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Pharmacokinetics and relative bioavailability of tablet of micronized glibenclamide in 4 Chinese healthy men

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ABSTRACT Pharmacokinetic characteristics and relative bioavailability of the regular preparation (RG) and micronized preparation (MG) of glibenclamide (Gli) were studied in 4 Chinese healthy men. Each volunteer entered 2 consecutive experiments at the same dose (10.5 mg) of RG and MG tablets given orally. Blood samples were drawn before and 0.5, 1, 2, 3, 4, 6, 8, 10, and 12 h after *po*. A selective HPLC method with a uv spectrophotometric detector (sensitivity; 16 ng · ml^{-1}) was established for determining serum Gli concentration. C_{max} for RG was 212 ±41 ng · ml^{-1}, for MG 529±73 ng · ml^{-1}. T_{max} for RG and MG were 3.5±0.6 and 2.2±0.3 h, respectively. The relative oral bioavailablity of MG was found to be 77%, increased approximiately 1.7-fold that of RG. The other pharmacokinetic parameters (V_d, Cl, $T_{\frac{1}{2}}$) were substantially the same after ingestion of GR and MG.

KEY WORDS glyburide; pharmacokinetics; dosage forms; high pressure liquid chromatoraphy; human experimentation

Glibenclamide (Gli, glyburide) is one of the most potent second generation oral antidiabetic drugs of sulfonylurea series. Because Gli has a hypoglycemic activity several hundred fold that of tolbutamide and a longer duration of action, individualizing Gli dosage by Therapeutic Drug Monitoring is important. Gas chromatographic[1] or radioimmunological[2] and a more efficient HPLC method[3,4] have been developed. The pharmacokinetic data were divergent[1,5]. It may be the uncontrolled experimental condition and the bioavilability difference of different preparations. A highly micronized preparation (MG, 3.5 mg/tablet) was made by H Trommsdorff Pharmaceutical Co. This paper compared the bioavailability characteristics of MG with the existing RG in Chinese health men.

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MATERIALS AND METHODS

Experiments in healthy volunteers Four young male graduate students, aged 26—28 a, were undertaken the 2 experiments of MG and RG. The washout period between these 2 experiments was at least 1 mouth. There was no evidence of illness suggested by history, clinical, and laboratory examination. Either MG or RG tablet containing 10.5 mg (base) was given *po* at 08 : 30 in about 1 h after a standardized carbohydrate breakfast. Blood samples were collected from antecubital vein before and 0.5, 1, 2, 3, 4, 6, 8, 10, and 12 h after the dose. The blood samples were allowed to clot and stood for 45 min. The serum samples were obtained by centrifugation at 300 × *g* for 20 min and stored at −18℃ until HPLC analysis[6].

Sets of apparatus Shimadz HPLC included bidirectional pump (LC—6A), variable wavelength uv detector (SPD—6AV), automatic injector (Rheodyne U6K) with 100 μl samples loop, and data processor (L—R4A). The Shimpack CLC—ODS reversed-phase column (150 × 6.0 mm ID) was used at 50℃. The mobile phase consisted of acetonitrile and water (55 : 45. vol : vol), with pH 4.7 and kept at 1.5 ml · min^{-1} flow rate. The uv absorbance wavelength

of the eluate was 230 nm.

Drug and reagents Standard substance (Gli and tolbutamide as internal standard) were produced by Research Laboratory of Shanghai Xing-Yi Pharmaceutical Factory; RG tablet — Xing-Yi Pharmaceutical Factory; MG — H Trommsdorff Pharmaceutical Co; acetonitrile (HPLC grade) and benzene (AR grade) both from Shanghai First Reagent Factory.

Stock solution was Gli 1 mg · ml^{-1} in methane, its working standard solution was of concentration 1—10 $\mu g \cdot ml^{-1}$ diluted with mobile phase. The stock of internal standard (IS) was also of concentration 1 mg · ml^{-1} in methane and during assay IS was diluted to 5 $\mu g \cdot ml^{-1}$ with mobile phase. All the stocks were kept at −4℃.

HPLC analysis Into samples of 0.5 ml blank serum, a series of Gli (10—350 ng) working standard solution were added, followed by the addition of IS working solution 50 μl and 0.5 ml HCl (50 mmol · L^{-1}) successively. The tubes were capped, and shaken gently. After 3 ml bnezene were added, the tubes were reshaken for 10 min and centrifuged at 300 × *g* for 5 min. The residue in the tubes were extracted once more by 3 ml benzene. The combined supernatant was evaporated to dryness over a 45℃ water bath with a stream of nitrogen. The residue was dissolved in 150 μl of mobile phase for HPLC analysis.

The serum samples collected from volunteers were assayed for Gli concentration in the same way without adding the Gli.

Calculation Gli concentration were processed by computer. The PK parameters were compared by *t* test.

RESULTS

Quality control of Gli HPLC assay A typical chromatogram of serum extract is shown in Fig 1. The retention time of IS and Gli were 4.3 and 7.9 min, respectively. The peaks were sharp, well separated, and not interfered by serum. The calibration curve was linear over the range of 10—350 ng (r = 0.9993) (Fig 2). The sensitivity was 16 ng per 1.0 ml serum extract. The precision and recovery of this HPLC assay were shown by Tab 1, during within-day replication the coefficient of variation ranged from 1.9% to 5.3% and the recovery 95.6% to 100.3% (n =4), while the between-day studies gave coefficient of variation from 3.4% to 3.9 and recovery 96.7% to 102.8% (n =5).

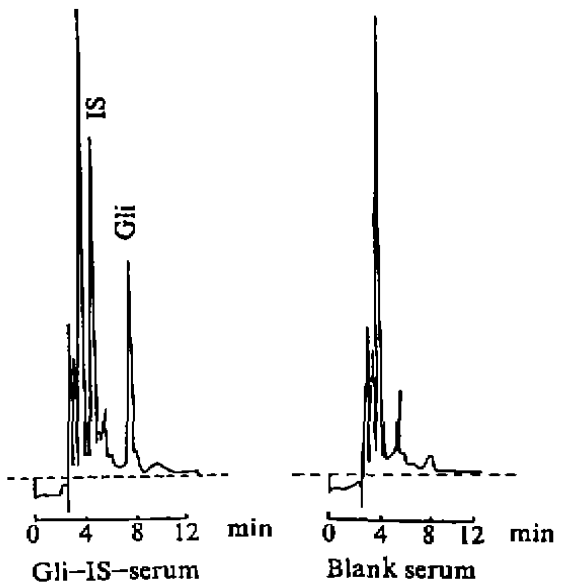

Fig 1. Chromatograms of blank serum and glibenclamide (Gli) 150 ng and internal standard (IS) tolbutamide 250 ng.

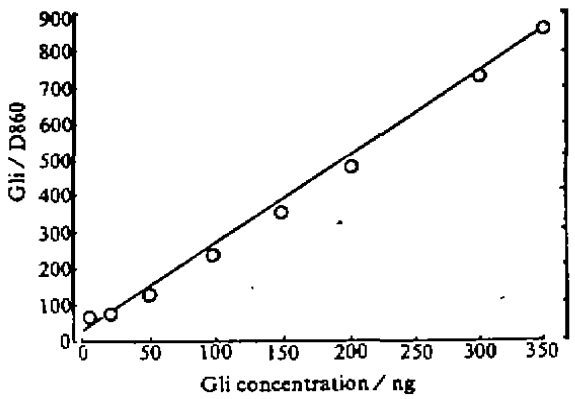

Fig 2. Peak height ratio (Gli/D860) and Gli concentration relationship.

Tab 1. Precision and recovery of Gli HPLC assay.

Added Gli/ ng · ml^{-1}	Recovered/ ng · ml^{-1} ($\bar{x}\pm s$)	Recovery/ %	CV/ %
Within-day			
50	49.5±1.4	99.0	2.8
150	143.4±7.7	95.6	5.4
300	299.4±6.2	99.8	2.1
Between-day			
50	51.4±1.8	102.8	3.5
150	149.9±6.0	99.9	4.0
300	289.9±9.9	96.7	3.4

PK characteristics after *po* of MG or RG

The Gli concentration-time curve was calculated individually for each of the 8 experiments. The profile of the Gli mean serum concentration time curve for the 2 occasions indicated a much higher serum available concentration for the micronized preparation than that for the regular preparation. (Fig 3).

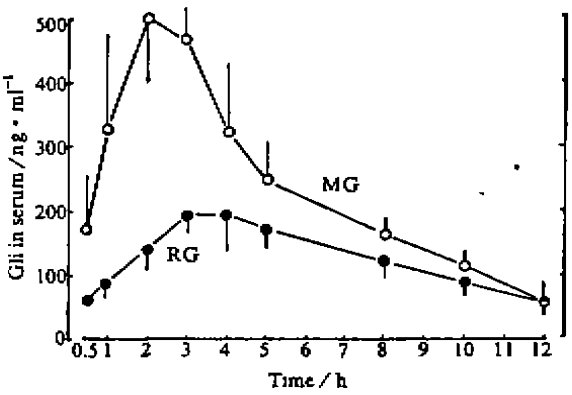

Fig 3. Gli concentration in serum of 4 healthy volunteers after *po* 10.5 mg of MG or RG.

Tab 2 summaraized all the PK data of the 2 Gli preparation measured on the same 4 volunteers. T_{max} for RG was 3.5±0.6 h, for MG shortened to 2.3±0.3 h ($P<0.05$); C_{max} for RG was 212±41 ng · ml^{-1}, for MG increased to 529±73 ng · ml^{-1} ($P<0.01$); $T_{\frac{1}{2}}$ for RG was 4.7±0.5 h, for MG 4.2±0.3 h ($P>$

Tab 2. Pharmacokinetics of Gli in 4 healthy men following oral administration of 10.5 mg of micronized preparation (MG) or regular preparation (RG). $^{*}P>0.05$, $^{**}P<0.05$, $^{***}P<0.01$ *vs* RG.

Body weight		63 kg	58 kg	76 kg	72 kg	$\bar{x}\pm s$
Lag-time/h	RG	0.22	0.34	0.23	0.34	0.28±0.07
	MG	0.03	0.11	0.07	0.11	0.08±0.04**
K_{el}/h	RG	0.15	0.15	0.17	0.13	0.15±0.02
	MG	0.16	0.19	0.16	0.19	0.18±0.02*
T_{max}/h	RG	3.00	3.89	4.16	2.92	3.49±0.63
	MG	1.96	2.18	2.58	2.35	2.27±0.26**
C_{max}/ng·ml^{-1}	RG	179	271	204	193	212±41
	MG	636	479	511	488	529±73***
$T_{\frac{1}{2}}$ /h	RG	4.62	4.50	4.14	5.40	4.7±0.5
	MG	4.42	3.73	4.38	3.66	4.2±0.3*
Cl /L·h^{-1}	RG	2.98	2.18	2.48	3.15	2.7±0.5
	MG	2.17	3.28	2.29	3.19	2.7±0.6*
V_d/L	RG	18.67	14.38	13.95	24.82	18.1±5.0
	MG	13.85	16.02	14.81	15.97	15.1±1.0*
AUC/h·ng·ml^{-1}	RG	1584	2166	1902	1500	1788±305
	MG	3700	2452	3504	2519	3044±150**

0.05); V_d for RG was 18±5 L, for MG 15±1 L ($P>0.05$); Cl for RG was 2.7±0.5 L·h^{-1}, for MG 2.7±0.6 L·h^{-1} ($P>0.05$); $AUC_{0-\infty}$ for RG was 1.8±0.3 μg·h·ml^{-1}, for MG raised to 3.0±0.2 μg·h·ml^{-1} ($P<0.05$).

DISCUSSION

Our HPLC method of measuring serum Gli concnetration is a modification from Wahlin-Boll[6]. A different mobile phase acetonitrile is used and our method overrides the old one by the advantage of excellent sensitivity, linearity, and reproducibility.

The increased ratio (170%) of AUC_{MG}/AUC_{RG} showed an obvious increase (1.7 fold) in the relative oral bioavailability of this new micronized preparation. According to Rupp *et al*[5] who referred the bioavailability of RG to be 45% of the dose ingested on this basis. This indicates a fairly complete gastro-intestinal absorption and very low first-pass metabolism rate in liver.

The PK of MG is also characterized by rapid and fairly complete absorption. Its maximum serum concentration is more than twice higher than RG, and T_{max} for the concentration to reach the peak is about 1 h shorter. By contrast, the other PK parameters, such as V_d, Cl, $T_{\frac{1}{2}}$ of the two preparations showed no substantial difference, thus indicating that they remain to be the same substance present in the circulation and encounter the same process of distribution and elimination.

In another study of this group on the dose-concentration relationship of MG, the comparison of $AUC_{0-\infty}$ and C_{max} was made in one subject who ingested 2 doses of MG 10.5 and 5.25 mg (50% dose-reduciton) on 2 occassions. The ratios of $AUC_{0-\infty}$ and C_{max} computed from the 2 experiments were demonstrated to be 2:1, suggesting perfect dose-concentration correlation of micronized preparation.

The increased bioavailability, higher blood concentrtion and shortened T_{max} of the new MG preparation are obviously beneficial to diabetic patients. Thus the dosage of MG can be minimized in order to reduce rate of side-effects and the patients's high blood sugar levels can be in due time well controlled.

In conclusion, the new micronized preparation of Gli was proved to be a new and good generation over the regular Gli preparation.

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格列苯脲微粉片剂在4名健康中国男性志愿者中的药物动力学和相对生物利用度

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摘要 改良了血清格列苯脲 Gli 浓度的反相 HPLC 法，测定了4名口服普通型和微粉型 Gli 制剂的志愿者血药浓度。对比两种剂型 Gli 药物动力学及相对生物

利用度。结果显示：微粉型和普通型 Gli 的 C_{max} 分别为 529±73 ng·ml^{-1} 和 212±41 ng·ml^{-1}；T_{max} 前者为 2.2±0.3 h，后者为 3.5±0.6 h。微粉型 Gli 的相对生物利用度(F)约为 77%。两种剂型的其它药动学参数，如 $T_{\frac{1}{2}}$，V_d，Cl 等无显著性差异。

关键词 格列苯脲；药物动力学；剂型；高压液相色谱法；人类实验

药代动力学

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Effects of lisinopril and captopril on calcium in rat heart

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ABSTRACT We studied the effects of lisinopril (Lis) and captopril (Cap), two angiotensin-converting enzyme inhibitors, on calcium in ischemia/reperfusion and normal rat hearts. Ischemia/reperfusion hearts were subjected to 15 min ischemia followed by 1 or 30 min reperfusion. Lis 0.1 μmol·L^{-1} and Cap 200 μmol·L^{-1} decreased the concentration of calcium in ischemia/reperfusion hearts (the content of calcium in reperfusion 1 min heart were reduced from 4.0±0.6 to 2.7±0.5 and 3.0±0.9 μmol/g dry wt respectively). In cultured cell of neonatal rat heart, both drugs inhibited the uptake of $^{45}Ca^{2+}$. The activity of Na^+, K^+-ATPase prepared from rat heart was increased (activity increased from 15.7±2.3 in control group to 21.2±2.0 and 22.0±3.1 μmol/h mg protein in Lis and Cap groups, respectively). This calcium lowering effects of Lis and Cap may be important in protecting the ischemia/reperfusion damage of myocardium.

KEY WORDS angiotensin-converting enzyme inhibitors; cultured cells; ventricular fibrillation; calcium radioisotopes; heart; myocardial reperfusion injury

Angiotensin-converting enzyme inhibitor (ACEI) have protecting effects against hypertension and heart failure clinically. Recently ACEI has been ascribed to decrease the size of myocardial infarction and alleviate the damage caused by ischemia/reperfusion in animal heart both *in vitro* and *in vivo*[1-3]. Our experiments have demonstrated that lisinopril (Lis) protected the myocardial ischemia/reperfusion injury in Langendorff rat heart[4,5]. Myocardial injury during ischemia/reperfusion was thought to be associated with massive accumulation of intracellular calcium and the increase of free radicals. In the present study, effects of Lis and Cap on calcium in the rat heart were studied.

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MATERIALS AND METHODS

Lis was a gift from Merck Sharp & Dobme Research Lab, NJ, USA; Cap was a product of Dandong Pharmaceutical Factory, China; $^{45}CaCl_2$ (999 MBq·g^{-1}) was purchased from China Institute of Atomic Energy, Beijing. Wistar rat of either sex weighing 250±*s* 30 g were provided by our Academy.

Effects on ventricular fibrillation (VF) in ischemia/reperfusion rat heart Working heart and Langendorff hearts were perfused with buffer contained: NaCl 117.2, KCl 5.37, $MgSO_4 \cdot 7H_2O$ 0.8, $NaH_2PO_4 \cdot 2H_2O$ 0.92, $CaCl_2$ 2.47, $NaHCO_3$ 25, EDTA 0.05, glucose 11.1 (mmol·L^{-1}), which was equilibrated with 95% O_2+5% CO_2, pH 7.4, at 36.5 and 37.5℃. The perfusion buffer was filtered through a G_3 filter before reaching the heart. Preload and afterload were held at 1.17 and 6.93 kPa, on the work-