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Estimation of CYP2D6*10 genotypes on citalopram disposition in Chinese subjects by population pharmacokinetic assay

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SUMMARY

What is known and objective: **There is great interindividual variability in citalopram (CIT) pharmacokinetics. We attempted to establish a population pharmacokinetic (PPK) model of CIT in Chinese healthy subjects, to evaluate the effect of genetic polymorphism on CIT pharmacokinetics and to compare the PPK and non-compartmental (NCA) assays in the estimation of CIT bioequivalence.**

Methods: **Blood samples of 23 healthy subjects were collected after administration of CIT; plasma concentration of CIT was analysed using LC/MS-MS. CYP2C19 and CYP2D6*10 genotypes were determined. PPK model was established by using nonlinear mixed-effect modelling (NONMEM). The model was evaluated using goodness-of-fit plots and relative error measurements. Bioequivalence of CIT was evaluated by both PPK and NCA method.**

Results and discussion: **The estimated population absorption rate constant (k_a), clearance (CL/F) and volume of distribution (Vd/F) in Chinese healthy subjects are 0.64 L/h, 12.7 L/h and 705 L, respectively. Different CYP2C19 and CYP2D6 genotypes have impacts on CIT pharmacokinetics. There is about 5.5% decrement of CL/F for each CYP2C19*2 or CYP2D6*10 allele. The 90% confidence interval of CIT bioavailability obtained from NCA and PPK model were 96.4–105.4% and 92.5–103.4%, respectively.**

What is new and conclusion: **The PPK of CIT is best characterized by a one-compartment disposition model with first-order absorption. CYP2C19 and CYP2D6 genotypes have impacts on the CL/F of CIT. Bioequivalence of CIT can be estimated by both NCA and PPK model.**

WHAT IS KNOWN AND OBJECTIVE

Citalopram (CIT) is a selective serotonin reuptake inhibitor, which is mainly used for the treatment of depression. After oral administration, maximum plasma concentrations are seen 2–4 h after dosing. The absolute bioavailability of an oral formulation is about 80%.[1,2] There is great interindividual variability in CIT pharmacokinetics. It was reported that there is a 7-fold variance in apparent oral clearance (CL/F) in patients receiving CIT monotherapy.[3,4] Hepatic metabolism is one of the most important causes for variability in CIT pharmacokinetics. CIT is converted into its major metabolite demethylcitalopram (DCIT) through *N*-demethylation, and a further demethylation at the same site results in didesmethylcitalopram (DDCIT), a minor secondary metabolite. The formation of these metabolites is catalysed by the CYP2C19, CYP2D6 and CYP3A4 isozymes.[5–8] CYP3A4 is responsible for an estimated 70% of the *N*-demethylation of CIT, whereas CYP2C19 contributes to about 7% of the catalysis.[5] CYP2D6 plays a still minor role in the metabolism of CIT and DCIT.[6,7]

Metabolic activity of CYP2C19 exhibits remarkable genetic polymorphism. People can be grouped into CYP2C19 extensive metabolizers (EMs) and poor metabolizers (PMs). The incidence of PM in Chinese (13–23%) is much higher than in Caucasian (2–5%).[9] *CYP2C19*2* and *CYP2C19*3* alleles can explain nearly all Chinese PMs. Genetic polymorphisms of CYP2C19 on the metabolism of CIT have been widely studied. Homozygous CYP2C19 nonfunctional allele carriers have an estimated 42% reduced clearance as compared with homozygous wild-type carriers. Lower doses of CIT in CYP2C19 PMs were recommended.[10] The frequencies of CYP3A4 alleles (*4, *5, *6, *18A and *19) are all lower than 1% in Chinese.[11,12] The importance of these alleles on CIT therapy is limited. CYP2D6 also shows remarkable genetic polymorphism in the Chinese population, and the frequency of the loss-of-function CYP2D6*10 allele in Chinese is about 37–70%.[13–15]

Population pharmacokinetic (PPK) study provides a quantitative estimation of the interpatient variability in pharmacokinetic response, the intrapatient variability and the influence of demographic, clinical and genetic factors on the pharmacokinetics. PPK is also suitable for modelling pharmacokinetic responses in a relatively large group of subjects with only relatively sparse samples for each subject. There are few studies of CIT PPKs especially on the influence of CYP2D6 genetic polymorphism.

The main objective of this study was to establish a PPK model for the Chinese population and to evaluate the effects of CYP2C19 and CYP2D6 polymorphisms on CIT pharmacokinetics. We also aimed to use the PPK model constructed to evaluate the bioequivalence of CIT formulations.

MATERIALS AND METHODS

Subjects

This study was conducted as a randomized, open-label, comparative, cross-over bioequivalence study. The protocol was approved

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[1]The author's contribution to this study is equal to the first one.

by the Ethics Committee of Ruijin Hospital. The trial was in accordance with the principles of the Declaration of Helsinki and the guidelines of Good Clinical Practice. All subjects gave written informed consent prior to enrolment in the study. The CYP2C19 genotype of 42 candidates was determined by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) assays. Subjects with CYP2C19 *1/*1, *1/*2 or *1/*3 alleles were considered as EMs. Finally, 24 male Chinese Han volunteers (age, 25.0 ± 2.9 years; weight, 65.2 ± 5.0 kg) of EM genotype were enrolled. Each subject was physically normal and had no antecedent history of significant medical illness or hypersensitivity to any drugs. Their health status was judged to be normal based on a physical examination with a screening of blood chemistry, complete blood cell count, urinalysis and electrocardiogram before the study. None of these subjects had taken any drugs for at least 2 weeks before the study.

Study protocol

All volunteers entered the study centre before 8:00 PM the day before the study. After an overnight fast, they received a single dose of 20-mg test or reference CIT tablets (reference formulation: Ciprimil, 20-mg tablets, lot no. 2127701; H. Lundbeck A/S, Copenhagen, Denmark; test formulation: Citalopram, 20-mg tablets, lot no. 7B7471; Salutas Pharma GmbH, Barleben, Genrmany) formulation with 240 mL of water at 8:00 AM. No water was allowed 1 h prior to and 2 h after drug administration. Standard meals were provided 4 and 10 h after dosing, respectively. No other food was permitted during the 'in-house' period. Xanthine-containing drinks including tea, coffee or cola were abandoned.

Two millilitres of blood samples was collected from an indwelling catheter in the arm into sterile EDTA anticoagulated tube before (0 h) and 0.5, 1, 2, 3, 4, 6, 8, 12, 24, 36, 48, 72, 96, 120 and 144 h after dosing. Systolic and diastolic arterial pressure and heart rates were recorded at each sampling time. The blood samples were centrifuged at 3000 *g* for 10 min, and plasma was separated and stored at −70 °C until analysis.

Genotyping of CYP2C19 and CYP2D6

Leucocyte DNA was extracted from blood samples by a modified phenol–chloroform extraction assay. The obtained DNA was dissolved in TE buffer and stored at 4 °C. CYP2C19 genotyping was conducted by the PCR-RFLP assay according to the previous studies with minor revision. Fragments containing CYP2C19*2 and *3 alleles were amplified. The final 50 μL of PCR mixture contained 50 ng of genomic DNA, 1× PCR buffer, 1.5 mM of $MgCl_2$, 0.2 mM of each dNTP, 0.4 μM of each primers (for CYP2C19*2, P1: TCA GAG GCT GCT TGA TAG AAA T, P2: AGT CAA TGA ATC ACA AAT ACG C; for CYP2C19*3, P3: TTC ATC CTG GGC TGT GCT, P4: AGG GCT TTG GAG TTT AGT GG. The primers were synthesized by Invitrogen Co. Ltd, Shanghai, China) and 2 U Taq DNA polymerase (Fermentas, Ontario, Canada). The reaction was carried out according to the following program: 7 min of initial denaturation at 94 °C; followed by 30 cycles of 94 °C for 45 s, 55 °C for 45 s and 72 °C for 45 s; with a final extension at 72 °C for 7 min. PCR products were analysed by electrophoresis with 2% agarose gels. The amplified DNA fragments containing CYP2C19*2 and *3 allele were digested with SmaI and BamHI (TaKaRa Biotech, Dalian, China) respectively, at 37 °C for 16 h. The digested fragments were analysed by electrophoresis with 4% agarose gels.

A tetra-primer method established in our laboratory was used to determine the genotype of C100T (CYP2D6*10).[15] Twenty-five microlitres reaction system contained 50 ng DNA, 1× PCR buffer, 1.5 mM $MgCl_2$, 0.2 mM dNTP, 0.4 μM for each of the outer and inner primers (outer1: TCA ACA CAG CAG GTT CAC TCA CAG CA, outer2: CTG TGG TTT CAC CCA CCA TCC AT, inner1: ACG CTG GGC TGC ACG CTA CC, inner2: AGT GGC AGG GGG CCT GGT GA), 0.04% Tween-20, 1.25 mM tetramethyl ammonium chloride and 0.5 U Heat start Taq DNA polymerase (Bio Basic Co. Ltd, Ontario, Canada). The reaction was performed according to the following program: 15 min at 94 °C; followed by 33 cycles of 94 °C for 30 s, 65 °C for 30 s and 72 °C for 45 s; with a final extension at 72 °C for 7 min. PCR products were analysed by electrophoresis with 2% agarose gels.

Determination of CIT and DICT in plasma

Plasma CIT and DCIT concentrations were analysed using a high-performance liquid chromatography tandem mass spectrometry (LC-MS/MS).[16] Briefly, to 200 μL plasma sample, 600 μL of methanol with internal standard (desipramine) was added to precipitate plasma protein. After vortexing for 5 min, the samples were centrifuged at 15 493 *g* for 10 min. Hundred microlitres of supernatant was mixed with 100 μL water, and 10 μL mixture was injected into the Angilent 1200 LC system and eluted with acetonitrile and 0.25% formic acid (30 : 70, v/v) at a flow rate of 300 μL/min. The separation was carried out using a Zorbax XDB C18 column (2.1 × 50 mm, 3.5 μm; Agilent Technologies Inc., USA) with temperature of 35 °C. HPLC system was coupled to an Agilent 6410 triple quadrupole mass spectrometer (Agilent Technologies Inc., Santa Clara, CA, USA) with an electrospray interface in positive ionization mode. Multiple reaction monitoring was used to quantify CIT (m/z 325 $[M + H]^+ \rightarrow 109$), DCIT (m/z 311 $[M + H]^+ \rightarrow 109$) and despramine (m/z 267 $[M + H]^+ \rightarrow 208$). Linearity calibration range of CIT and DCIT was 0.2–100 and 0.25–50 ng/mL, respectively. The recovery of CIT and DCIT was 105.3% and 99.7%, respectively. The relative standard deviation of CIT and DCIT was lower than 11.4% and 8.3%, respectively.

Pharmacokinetic analyses

Non-compartmental (NCA) pharmacokinetic analysis. Plasma concentrations of CIT and DCIT obtained from subjects were analysed using NCA assay by WinNonlin 5.01 (Pharsight Corporation, Mountain View, CA, USA). The C_{max} and the T_{max} were obtained directly from the data. Area under concentration–time curve (AUC) was estimated by means of trapezoidal method and extrapolation of the area to infinite time.

Compartmental pharmacokinetic analysis. Structure model: Population pharmacokinetic model of CIT was constructed using NONMEM (Version 6; GloboMax, Hanover, MD, USA). One- and two-compartment structure models were evaluated during model construction. Concentration data of CIT were log-transformed to ensure the random effects are sufficiently distributed around zero. The first-order conditional estimation method was applied for the modelling. Model selection was based on the objective function value (OFV), parameter estimates and standard errors. OFV is proportional to −2 log likelihood of the relevant models. Lower

value indicates a better model. The distribution of empirical Bayes estimates was also important factors for model selection.

Interindividual and residual error model: The interindividual variability of the parameters was assessed using an exponential function: $P_i = TV(P_i) \cdot e^{\eta_i}$

where P_i was the individual value, $TV(P_i)$ was the population value for the parameters described in the equation and η_i was the random deviation of P_i from $TV(P_i)$. The values of η_i were assumed to be independently normally distributed with a mean of 0 and a variance of ω^2.

As the concentration data were log-transformed, an additive model was used for residual error analysis of CIT as: $\ln C_{obs} = \ln C_{pred} + \varepsilon$.

where C_{obs} is the observed concentration, C_{pred} is the predicted concentration, and ε is a residual error with a mean of 0 and a variance of σ^2.

Covariates: Subjects' demographic data such as age, body weight, physiological characteristics such as clearance of createnine (CLcr) and genetic polymorphisms were evaluated as the possible covariates of CIT pharmacokinetic model. The influence of continuous covariates on the pharmacokinetic parameter TV(P) was modelled according to the following equations:

$$TV(P) = \theta_P \times (\text{covariate})$$

$$TV(P) = \theta_P + \theta_C \times (\text{covariate})$$

$$TV(P) = \theta_P \times (e^{\text{covariate} \times \theta_c})$$

$$TV(P) = \theta_P \times (\text{covariate}/\text{means of covariate})^{\theta_c}$$

For CYP2C19 and CYP2D6 genotypes, discrete numbers were given to each index: 0 and 1 for CYP2C19 *1/*1 and *1/*2 (or *1/*3) subjects, respectively; 0, 1, 2 for CYP2D6 *1/*1, *1/*10 and *10/*10 subjects, respectively. We also considered the influence of CYP2C19 and CYP2D6 genotype simultaneously: 0–5 was assigned to 2C19*1/*1- 2D6*1*1, 2C19*1/*1- 2D6*1/*10, 2C19*1/*2- 2D6*1/*1, 2C19*1/*1- 2D6*10/*10, 2C19*1/*2- 2D6*1/*10 and 2C19*1/*2- 2D6*10/*10, respectively. Pharmacokinetic parameter TV(P) was modelled according to the following equations:

$$TV(P) = \theta_P + \theta_C \times (\text{covariate})$$

$$TV(P) = \theta_P \times (e^{\text{covariate} \times \theta_c})$$

$$TV(P) = \theta_P \times \theta^{\text{covariate}}$$

where TV(P) is the typical value of the pharmacokinetic parameters, θ_P is the population estimation of the parameter, and θ_c is the factor contributed by the covariate.

Formulation factors on the pharmacokinetics of CIT were also tested.[17] The population values of pharmacokinetic parameters for reference and test formulations are as follows:

$$k_{aT} = \theta_{ka} \cdot k_{aR}$$

$$k_a = (1 - \text{TRET}) \cdot k_{aR} + \text{TRET} \cdot k_{aT}$$

$$F_1 = (1 - \text{TRET}) \cdot 1 + \text{TRET} \cdot F_T$$

k_{aT} and k_{aR} are PPK values of absorption constant of test and reference formulation. F_T is the relative bioavailability of test formulation of CIT. TRET is the formulation indicator variable, TRET = 0 indicated reference product and TRET = 1 indicated test product.

A forward inclusion and backward elimination techniques were used for the final regression model. Each candidate covariate was screened in turn by adding it to the base model. Weighted residuals and the change in the OFV were noted in the model-building process. Changes in the OFV approximate the chi-square distribution with the degrees of freedom (d.f.) equal to the number of covariates introduced. A covariate was considered statistically significant when the OFV decreased by 3.84 or greater ($P < 0.05$, d.f. = 1) when added to the base model (forward inclusion). The full model included all covariates that showed a significant decrease in OFV. Hence, each covariate remaining in the model was removed in turn by fixing its value to zero. This procedure was repeated until the value of the objective function failed to increase by more than the critical value of 6.63 ($P < 0.01$, d.f. = 1; backward elimination). Individual pharmacokinetic parameters, arithmetic means and standard deviations were calculated using the NONMEM Bayesian estimates from POSTHOC output.

Model validation: As there are relatively few subjects in this study, the data-splitting method was not used for model testing. Instead, the stability and performance of final model were assessed through an internal validation method that involved a nonparametric bootstrap with resampling and replacement. In this study, 400 bootstrap samples were generated, and the PPK parameters were estimated for each of the 400 samples using the final model. The mean and standard error of parameter estimates from the bootstrap analyses were then compared with the NONMEM estimates from the final model.

Statistical analysis

Pharmacokinetic parameters of CIT were shown as means ± standard deviations (SD). Kruskal–Wallis test was used to compare C_{max}, AUC and Cl among different CYP2C19 and CYP2D6 genotype groups. Mann–Whitney *U*-test was used to compare C_{max}, AUC and Cl between two CYP2D6*10 genotype groups. $P < 0.05$ was considered to be statistically significant.

Analysis of variance (ANOVA) was performed on the log-transformed C_{max}, AUC from NCA analysis. Two one-sided tests were carried out for bioequivalence, and 90% confidence intervals (CI) were calculated. Bioequivalence was determined, if the 90% CI of the geometric mean was within 80–125%.

RESULTS AND DISCUSSION

Genetic polymorphism of CYP2C19 and CYP2D6 on CIT pharmacokinetics

Twenty three of 24 subjects finished the study and one subject discontinued for personal reason. 736 CIT plasma concentrations were obtained. In 23 subjects, there are 9 *1/*1 and 14 *1/*2 subjects for CYP2C19. There is difference in the concentration time curve of CIT among various CYP2C19 genotypes (Fig. 1). However the difference of CL/F between different CYP2C19 genotype was not statistically significant ($P = 0{\cdot}071$; Fig. 2A). Studies have shown that the disposition of CIT is associated with the polymorphism of CYP2C19[9]. Yu *et al.* [18] suggested gene-dose effect in CYP2C19 CIT *N*-demethylation. The $t_{1/2}$ and CL/F of CIT, as well as the AUC, C_{max} and T_{max} of DCIT in PMs were significantly different from those in homozygous EMs ($P < 0{\cdot}05$) and heterozygous EMs ($P < 0{\cdot}05$). Yin *et al.* [10] used CYP2C19 genotypes as

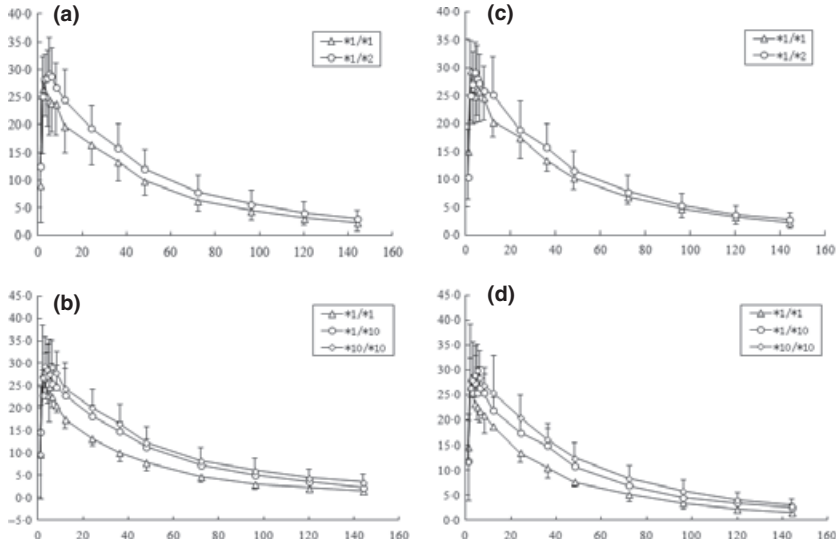

Fig. 1. Concentration–time curve of citalopram in test and reference formulation with various CYP2C19 and CYP2D6*10 genotypes. (a, c) various CYP2C19 genotypes of test and reference formulation; (b, d) various CYP2D6*10 genotypes of test and reference formulation.

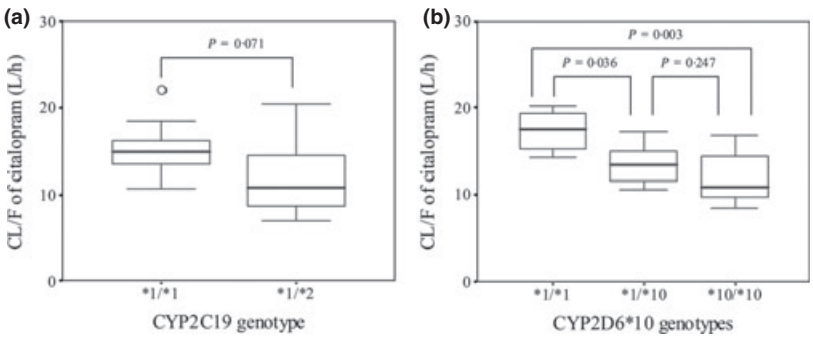

Fig. 2. Clearance (CL/F) of 23 Chinese subjects after oral administration of 20-mg citalopram among different CYP2C19 (a) and CYP2D6*10 (b) genotypes. °: Outliers.

the covariate of CL/F in PPK modeling of CIT in 53 Chinese patients. They found that CYP2C19 PM patient had a 42·9% and 33·3% lower CL/F than homozygous and heterozygous EM patients, respectively. To minimize the influence of CYP2C19 genotypes on exposure to CIT, we excluded subjects carrying the homozygous CYP2C19 mutant alleles from this study. It seemed that the CL/F of CYP2C19 *1/*1 subjects is higher than that of *1/*2 subjects.

There were 4 *1/*1, 7 *1/*10 and 12 *10/*10 subjects for CYP2D6. CL/F in CYP2D6 *1/*1 group was significantly higher than *1/*10 and *10/*10 groups ($P < 0·05$; Fig. 2B). When different CYP2C19 and CYP2D6 genotypes were considered simultaneously (2C19-2D6), the pharmacokinetic parameters are summarized in Table 1. The ratio of C_{max}, $AUC_{0-\infty}$ of 3 CYP2D6*10 groups with CYP2C19*1/*1 genotype was 1 : 1·23 : 1·15 and 1 : 1·22 : 1·25, respectively; for CYP2C19*1/*2 group the ratios were 1 : 1·18 : 1·24 and 1 : 1·48 : 1·93, respectively.

A previous study used human CYP3A4, CYP2C19 and CYP2D6 isozymes expressed by cDNA in the metabolism of CIT *in vitro*. The results showed that CYP2C19 and CYP3A4 had much higher K_m values than CYP2D6 (166, 204 and 20 μM, respectively). Nevertheless, the V_{max} of CYP2C19 and CYP3A4 is also 10 times higher than that of CYP2D6. Thus, intrinsic clearance values (V_{max}/K_m) of CIT was similar for the three isozymes[7]. CYP2D6 plays a minor role in the biotransformation of CIT due to its lower expression level than CYP2C19 and CYP3A4. *In vivo* studies of CYP2D6 polymorphism on CIT pharmacokinetics are scarce. Sindrup *et al.* [6] found that $AUC_{0-120\ h}$ and $t_{1/2}$ of CYP2D6 PM seemed higher than those of EM subjects (5303·8 ± 1962·3 vs. 4244·1 ± 1021·6 nmol·h/L for AUC, 38·0 ± 12·2 vs. 31·6 ± 6·2 h for $t_{1/2}$), but the difference was not statistically significant. On the other hand, Herrlin *et al.* [19] investigated the pharmacokinetics of the CIT, DCIT and DDCIT enantiomers in Swedish subjects. The results suggested that clearance of DCIT but not of CIT was influenced by CYP2D6 phenotype. It our study it seemed there is a gene dose effect of CIT pharmacokinetics among different CYP2D6*10 genotypes and the effect is more significant in subjects with CYP2C19*1/*2 genotype. It can be inferred that CYP2D6

Table 1. Non-compartment pharmacokinetic parameters of citalopram in various CYP2C19 and CYP2D6 genotype

	Reference					Test				
	$t_{1/2}$ (h)	T_{max} (h)	C_{max} (ng/mL)	$AUC_{0-\infty}$ (ng·h/mL)	CL (L/h)	$t_{1/2}$ (h)	T_{max} (h)	C_{max} (ng/mL)	$AUC_{0-\infty}$ (ng·h/mL)	CL (L/h)
CYP2C19*1/*1	48.0 ± 5.7	3.2 ± 1.1	31.6 ± 10.3	1356 ± 276	15.6 ± 3.3	43.2 ± 5.0	2.4 ± 1.1	30.3 ± 4.5	1396 ± 244	15.0 ± 2.5
CYP2D6*1/*1	43.4	2.5	27.8	1123	18.4	39.9	1.5	31.9	1226	16.6
	48.2, 38.7	2.0, 3.0	27.2, 28.5	1328, 919	15.1, 21.8	48.0, 31.8	1.0, 2.0	30.6, 33.2	1382, 1070	14.5, 18.7
CYP2D6*1/*10	50.4	4.0	34.2	1374	14.6	45.2	2.5	28.4	1336	15.2
	50.9, 49.8	3.0, 5.0	32.4, 36.0	1463, 1284	13.7, 15.6	46.8, 43.7	3.0, 2.0	28.9, 28.0	1515, 1157	13.2, 17.3
CYP2D6*10/*10	48.9 ± 6.3	3.2 ± 1.1	32.0 ± 14.1	1402 ± 314	14.8 ± 3.2	43.6 ± 3.0	2.8 ± 1.3	30.3 ± 6.1	1449 ± 270	14.2 ± 2.6
CYP2C19*1/*2	51.5 ± 19.7	4.5 ± 2.7	34.1 ± 5.1	1836 ± 564	11.7 ± 3.8	45.3 ± 15.1	3.7 ± 2.7	33.6 ± 6.5	1770 ± 407	12.1 ± 3.2
CYP2D6*1/*1	34.1	2.0	28.7	1142	18.0	35.9	2.5	27.9	1207	17.1
	38.6, 29.6	2.0, 2.0	26.9, 30.6	962, 1322	20.8, 15.1	42.2, 29.6	3.0, 2.0	25.4, 30.4	992, 1423	20.2, 14.1
CYP2D6*1/*10	46.4 ± 5.3	6.0 ± 3.5	34.0 ± 2.1	1694 ± 195	11.9 ± 1.5	51.7 ± 20.1	3.2 ± 1.8	31.2 ± 2.8	1609 ± 230	12.6 ± 1.8
CYP2D6*10/*10	60.0 ± 24.6	4.1 ± 1.8	35.7 ± 6.2	2205 ± 566	9.8 ± 3.3	43.4 ± 12.0	4.4 ± 3.5	36.9 ± 7.5	1996 ± 350	10.3 ± 2.2
Total	50.1 ± 15.6	3.9 ± 1.4	33.1 ± 7.4	1648 ± 504	13.2 ± 4.1	44.5 ± 12.1	3.2 ± 2.3	32.3 ± 5.9	1595 ± 381	13.2 ± 3.2

genetic polymorphism may play a more important role in CIT metabolism in subjects with poor CYP2C19 and/or CYP3A4 metabolic activity.

PPK model of CIT and the influence of genetic polymorphism

Different structure models (1 and 2 compartment model, with or without lag time) were tested. The best structural model consisted of a 1-compartment model with a single first-absorption process and first-order elimination process. The absorption rate constant (k_a) for the 23 healthy subjects was 0·64 ± 0·081 L/h, but with large inter-individual variation (62·2%). T_{max} obtained using NCA method also showed great variability (T_{max}: 2·12 h). Yin *et al.* [10] tested different k_a (0·25–2·5 L/h), and found that 0·75 L/h was the best, an estimate comparable to that obtained in our study. Although CIT is completely absorbed in the intestine[20], the duration of absorption shows great inter-individual variability. In previous studies, Bies *et al.* [21] and Friberg *et al.* [22] found great inter-individual variability of k_a (CV=45·1% and 51·0%, respectively).

Since CIT was administered orally, the clearance (CL/F) and distribution volume (Vd/F) included bioavailability (F). CL/F (mean ± SE) was estimated to be 12·7 ± 0·49 L/h; the Vd/F was 705 ± 19·3 L (Table 2). The Vd/F estimate in the present study is lower than estimates reported by investigators in Denmark (1310·2 ± 165·66 L)[2], Sweden (1364 ± 155 L)[23] and Australia (1297 L)[22], but is comparable to that reported for Chinese subjects (670 ± 244 L)[10]. There may be an ethnic difference in Vd/F of CIT between Caucasian and Chinese subjects.

A previous study in Caucasians[21] found that body weight and age influence CL/F and Vd/F significantly. The CL/F increased 0·14 L/h for every kilogram of body weight and decreased 0·23 L/h for every year of age. The study of Yin *et al.* [10] in Hong-Kong Chinese patients also found that CL/F increased by an estimated 0·11 L/h for every kilogram body weight. We tested body weight, age and CLcr as covariates, but none neither of these factors was significant. This may be due to that the subjects included in this study being healthy with normal renal function and similar body weight and age.

When CYP2C19 genotype was tested as a covariate, CL/F of CYP2C19*1/*2 subjects was about 14% lower than that of *1/*1 subjects. However, the OFV decreased by only about 4·0, CYP2C19 is not a significant covariate with CL/F. The result is consistent with previous pharmacokinetic studies on CIT which showed that CYP2C19 *1/*1 (homozygous EMs), *1/*2 or *1/*3 (heterozygous EMs) can be categorized as one group.

We classified subjects according to both CYP2C19 and CYP2D6 genotype. 6 different genetic polymorphism groups, 0–5 could be identified. The genotype was used as a covariate for CIT CL/F. OFV decreased by 13·8, and inter-individual variability of CL/F decreased from 23·0% to 18·0% (Table 2). Subjects with CYP2D6*10/*10 and CYP2C19*1/*2 genotype had CL/F 27·4% lower than subjects with CYP2D6*1/*1 and CYP2C19*1/*1 genotypes. The results suggest that CYP2D6*10 allele has an impact on the pharmacokinetics of CIT, especially in subjects with a CYP2C19 defective allele.

Model validation

The assessment of the predictive performance of the final model is represented in scatter plots of observed CIT concentration (DV) versus population predicted CIT concentrations (PRED, Fig. 3A)

Table 2. The citalopram pharmacokinetic parameters obtained by nonlinear mixed-effect modelling

	Basic model		Final model		
	Mean (SE)	95% CI	Mean (SE)	95% CI	Bootstrap
Parameters (θ)					
CL/F (θ_1) (L/h)	12.7 (0.49)	12.5–12.9	15.6 (0.43)	15.4–15.8	16.0 (0.69)
Vd/F (θ_2) (L)	705 (19.3)	697.1–712.9	705 (19.2)	697.2–712.8	699 (18.8)
k_a (θ_3) (L/h)	0.64 (0.081)	0.61–0.67	0.63 (0.081)	0.60–0.66	0.61 (0.058)
Genotpe (θ_4)	NA	NA	−0.054 (0.017)	−0.061 to −0.047	−0.25 (0.034)
Inter-individual variability (η)					
ωCL, %	23.0 (11.6)	18.3–27.7	18.0 (10.0)	13.9–22.1	14.8 (1.71)
ωVd, %	8.89 (8.41)	5.5–12.3	9.29 (8.31)	5.9–12.7	10.7 (2.31)
ωk_a, %	62.2 (32.2)	49.0–75.4	62.4 (32.4)	49.2–75.6	59.9 (5.5)
Residual error (ε)					
σ, %	32.1 (15.0)	26.0–38.2	31.9 (14.1)	26.1–37.7	32.4 (3.35)
Objective function value	−696.2		−710.0		

As interindividual variability of Vd/F is lower than 0.01%, it was fixed to 0.

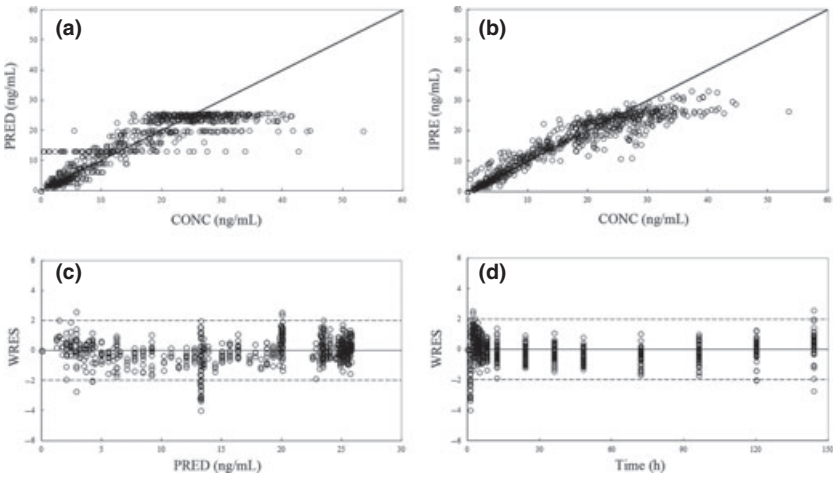

Fig. 3. Goodness-of-fit of citalopram population pharmacokinetic model. (a) Population-predicted concentration (PRED) vs. observed concentration (DV); (b) individual-predicted concentration (IPRE) vs. DV; (c) Weighted residual error (WRES) vs. PRED; (d) WRES vs. time.

and individual predicted CIT concentrations (IPRE, Fig. 3B); weighted residual (WRES) vs. PRED (Fig. 3C) and time (Fig. 3D). Residuals of most concentration data were randomly distributed within 2 standard deviations (SD), which designated good agreement. The average bias of CIT was 31·9% (95% confidence interval (CI): 26·1–37·7%). Some lack of fit is evident in the plot. Only 20 out of the 736 samples are out of 2 SD.

392 of the 400 bootstrap runs successfully, two runs terminated with rounding errors, and the other six runs terminated without covariate step. The bootstrap indicated good results with the results obtained from final model. The results of k_a, CL/F and Vd/F calculated by NONMEM are in the center of histogram of 392 runs of bootstraps (Fig 4).

Bioequivalence analysis by NCA and PPK modeling

Although the most popular method for the assessment of bioequivalence of different formulations is NCA, previous studies suggested that a PPK model may be better for studying drugs with more complicated plasma concentration-time profiles and may be useful for studying bioequivalence study in patients[24]. With the PPK method, a set of parameter estimates can be obtained. For example, k_a between different formulations can be compared using the PPK approach. Besides, the C_{max} values obtained by PPK are likely to be less biased than those observed using the NCA method. With Bayesian methodology, bioequivalence can be assessed [17, 25]. In this study, NCA analysis showed the 90% CI of the $AUC_{0-\infty}$ and C_{max} are within 80–125%, which indicated bioequivalence of the test drug to the reference drug. Bioequivalence estimation through PPK modeling assay showed similar results. We obtained individual C_{max} and AUC of the reference and test formulations of CIT using the Bayesian method. The 90% CIs of these parameters are similar to those obtained using NCA (Table 3). T_{max} obtained using NCA and PPK method was compared by Wilcoxon test, and both methods showed that there was no difference between the reference formulation and the test

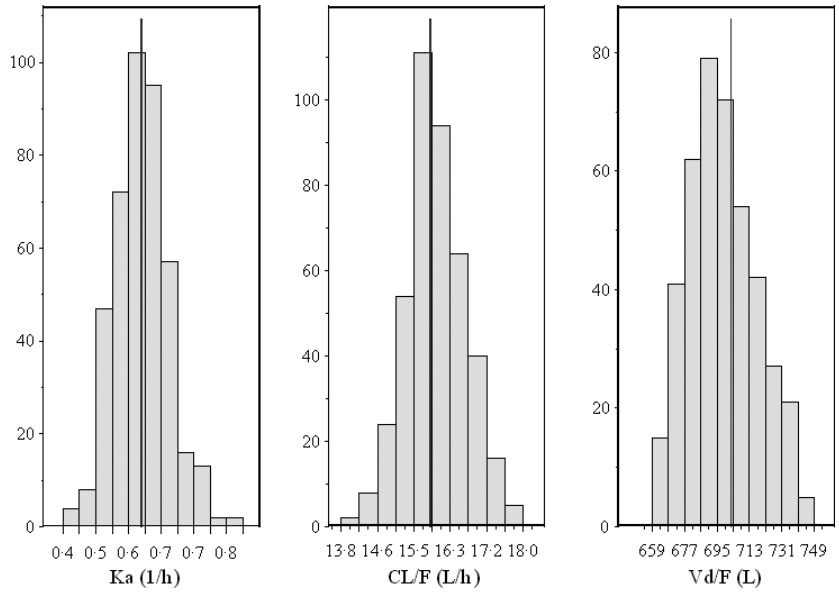

Fig. 4. Histogram of k_a, CL/F and Vd/F of citalopram from 392 bootstraps. Vertical line means the estimated parameters from population pharmacokinetic model we established.

Table 3. The bioequivalence of citalopram obtained from non-compartmental and nonlinear mixed-effect modelling (NONMEM) assay

	Non-compartment			NONMEM		
	Test	Reference	BA (%)	Test	Reference	BA (%)
C_{max} (ng/mL)	32.3 ± 5.9	33.1 ± 7.4	92.5–103.6	26.2 ± 2.5	26.4 ± 3.0	92.9–101.1
AUC (ng h/mL)	1595 ± 381	1648 ± 504	96.4–105.4	1575 ± 265	1616 ± 363	92.5–103.4
T_{max} (h)	3.22 ± 2.26	3.60 ± 1.44		2.77 ± 1.39	2.86 ± 0.91	
θ_{ka}				106.0% (84.8–127.2%)		
F_T				99.1% (89.4–108.9%)		

formulation. We also assessed the bioequivalence by introducing the formulation factors. The ratio of k_a between test and reference formulation (θ_{ka}) and bioavailability (F1) of test drug was used to assess potential differences between the 2 CIT formulations on the extent of absorption and the exposure. The point estimate (with 90% CI) for θ_{ka} and F_T were 106·0% (84·8–127·2%) and 99·1% (89·4%108·9%). It can be inferred that test and reference formulations had a similar rate of absorption and exposure.

WHAT IS NEW AND CONCLUSIONS

In this study, we studied the influence of CYP2C19 and CYP2D6*10 genotypes on the pharmacokinetic characteristics of CIT in healthy Chinese subjects by using PPK method for the first time. We found pharmacokinetics of CIT in Chinese subjects is best characterized by a one-compartment disposition model with first-order absorption, CYP2C19 and CYP2D6*10 alleles play significant roles in the metabolism of CIT. CIT pharmacokinetic parameter values (e.g. CL/F, Vd/F, AUC, etc.) estimated using NONMEM are similar to those obtained with NCA analysis. The PPK method could be used to assess the bioequivalence of CIT.

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