazide were calculated by non-compartmental pharmacokinetic methods. The peak plasma gliclazide concentration (C_{max}) and the time required to reach the peak (T_{max}) were read directly from the concentration-time graph of each subject. The area under the plasma gliclazide concentration-time curve from time zero to 24 h ($AUC_{gliclazide\text{-}24}$) was calculated using a linear trapezoidal rule. The elimination rate constant (k_e) was first estimated by log-linear least squares regression analysis of the last 4 concentration-time data pairs (7-24 h). The elimination half-life ($t_{1/2}$) was calculated using the relationship:

$$t_{1/2} = \frac{0.693}{k_e}$$

The pharmacodynamic measures were also evaluated from the change of glucose concentration from baseline (ΔPG). When it was necessary, the area under the ΔPG time curve from time zero to each time ($AUC_{\Delta PG}$), calculated by the linear trapezoidal rule, was used to correlate pharmacokinetics of gliclazide. In other cases, the difference of incremental glucose concentration ($D_{\Delta PG}$) between control (i.e., no drug administration) and drug administration group was calculated by subtracting mean ΔPG of test group from mean ΔPG of control group at each time point.

All data were expressed as means±standard deviation. Statistical analysis was carried out by parametric methods using SPSS 10.0 for Windows (SPSS Inc., Chicago, Illinois, USA). Two-sample comparisons were performed using students *t*-tests. The correlations between variables were assessed using linear regression. A p value below 0.05 was considered to be statistically significant.

RESULTS AND DISCUSSION

Pharmacokinetic analysis

All subjects completed study without any adverse effects and there were no episode of hypoglycemia. The temporal profile of mean plasma gliclazide concentration is shown in Fig. 2. The pharmacokinetic parameters of gliclazide (Diamicron®, 80 mg) in Korean are summarized in Table II. C_{max}, T_{max}, and $t_{1/2}$ are 4.69±1.38 mg/L, 3.45± 1.11 h, and 9.99±2.15 h respectively. These values are comparable to those of previous study (Glowka *et al.*, 1998).

Hypoglycemic effect of gliclazide

Temporal profile of plasma glucose level is shown in Fig. 3. In the control (i.e., no gliclazide administration) group, plasma glucose concentration reached the maximum at 0.75 h, was returned to basal concentration at 1.5 h and

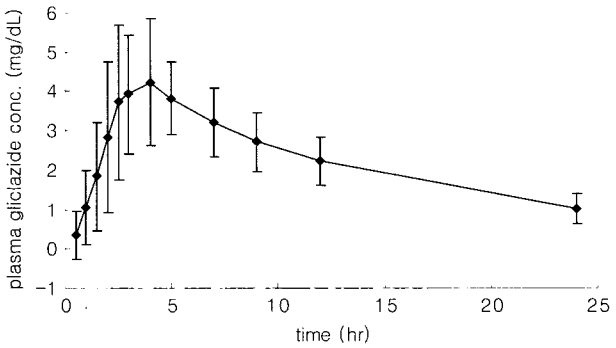

Fig. 2. Mean plasma gliclazide concentration-time profile of healthy subjects following oral administration of gliclazide 80 mg. Data are expressed as mean standard deviation.

Table II. Comparisons of demographic data and pharmacokinetic parameters between Korean and European

	Korean	European*
Number of subjects	11	10
Age (year)	24.5 ± 2.2	24.9 ± 5.9
Body weight (kg)	69.6 ± 5.7	65.2 ± 9.5
Gliclazide Formulation	Diamicron® 80 mg	Diabezid® 80 mg
$AUC_{0\text{-}\infty}$ (mg · h/L)	68.39 ± 20.29	47.36 ± 4.0
C_{max} (mg/L)	4.69 ± 1.38	4.56 ± 0.63
T_{max} (h)	3.45 ± 1.11	4.10 ± 0.44
$t_{1/2}$ (h)	9.99 ± 2.15	9.90 ± 0.82

*The value from Glowka *et al.* (1998)

was maintained thereafter until 4 h (the last collection time) after sugar administration. In gliclazide administration group, plasma glucose concentration reached the maximum at 0.75 h and returned to basal concentration at 1 h. The concentration was further decreased at 1.5 h after the administration, maintained thereafter until 3 h after sugar administration and plasma glucose concentration was

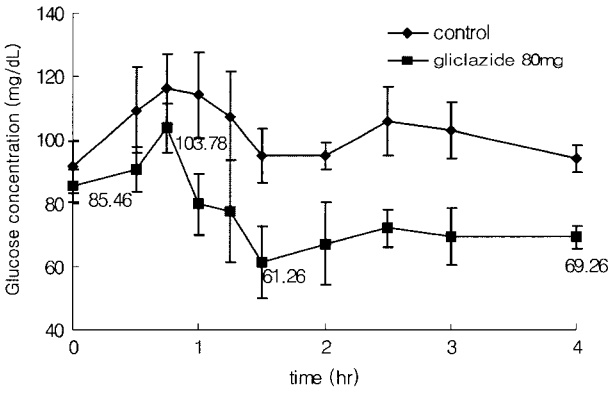

Fig. 3. Mean plasma glucose concentration-time curves in healthy subjects in gliclazide and control (i.e., no drug administration) groups. Data are expressed as mean standard deviation. Numbers represent mean plasma glucose concentrations of each time.

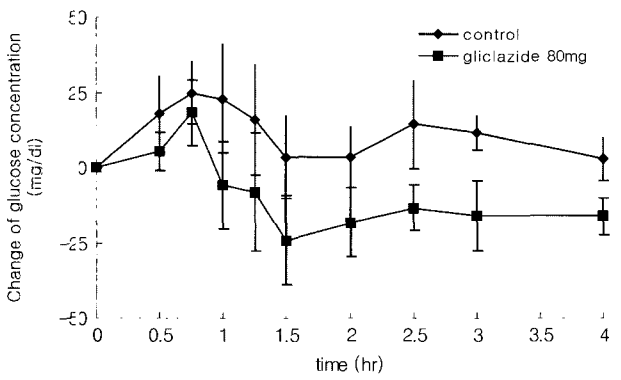

Fig. 4 Mean change of plasma glucose concentration-time curves in healthy subjects in gliclazide and control (i.e., no gliclazide administration) groups. Data are expressed as mean standard deviation. Numbers represent mean plasma glucose concentrations of each time.

increased at 4 h. As a result, plasma glucose concentration at 4 h is significantly higher than that of 1.5 h (Fig. 3). Change of glucose concentration from baseline (ΔPG) profile is depicted in Fig. 4. The maximum ΔPG ($C_{max,\Delta PG}$) of control group was 33.31±7.79 mg/dL and was significantly different from that of gliclazide administration group (18.71±10.26) (Table III). Gliclazide significantly inhibited the increase of plasma glucose concentration from 1 h to 4 h after the sugar consumption of 12 g (Fig. 3). Gliclazide decreased significantly the $AUC_{\Delta PG}$s at 1, 2, 3 and 4 h after the sugar consumption of 12 g (Table IV). The time

Table III. Pharmacodynamic parameters of plasma glucose after gliclazide administration and no gliclazide administration (i.e., control groups)

Gliclazide administration group			Control group		
Subject	$C_{max,\Delta PG}$ (mg/dL)	$T_{max,\Delta PG}$ (h)	Subject	$C_{max,\Delta PG}$ (mg/dL)	$T_{max,\Delta PG}$ (h)
1	31.46	0.75	1	30.32	0.75
2	26.03	0.75	2	35.76	1
3	26.84	0.75	3	41.97	0.5
4	14.32	0.75	4	37.31	1
5	26.01	0.75	5	50.16	1
6	5.84	0.75	6	26.75	1
7	30.31	0.75	7	24.11	0.75
8	7.0	0.5	8	24.91	0.75
9	18.6	0.75	9	29.11	1
10	2.73	0.5	10	32.66	1
11	16.66	0.75			
Mean±S.D	18.71±10.26*	0.70±0.10*	Mean±S.D	33.3±17.79	0.88±0.17

ΔPG : Change of glucose concentration from baseline.
$C_{max,\Delta PG}$: maximum ΔPG.
$T_{max,\Delta PG}$: time to maximum ΔPG.
*p<0.05, student *t*-tests.

Table IV. The area under the increase of glucose concentration-time curve ($AUC_{\Delta PG}$) of glicalzide administration group and control (no glicalzide administration) group

Time (h)	Glicalzide administration group	Control group	Difference
1	5.78 ± 5.95 (mg · h/dL)	15.81 ± 6.88 (mg · h/dL)	S
2	-10.60 ± 17.22 (mg · h/dL)	24.91 ± 18.58 (mg · h/dL)	S
3	-25.85 ± 20.43 (mg · h/dL)	35.88 ± 27.32 (mg · h/dL)	S
4	-41.96 ± 26.86 (mg · h/dL)	42.99 ± 30.16 (mg · h/dL)	S

Mean ± SD
S: significantly different, $p<0.05$, student *t*-tests

to maximum ΔPG ($T_{max,\Delta PG}$) of placebo group was 0.88± 0.17 h, which was significantly different from that of giclazide administration group (0.70±0.10 h) (Table III). Six out of 10 subjects showed maximum ΔPG at 1 h after the sugar consumption in the control group. In contrast, in gliclazide administration group, 9 of 11 subjects showed maximum ΔPG at 0.75 h and there was no subject with $T_{max,\Delta PG}$ of 1 h (Table III). The ΔPG of the control group was not significantly different from that of gliclazide administration group at 0.75 h, and $T_{max,\Delta PG}$ and $C_{max,\Delta PG}$ were apparently different between two groups. These observations indicated that the hypoglycemic effect of gliclazide was appeared at 1 h. At 1 h, the plasma concentration of gliclazide was 1.05±0.94 mg/L but the difference of ΔPG between two groups was at the maximum. The $D_{\Delta PG}$ was maintained maximum (28.82 mg/dL) up to 3 h and was decreased to 18.89 mg/dL at 4 h when the plasma concentration of gliclazide was maximum (Fig. 5). The correlations between pharmacodynamic parameters ($D_{\Delta PG}$ and $AUC_{\Delta PG}$) and pharmacokinetic parameter ($AUC_{gliclazide}$) were studied with linear regression analysis at 1, 1.5, 2, 2.5, 3, 4 h after the administration. For the correleation between $D_{\Delta PG}$ and $AUC_{gliclazide}$, the degree of correlation was highest ($r=0.636$, $p<0.05$) at 1 h and was decreased gradually; At 2 h after the administration, the

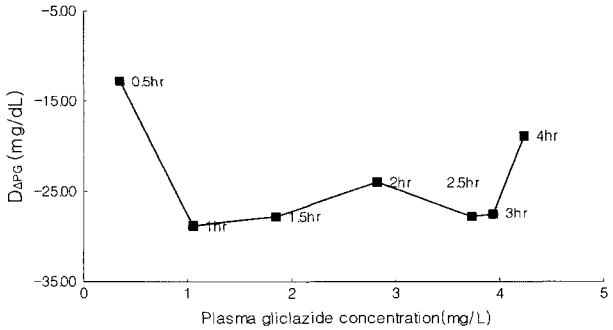

Fig. 5. Relationship among plasma gliclazide concentration, difference of incremental glucose concentration between control and drug administration group ($D_{\Delta PG}$) and time. The numbers given in the figure represent the time after gliclazide administration.

Table V. The correlation coefficients between pharmacodynamic parameters (viz, $D_{\Delta PG}$ and AUC_{DPG}) and $AUC_{gliclazide}$ at 1, 1.5, 2, 2.5, 3, 4 h after the gliclazide administration.

Time (h)	$AUC_{gliclazide}$ vs. $D_{\Delta PG}$	$AUC_{gliclazide}$ vs. $AUC_{\Delta PG}$
1	0.636*	0.571*
1.5	0.542*	0.615*
2	0.014	0.642*
2.5	0.265	0.640*
3	0.133	0.583*
4	0.177	0.460*

ΔPG: Change of glucose concentration from baseline.
$AUC_{\Delta PG}$: Area under the increase of glucose concentration-time curve.
$AUC_{gliclazide}$: Area under gliclazide concentration-time curve.
*$p<0.05$

correlation did not reach a statistical significance (Table V). For the correlation between $AUC_{\Delta PG}$ and $AUC_{gliclazide}$, the degree of correlation was highest ($r=0.642$, $p<0.05$) at 2 h and was decreased gradually to 4 h (r=0.460) (Table V). Therefore, the correlation of $AUC_{\Delta PG}$ and $AUC_{gliclazide}$ was apparently better than that of $D_{\Delta PG}$ and $AUC_{gliclazide}$ at every time except 1 h after the administration (Table V). These observations indicated that the hypoglycemic effect of gliclazide was already appeared at 1 h and that homeostasis mechanism may occur at 1.5 h after the administration in healthy volunteers. Campbell reported that serum gliclazide concentration of 1.5 mg/mL represents the threshold for hypoglycemic effect (Campbell *et al.*, 1980). In contrast, plasma gliclazide concentration of 1.05±0.94 mg/mL showed hypoglycemic effect in this study. The underlying mechanism for the discrepancy in the gliclazide concentration required for the hypoglycemic effect was directly studied. However, racial difference and/or experimental difference (e.g., pharmacodynamic index) may have contributed for the dissociation. This aspect of gliclazide pharmacodynamics is currently under investigation in this laboratory.

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