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ORIGINAL RESEARCH

*CYP2C19*2* Polymorphism Is Associated with Impaired Oral Clearance of Gliclazide in Healthy Chinese

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Background: Previous studies suggest gliclazide is metabolised primarily by CYP2C19 rather than CYP2C9, unlike other sulphonylureas. *CYP2C19 *2* and *3* polymorphisms are more common in Asians.

Methods: We investigated the effect of CYP2C19 polymorphisms on gliclazide pharmacokinetics in 15 healthy male Chinese subjects after a single 80mg oral dose.

Results: In *CYP2C19* poor metabolisers (**2/*2*, n=4), plasma area-under-the-curve was higher by nearly two-fold compared with intermediate metabolisers (**2* and **3* heterozygotes, n=7) and extensive metabolisers (**1/*1*, n=4) ($p<0.001$). Apparent oral clearance was mean (SD) 0.70 (0.12), 1.22 (0.22) and 1.52 (0.47) mL/min/kg in poor, intermediate and extensive metabolisers, respectively ($p = 0.005$).

Conclusion: *CYP2C19*2* polymorphism is associated with increased total gliclazide concentration and reduced oral clearance. Pharmacogenetic studies are warranted on the impact of CYP2C19 polymorphisms on treatment response and hypoglycaemia.

Keywords: gliclazide, pharmacogenetics, CYP2C19

Introduction

Gliclazide is a second-generation sulphonylurea which acts on the sulphonylurea receptor SUR-1 thereby stimulating insulin release.[1] Gliclazide is a first choice sulphonylurea in many countries as it is perceived to have a lower hypoglycemic risk due to its shorter action and inactive metabolites that are renally eliminated.[2] Based on our local data, nearly 50% of type 2 diabetic patients have been treated with gliclazide at some point.[3] Sulphonylureas undergo extensive hepatic metabolism mainly via CYP2C9 and CYP2C19 into several inactive metabolites, such as methylhydroxygliclazide and carboxygliclazide with gliclazide. The impact of CYP2C9 polymorphisms on the pharmacokinetics of a number of first- and second-generation sulphonylureas has been studied including glipizide[4], glibenclamide[5] and glimepiride.[6] Metabolism of the above sulphonylureas is predominantly influenced by CYP2C9 rather than CYP2C19[4,5]. The pharmacokinetics of gliclazide appeared to be primarily influenced by CYP2C19 rather than CYP2C9, in contrast to other sulphonylureas.[7,8] This has been shown in two previous studies of Chinese subjects.[7,8] Individuals with *CYP2C19*2* (rs4244285) and **3* (rs4986893) alleles produce a non-functional enzyme that is associated with reduced gliclazide oral clearance.[7,8]

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The allele frequency of *CYP2C19*2* is 40% in Asians as compared with 18% in Caucasian populations.[9,10] The *CYP2C19*3* occurs almost exclusively in East Asians with a mean allele frequency of 7%.[10] Therefore, genetic variation in CYP2C19 is potentially of great importance in treatment efficacy and hypoglycaemic risk among Asian patients with diabetes. Polymorphisms in CYP2C19 have been shown to account for ethnic differences in the efficacy of clopidogrel.[11] By contrast, the allele frequency of *CYP2C9*3* (rs1057910) which is known to affect sulphonylurea efficacy[12] occurs in less than 4% of Asians.[9]

The aim of the current study was to investigate the effect of *CYP2C19*2, *3* as well as *CYP2C9*3* alleles on gliclazide pharmacokinetics in healthy Chinese subjects.

Patients and Methods

Subjects

Fifteen healthy male Chinese subjects took part in the study. Subjects were all non-smokers with body mass index between 18 and 27 kg/m^2 and in good health as determined from medical history, physical examination, electrocardiogram and routine chemistry and haematology. Subjects were excluded if they were regular consumers of alcohol, tobacco or drug use in any form, used any antidiabetic medications, including gliclazide 4 weeks before the study.

Subjects were required not to take any prescription or non-prescription medication 2 weeks before and throughout the study. They were instructed to abstain from alcohol, grapefruit juice, caffeine or xanthine-containing food or beverages for 72 hrs prior to the study and during each study session.

Determination of Genotypes

A 10 mL blood sample was obtained from each subject and DNA was extracted. The *CYP2C19* wild type **1* and mutant alleles *CYP2C19*2* in exon 5 and *CYP2C19*3* in exon 4, *CYP2C9*3* in exon 7 single nucleotide polymorphisms were identified by polymerase chain reaction (PCR) amplification. The genotyping for the polymorphisms was performed using Taqman SNP genotyping assays (Applied Biosystems, Foster City, CA). PCR and fluorescent detection were performed using the ViiA7 real-time PCR detection system (Applied Biosystems, Life Technologies). Subjects were divided into CYP2C19 extensive metabolisers (EM) (*CYP2C19*1/*1*), CYP2C19 intermediate metabolisers (IM) or heterozygotes (**1/*2 or *1/*3*) and CYP2C19 poor metabolisers (PM) (**2/*2, *2/*3 or *3/*3*).

Study Design

This study was performed as part of a single dose, two-treatment, two-period, two-sequence crossover bioavailability and bioequivalence study with a washout period of 1–2 weeks. The plasma drug concentrations used in the analysis were that from the reference product (Diamicron Tablet 80mg, Servier). After a 10 hr overnight fast, each subject received a single oral dose of gliclazide (Diamicron Tablet 80mg, Servier). Venous blood samples were collected pre-dose (0 hrs) and at 0.5, 1, 1.5, 2, 2.5 3, 3.5, 4 4.5, 5, 5.5, 6, 8 10, 12, 12, 24, 48 and 72 hrs post-dosing (19 timepoints). To minimise the risk of hypoglycemia, 20% glucose solution was given orally to each subject 2 hours after drug administration. All blood samples were collected in lithium heparin tubes and centrifuged immediately at 4000 rpm for 10 mins at 4°C. The separated plasma samples were stored at −80° C until analysis.

Determination of Gliclazide Concentration in Plasma

Plasma concentrations of gliclazide were determined by a high-performance liquid chromatography-mass spectrometric (HPLC LC/MS) assay.[13] The method was referenced to the Bioanalytical Method Guidance as stated in the United States Food and Drug Administration. The calibration standards and quality control samples were prepared by spiking known amounts of gliclazide in drug-free plasma. The HPLC system consisted of a Waters Alliance 2690 separation module, Millenium chromatography management system and Waters 996 photodiode array detector (Waters, Milford, Mass).

Pharmacokinetic Data Analysis

The pharmacokinetic parameters of gliclazide were calculated via the noncompartmental method with the WinNonlin program (Pharsight Corporation). The peak plasma concentration (C_{max}) and time to peak drug concentration (T_{max}) were obtained directly from the original concentration-time data. The terminal elimination rate constant was estimated by linear regression of the terminal portion of concentration–time curve and the elimination half-life $t_{1/2}$ was calculated as $0.693/\lambda_z$. The area under the plasma concentration-time curve (AUC) was calculated by the linear trapezoidal rule. $AUC_{0\text{-last}}$ was calculated as the concentration-time curve from time zero to the last sampling time. AUC from time zero to infinity ($AUC_{0\text{-inf}}$) was calculated as $AUC_{0\text{-last}} + C_t/\lambda_z$. The apparent oral clearance (CL/F) was calculated as $Dose/AUC_{0\text{-inf}}$.

Statistical Analysis

Data were expressed as mean (SD). The pharmacokinetic parameters of gliclazide among the three CYP2C19 groups were compared by one-way analysis of variance and post hoc Bonferonni test for multiple comparisons. Analysis was performed with IBM SPSS (version 24.0, Chicago Illinois).

Results

A total of 15 subjects with genotypes available completed the study. Their demographic details and CYP2C19, CYP2C9 genotypes are shown in Supplementary Table S1.

Effect of CYP2C19 Polymorphisms on Gliclazide Pharmacokinetics

For CYP2C19, four subjects were EM (**1/*1*), 7 subjects were IM (5 with **1/*2* and 2 with **1/*3* genotypes) and 4 subjects were PM (all with **2/*2* genotypes). Age and body weights were similar between these three groups. The mean age (SD) of subjects in groups EM, IM and PM were 22.7 (3.06) years, 20.7 (1.1) years and 24.5 (5.2) years. The mean body weights were, respectively, 64.6 (13.1) kg, 64.6 (7.3) kg and 68.9 (5.0) kg.

There were no differences between T_{max} and C_{max} in the three CYP2C19 genotype groups (Table 1). $AUC_{0\text{-last}}$ and $AUC_{0\text{-inf}}$ were higher in the PM group than the IM and EM groups. The $t_{1/2}$ was 20.6 (1.88) hours in the PM group as compared with 13.6 (2.0) hours in the IM group and 11.3 (0.87) hours in the EM group (EM vs PM, $p < 0.001$ and IM vs PM, $p < 0.0001$). The oral clearance was lower in the PM group, as compared with IM and EM groups, respectively (EM vs PM p=0.014, IM vs PM p=0.018). The oral clearance of the three groups is shown in Figure 1. The mean plasma concentration-time profiles are shown in Supplementary Fig S1.

Only one subject (subject 4 in Supplementary Table S1) was heterozygous for the *CYP2C9*3* variant. The pharmacokinetic analysis with CYP2C19 genotype groups was repeated with this individual excluded which yielded similar results (data not shown).

Conclusions

We report that *CYP2C19 *2/*2* PMs had higher plasma concentrations of gliclazide over time, two-fold longer drug half-life and reduced oral drug clearance as compared with IMs and EMs after a single oral dose. Our findings are consistent with previous studies on CYP2C19 and gliclazide pharmacokinetics. In a study of 18 Chinese healthy individuals by Shao et al, *CYP2C19* PM (either **2/*3* or **3/*3* genotypes) exhibited higher AUC, lower oral clearance and prolonged half-life (33 vs 19 hrs) as compared with *CYP2C19* EM **1* homozygotes or heterozygotes (**1/*2* or **1/*3*) following a single dose of gliclazide 80mg.[7] In a 6-day multi-dose pharmacokinetic study of gliclazide 30 mg MR, Zhang and colleagues found the $AUC_{0\text{-inf}}$ was fourfold higher in CYP2C19 PMs as compared with *CYP2C19*1* heterozygotes and *CYP2C19*1* homozygotes, suggesting such pharmacokinetic differences may be further exaggerated with multiple dosing of modified release preparations.[8]

The impact of CYP2C9 variants on sulphonylurea efficacy has been shown in several studies.[12,14,15] A large retrospective study in 1073 incident users of sulphonylureas in Scotland found *CYP2C9*2* and **3* variants were associated with 0.5% greater reduction in HbA1c.[12] Similarly, an increased risk of hypoglycemia with sulphonylureas has been reported among CYP2C9 slow metabolisers.[16–18] CYP2C9 polymorphisms (**2* or **3*) are common in Caucasians but uncommon in Chinese with allele frequencies of 0.1% and 3%, respectively.[19] Conversely, to date,

Table 1 Comparison of Pharmacokinetic Parameters of Gliclazide Among Different CYP2C19 Genotype Groups

Parameters	Genotype Group			One Way ANOVA	Multiple Comparisons *p value*		
	EM n=4	IM n=7	PM N=4	*p* =	EM vs IM	EM vs PM	IM vs PM
T_{max} (hr)	3.13(1.03)	3.29(0.82)	3.88(0.95)	0.51	N/A	N/A	N/A
C_{max} (ng/mL)	4.48(1.44)	4.49(0.67)	4.75(0.44)	0.89	N/A	N/A	N/A
$AUC_{0\text{-last}}$ (ng.h/mL)	56.3(18.8)	65.8(14.4)	106.2(14.4)	0.001	0.99	0.003	0.002
$AUC_{0\text{-inf}}$ (ng.h/mL)	57.0(19.0)	67.8(15.8)	117.3(17.7)	<0.001	0.95	0.002	0.001
$t_{1/2}$ (hr)	11.5(1.0)	13.6(2.0)	20.6(1.88)	<0.001	0.24	<0.0001	<0.001
CL/F (mL/min/kg)	1.52(0.47)	1.22(0.22)	0.70(0.12)	0.005	0.35	0.037	0.004

Note: Data mean (SD).

Abbreviations: EM, extensive metaboliser; IM, intermediate metaboliser; PM, poor metaboliser; T_{max}, time to peak plasma concentration; C_{max}, peak plasma concentration; AUC_{last}, area under concentration–time curve to last measurement; AUC_{inf}, area under concentration–time curve to infinity; $t_{1/2}$, termination elimination half-life; CL/F, apparent oral clearance.

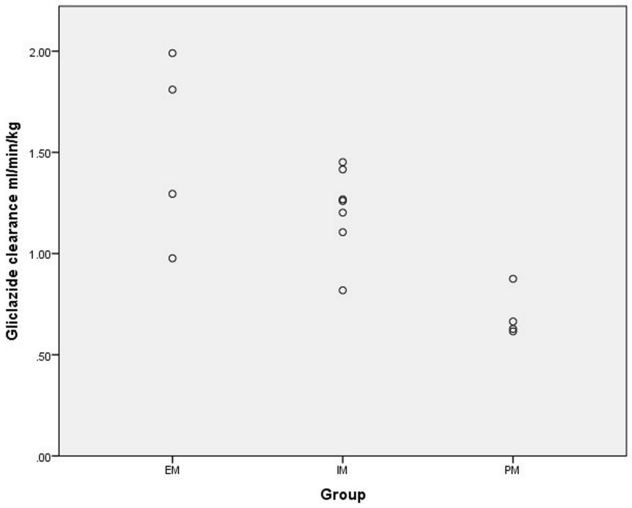

Figure 1 Oral clearance of gliclazide in different CYP2C19 genotype groups.
Abbreviations: EM, extensive metaboliser; IM, intermediate metaboliser; PM, poor metaboliser.

there have been no large-scale studies on the impact of CYP2C19 polymorphisms on sulphonylurea treatment efficacy or hypoglycaemic risk. This combined pharmacogenomic information may help to individualise choice of sulphonylureas (gliclazide versus other agents) based on the patient's CYP2C9, CYP2C19 genotypes and clinical factors. Polypharmacy is common in patients with type 2 diabetes.[20] Concomitant prescribing of CYP2C19 inhibitors, such as proton pump inhibitors and clopidogrel, may further affect the pharmacokinetics of gliclazide in patients who are CYP2C19 PMs.

Our study had a number of limitations. The sample size is small being part of a bioavailability and bioequivalent study where genotypes were retrospectively determined. Not all combinations of CYP2C19 risk alleles are represented in this study. Only one individual had a CYP2C9 variant and we were not able to examine the influence of CYP2C19 over CYP2C9 with respect to gliclazide pharmacokinetics. Only male subjects were investigated and we did not make any simultaneous pharmacodynamic assessments. Our preliminary findings require confirmatory testing in larger pharmacogenomic studies.

In summary, in this exploratory analysis, we have shown that *CYP2C19*2* polymorphisms were associated with increased total gliclazide concentration, prolonged drug half-life and reduced oral clearance. Given the popularity of sulphonylureas and that as much as one-fifth of Asians may be CYP2C19 PMs,[21] further pharmacogenetic studies are needed to evaluate the impact of these polymorphisms on gliclazide treatment response and hypoglycaemic risk.

Ethical Statement

Written informed consent was obtained from all participants. The study has been approved by the Joint Clinical Research Ethics Committee of the Chinese University of Hong Kong and New Territories East Cluster before the start of the study. The study was conducted in accordance with International Council for Harmonisation (ICH) Good clinical practice. The study was registered at Clinical Trials.gov NCT02643329. This study has been presented at the International Diabetes Federation Congress, December 2017, Abu Dhabi.

Data Sharing Statement

The deidentified patient data generated during and/or analysed during the current study are available from the corresponding author on reasonable request 9 months after publication and up to 3 years post-publication.

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Disclosure

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