Pharmacogenetic and safety analysis of cinacalcet hydrochloride in healthy Chinese subjects

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Background: Our study aims to explore the effect of genetics on the pharmacodynamics (PD) and pharmacokinetics (PK) of cinacalcet in healthy Chinese subjects; to investigate the effect of dietary factors on cinacalcet, and to evaluate the safety of cinacalcet under fasting and non-fasting conditions using a bioequivalence trial.

Methods: We investigated the relationship of cinacalcet PK with single nucleotide polymorphisms (SNPs) of CYP3A4, CYP1A2 and CYP2D6, and of cinacalcet PD with SNPs of calcium-sensitive receptors (CASR) and vitamin D receptors (VDR) in 65 healthy Chinese subjects recruited to participate in this study. Our study was a phase I, open-label, randomized, two-period, two-sequence crossover, a single-center clinical study designed under both fasting and non-fasting conditions to investigate the effect of dietary factors on cinacalcet. Plasma cinacalcet concentrations were analyzed using a validated HPLC-MS/MS assay. Clinical laboratory tests evaluated safety. Thirteen SNPs of CASR, VDR, and CYP genes were selected for pharmacogenetic analysis.

Results: CYP3A4 rs4646437 was found to be associated with the PK of cinacalcet under fasting conditions ($P<0.01$). Subjects carrying T alleles of rs4646437 appeared to metabolize cinacalcet poorly. The C_{max} and AUC of subjects in the non-fasting group were significantly higher ($P<0.0001$) than those in the fasting group. The T_{max}, CL/F, and Vd/F in the fasting group were significantly higher ($P<0.0001$) than those in the non-fasting group. In the fasting group, the geometric least square mean ratios (T/R) of the C_{max} and AUC_{0-t} were 109.89% and 105.33%, and the corresponding 90% CIs were 98.36–122.79% and 98.04–113.15%, respectively. In the non-fasting group, the T/R of the C_{max} and AUC_{0-t} were 100.74% and 99.09%, and the corresponding 90% CIs were 92.65–109.54% and 94.79–103.58%, respectively. All adverse events (AEs) were mild, and no serious adverse events (SAEs) occurred during the bioequivalence trial.

Conclusions: Following our investigation, we reached the following conclusions: CYP3A4 rs4646437 may affect cinacalcet PK; the reference and test preparations of cinacalcet were bioequivalent under fasting and non-fasting conditions and were safe to use; and dietary factors had a significant effect on the PK of cinacalcet, in that exposure to the drug increased when cinacalcet was taken after eating.

Keywords: Cinacalcet; bioequivalence; pharmacokinetics (PK); calcium-sensitive receptors (CASR); CYP3A4

Submitted Feb 05, 2020. Accepted for publication Aug 30, 2020.

doi: 10.21037/atm-20-1329

View this article at: http://dx.doi.org/10.21037/atm-20-1329

Introduction

Secondary hyperparathyroidism (SHPT) is a common complication in patients with chronic renal failure (1,2). Typical presenting symptoms of SHPT include elevated parathyroid hormone (PTH) levels and hyperplasia of the parathyroid glands, which can lead to bone injury, refractory pruritus, anemia, nervous system damage, and cardiovascular diseases (3,4). Studies have suggested that long-term elevated levels of PTH increase the risk of death in patients with chronic kidney disease (CKD) who require dialysis (5,6). Treatment of SHPT should aim to reduce parathormone concentrations through the administration of calcimimetics or active vitamin D and to stabilize calcium and phosphate metabolism (7).

Cinacalcet is the first of a new class of compounds called calcimimetics, which activates calcium-sensitive receptors (CASR) in the parathyroid gland and reduces the secretion of PTH (8,9). After binding with CASR, cinacalcet enhances its sensitivity to extracellular calcium ions, which inhibits the secretion of PTH (10). These calcium-lowering effects of cinacalcet overcome the limitations of standard hypercalcemia therapy (11). In addition to inhibiting the secretion of PTH, cinacalcet can also reduce the levels of serum calcium and phosphorus in SHPT patients. Treatment with cinacalcet can also alleviate the symptoms of bone metabolism disorders and prevent vascular and soft tissue calcification, and is well-tolerated by patients, producing minimal adverse events (AEs). Cinacalcet is now used widely in the clinical treatment of SHPT patients with CKD on maintenance dialysis (7).

Additionally, some articles have shown that cinacalcet is more effective when used in patients with more severe SHPT, indicating this drug's efficacy is related to SHPT severity (12). Cinacalcet hydrochloride has been used successfully in US and Japan in the treatment of SHPT while maintaining serum levels of calcium and phosphorus (13). Cinacalcet also reduces the incidence rate of calcific uremic arteriolopathy (CUA; calciphylaxis) in patients undergoing dialysis (14). Furthermore, the usage of cinacalcet alleviates hyperparathyroidism secondary to lithium therapy in patients with the bipolar affective disorder (15).

However, cinacalcet overdose can result in hypocalcemia, and the response to cinacalcet varies significantly between individuals (16). Commonly, serum calcium decreases following the initiation of cinacalcet treatment (17); therefore, a patient's serum calcium levels should be regularly monitored while they are medicated to avoid hypocalcemia. Calcium supplementation should be considered when hypocalcemia occurs (18,19). Given the high protein binding rate of cinacalcet, hemodialysis is not an effective treatment for overdose (20). Due to the significant variation in individual response to this drug, it is necessary to develop individual treatment plans for patients taking cinacalcet.

The elimination of cinacalcet is principally mediated by oxidative metabolism, partly by cytochrome P450 (CYP) 3A4 (21). It has been reported that cinacalcet is metabolized primarily by CYP3A4, CYP2D6, and CYP1A2, of which CYP3A4 is the dominant metabolic enzyme (7). As cinacalcet is also a strong inhibitor of CYP2D6 (22), when it is co-administered with drugs metabolized by CYP2D6 with narrow therapeutic targets, the dosage of cinacalcet may need adjustment (23). However, there is no literature describing the effect of single nucleotide polymorphisms (SNPs) of CYP enzymes on the pharmacokinetics (PK) of cinacalcet. Reports on the effect of SNPs are limited to CASR, which acts as the interaction site of cinacalcet and other calcium regulatory receptors, including vitamin D receptors (VDR) (16,24-27).

This study was conducted to investigate the effect of dietary factors on cinacalcet and to evaluate the safety of this drug in a bioequivalence trial using two types of cinacalcet hydrochloride tablets. Further, SNPs of CASR and VDR have been reported to affect cinacalcet pharmacodynamics (PD). In the study, we investigated whether these SNPs had any impact on cinacalcet response in healthy Chinese subjects after receiving a single dose under fasting conditions. We selected SNPs of CYP3A4, CYP2D6, and CYP1A2 enzymes, which are commonly reported to affect the metabolism of other drugs, to explore whether they have any effect on the PK of cinacalcet under fasting conditions (28-32). We present the following article in accordance with the CONSORT reporting checklist (available at http://dx.doi.org/10.21037/atm-20-1329).

Methods

Subjects

One hundred thirty-four healthy subjects aged 18–65 years (inclusive) with a body mass index (BMI) in the range of 19–26 kg/m^2 (inclusive) were enrolled in this study. Subjects that had clinically significant abnormalities in electrocardiogram results, blood chemistry, or urinalysis were excluded from this study, as were those who returned

Table 1 Demographic characteristics

Characteristics	Fasting group (n=65)	Fed group (n=63)
Age (years), mean ± SD (range)	25.4±4.2 (18 to 40)	25.5±4.6 (18 to 39)
Sex, n (%)		
Male	49 (71.0)	45 (70.3)
Female	20 (29.0)	19 (29.7)
Height (cm), mean ± SD (range)	169.15±7.92 (152.9 to 186.8)	170.11±8.13 (149.3 to 188.1)
Weight (kg), mean ± SD (range)	65.03±6.62 (50.3 to 80.8)	64.01±7.13 (49.3 to 82.1)
BMI (kg/m^2), mean ± SD (range)	22.74±1.89 (19.2 to 25.9)	22.07±1.77 (19 to 26)

positive pregnancy tests. None of the subjects consumed excessive alcohol or smoked, and none had taken any drugs for at least two weeks before the study. Baseline hematology and clinical biochemistry laboratory parameters were within normal limits for all participants. After receiving oral and written explanations, subjects gave written informed consent before the commencement of the study.

In the fasting group, of the 69 enrolled, 65 subjects completed the study. Four withdrew due to AEs after the first cycle of administration. In the non-fasting group, of the 65 subjects enrolled, two withdrew, and 63 completed the study. One subject withdrew on his initiative before drug administration, and another was found not to match their given identity. The demographic characteristics of the study participants are shown in *Table 1*.

Study design

This study (China Food and Drug Administration registration: 2016L07520; Drug clinical trial registration number: CTR20160747; full trial protocol (including dates defining the periods of recruitment and follow-up): http://www.chinadrugtrials.org.cn/clinicaltrials.searchlistdetail.dhtml) was a phase I, open-label, randomized, two-period, two-sequence crossover, single-center clinical trial designed to evaluate the bioequivalence of cinacalcet hydrochloride 25 mg tablets The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by ethics board of the First Affiliated Hospital of Nanjing Medical University (No. 2016-MD-208) and informed consent was taken from all the patients. Each subject participated in two phases, a test administration and a reference administration phase (*Figure 1*), under fasting or non-fasting conditions (33). The test preparation was provided by Jiangsu Jiayi Medicine Co., Ltd., and the reference preparation, REGPARA, came from Kyowa Hakko Kirin Co., Ltd. Each subject underwent two cycles of administration, randomized to receive the test preparation (T) or the reference preparation (R) at a dose of 50 mg. The random table (including random allocation sequence) was generated by the statistical analysis company using SAS software Proc Plan process. Block randomization method was used to randomize. The order of each subject receiving the test preparation or reference preparation was determined by the random table after the sponsor completed the recruitment of the subjects. The intervening period between the two cycles was not less than 10 days. For the non-fasting group, test and reference preparations were administered 30 minutes after high-fat, high-calorie breakfast with the recommendations of regulatory guidelines.

This study didn't include any interim analyses. The trial would be stopped if the following conditions occur: (I) major mistakes in the clinical trial protocol were found in the trial, making it difficult to evaluate the drug; (II) the sponsor requested suspension under the premise of fully protecting the rights and safety of subjects; (III) the State Drug Administration or the ethics committee ordered the suspension of the trial for some reason; (IV) the clinical trial cannot be continued due to other reasons.

Bioanalytical assay

A confirmatory method was developed with high-performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS) to detect the concentration of cinacalcet in plasma. Cinacalcet hydrochloride-d_3 was used as an internal standard. The analyte and internal standard were extracted by protein precipitation and separated from the matrix by a reversed-phase

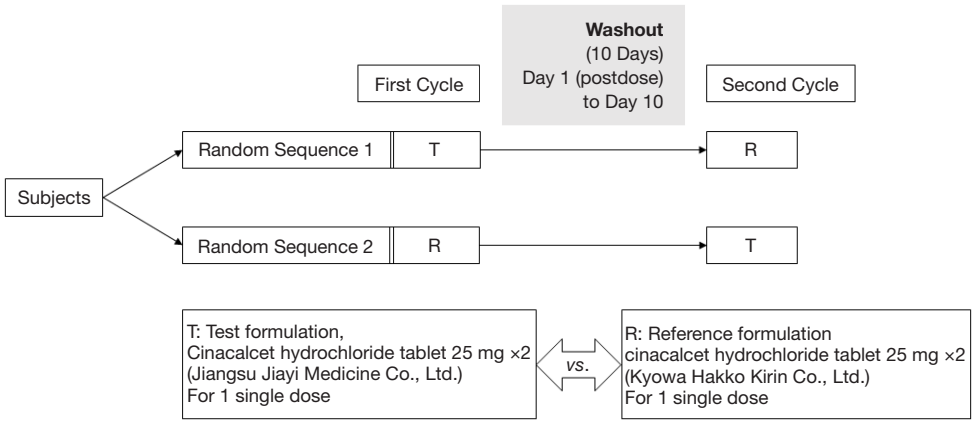

Figure 1 Schematic illustration of study design. 4 mL of peripheral blood was collected via an indwelling cannula into tubes holding the anticoagulant K2-ethylenediaminetetraacetic acid. During each treatment period, samples were collected at the following time points: 0, 0.25, 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, 6, 8, 12, 24, 48, 72 and 96 h after each single-dose administration. After collection, the blood samples were centrifuged within 30 min at 4 ℃ at 3,000 ×g for 8 min, and the supernatant plasma samples were stored at –70 ℃ until further analysis.

chromatographic column. A tandem quadrupole mass spectrometer with electrospray ionization mode (ESI) was used to conduct the quantitative analysis. The dynamic range of the assay was 0.100–30.0 ng/mL. The intra-assay precision and accuracy were less than 8.0% and 97.3–105.0%, respectively. The inter-assay precision and accuracy were less than 5.8% and 97.7–103.0%, respectively.

This trial was an open trial. Except for biological sample analysts, other personnel such as clinical researchers, participants, project management personnel, project supervisors, statistics and data management analysts were not blinded, only sample analysis personnel used blind analysis.

Safety and pharmacodynamic parameter evaluations

AEs evaluated safety, vital signs, physical examination, clinical laboratory tests (complete blood count, blood biochemistry, and urinalysis), coagulation check, and 12-lead electrocardiogram. The severity of all AEs and serious adverse events (SAEs) were assessed by clinicians and their potential relationship to medications.

To assess plasma intact PTH (iPTH) and serum calcium concentrations, blood samples were collected before dosing and at the fourth and 96th hour after drug administration. iPTH and serum calcium levels were measured by a fully automatic biochemistry analyzer (Beckman Coulter, AU5800).

Statistical analysis

Standard non-compartmental PK parameters (C_{max}, T_{max}, $t_{1/2}$, AUC_{0-t}, $AUC_{0-\infty}$, etc) were calculated by WinNonlin 7.0. Bioequivalence was assessed separately in the fasting and non-fasting groups. A 90% CI of the ratio of geometric means in the range of 80.00–125.00% between the test and reference preparations would be considered bioequivalent. SAS 9.4 was used for the statistical analyses. Statistically significant differences were determined by one-way analysis of variance (ANOVA) with an unpaired two-tailed heteroscedastic *t*-test. Statistical significance was set at $P<0.05$.

Genotype selection and analysis

Blood samples were obtained from the 65 fasting group subjects before the administration of cinacalcet for genotyping purposes. DNA was extracted from each subject's leukocytes using a commercially available kit (Relax Gene Blood DNA System; TIANGEN BIOTECH (BEIJING), China) (34). DNA samples were sent to Sangon Biotech for sequencing with a DNA sequencer (ABI3730, AB Sciex). Thirteen SNPs in five genes were selected for sequencing. Of these genes, *CASR* and *VDR* are involved in PTH and calcium regulation (16), and *CYP3A4*, *CYP2D6*, and *CYP1A2* are known as the major enzymes involved in cinacalcet hydrochloride metabolism (7). *Table 2* sets out information on the 13 selected SNPs in the *CASR*, *VDR*,

Table 2 The information of 13 SNPs in *CASR*, *VDR*, *CYP3A4*, *CYP2D6* and *CYP1A2* genes in Chinese healthy subjects (n=65)

Gene	SNP	Chromosome	Alleles	SNP location	Global MAF	Genotype frequency (%), n=65		
						AA	AB	BB
CASR	rs10190	3:122286389	C/T	3'-UTR	T =0.4669/2338	26.2	52.3	21.5
	rs1802757	3:122286284	C/T	3'-UTR	T =0.1983/993	27.7	53.8	18.5
	rs2221266	3:122277969	C/T	Intron	T =0.4619/2313	27.7	52.3	20.0
VDR	rs7975232	12:47845054	A/C	Intron	C =0.4846/2427	7.7	40.0	52.3
CYP2D6	rs16947	22:42127941	G/A	Intron	A =0.3592/1799	70.8	26.2	3.1
CYP1A2	rs2470890	15:74755085	C/T	Syn	T =0.2362/1183	80.0	20.0	0.0
	rs2472304	15:74751897	G/A	Intron	A =0.2380/1192	80.0	20.0	0.0
	rs2069514	15:74745879	G/A	Promoter	A =0.2089/1046	55.4	36.9	7.7
CYP3A4	rs2242480	7:99763843	C/T	Intron	T =0.4217/2112	61.5	33.8	4.6
	rs2246709	7:99768096	A/G	Intron	G =0.3678/1842	32.3	52.3	15.4
	rs4646437	7:99767460	C/T	Intron	A =0.3632/1819	83.1	15.4	1.5
	rs4646440	7:99763247	C/T	Intron	A =0.1486/744	66.2	29.2	4.6
	rs12333983	7:99756491	T/A	downstream	A =0.3916/1961	56.9	38.5	4.6

AA, homozygote major allele; AB, heterozygote allele; BB, homozygote minor allele; SNP, single nucleotide polymorphism.

CYP3A4, *CYP2D6*, and *CYP1A2* genes in the 65 healthy subjects.

Results

Safety and tolerability

In the fasting group, a total of 84 AEs occurred in 42 subjects, including 50 after receiving the test preparation and 34 after receiving the reference preparation. Four subjects withdrew due to AEs after the first cycle of administration. One of these subjects exhibited prolonged electrocardiogram (ECG) QRS wave duration, indicating an intraventricular block; two subjects had low fibrinogen, and one subject displayed abnormal coagulation function. Clinicians believed it was not appropriate for these subjects to continue with the experiment. In the non-fasting group, 71 AEs occurred in 31 subjects, including 45 after receiving the test preparation and 26 after receiving the reference preparation. One subject vomited after receiving the test preparation in the first cycle, and one subject vomited after receiving the test preparation in the second cycle. All AEs were mild, and no SAEs occurred in this study. These results show that the test and reference preparations were both safe under fasting and non-fasting conditions. *Table 3* sets out the specific symptoms of AEs during preparation administration.

PK and bioequivalence

The mean cinacalcet plasma concentration-time profiles for the test and reference preparations under fasting and non-fasting conditions are presented in *Figure 2*. The corresponding PK parameters are summarized in *Table 4* and *Figure 3*.

Statistical comparisons of cinacalcet hydrochloride between the test and reference preparations demonstrated that the geometric least square mean ratios (T/R) of C_{max}, AUC_{0-t}, and $AUC_{0-\infty}$ values in the fasting group were 109.89%, 105.33%, and 108.83% respectively, and the corresponding 90% CIs were 98.36–122.79%, 98.04–113.15%, and 98.90–119.75%. In the non-fasting group, the T/R of C_{max}, AUC_{0-t}, and $AUC_{0-\infty}$ values were 100.74%, 99.09% and 100.26% respectively, and the corresponding 90% CIs were 92.65–109.54%, 94.79–103.58%, and 96.00–104.71%. All CIs fell within the conventional 80.00–125.00% acceptance limits for bioequivalence (*Table 5*). There was no significant difference in T_{max} found between the two treatments under either fasting or non-fasting conditions (33). Additionally, the C_{max} and AUC of

Table 3 Specific symptoms of AEs during administration

AEs	Fasting group, n (%)		Fed group, n (%)	
	T (n=68)	R (n=66)	T (n=63)	R (n=63)
Increased white blood cell count (WBC)	0 (0.0)	0 (0.0)	1 (1.6)	0 (0.0)
Increased plasma alanine aminotransferase (AAT)	1 (1.5)	0 (0.0)	1 (1.6)	0 (0.0)
Increased red blood cell count (RBC)	1 (1.5)	0 (0.0)	0 (0.0)	0 (0.0)
Positive urine white blood cell	2 (2.9)	4 (6.1)	0 (0.0)	0 (0.0)
Positive urine leukocyte esterase	2 (2.9)	4 (6.1)	0 (0.0)	0 (0.0)
Positive urine albumin	2 (2.9)	1 (1.5)	1 (1.6)	0 (0.0)
Positive urine red blood cell	0 (0.0)	2 (3.0)	0 (0.0)	0 (0.0)
Positive urine occult blood	4 (5.9)	1 (1.5)	0 (0.0)	1 (1.6)
Extension of QRS waves in electrocardiogram (ECG)	1 (1.5)	0 (0.0)	0 (0.0)	0 (0.0)
Abnormal ECG	1 (1.5)	1 (1.5)	0 (0.0)	0 (0.0)
Increased neutrophils count	0 (0.0)	0 (0.0)	1 (1.6)	0 (0.0)
Increased aspartate aminotransferase	0 (0.0)	0 (0.0)	1 (1.6)	0 (0.0)
Hypocalcemia	13 (19.1)	11 (16.7)	8 (12.7)	4 (6.3)
Reduction of hemoglobin	1 (1.5)	0 (0.0)	0 (0.0)	0 (0.0)
Decrease of iPTH	9 (13.2)	6 (9.1)	8 (12.7)	4 (6.3)
Increase of iPTH	7 (10.3)	2 (3.0)	0 (0.0)	1 (1.6)
Decreased fibrinogen	4 (5.9)	1 (1.5)	0 (0.0)	0 (0.0)
Increased lipoprotein(a)	1 (1.5)	0 (0.0)	0 (0.0)	0 (0.0)
Increased serum creatine phosphokinase	0 (0.0)	0 (0.0)	1 (1.6)	0 (0.0)
Fighting in subjects	0 (0.0)	1 (1.5)	0 (0.0)	0 (0.0)
Coagulation disorder	1 (1.5)	0 (0.0)	0 (0.0)	0 (0.0)
Vomiting	0 (0.0)	0 (0.0)	2 (3.2)	0 (0.0)
All	50 cases in 31 subjects (45.6%)	34 cases in 20 subjects (30.3%)	24 cases in 13 subjects (20.6%)	10 cases in 5 subjects (7.9%)

subjects in the non-fasting group were significantly higher (P<0.0001) than those in the fasting group. The T_{max}, CL/F, and Vd/F in the fasting group were significantly higher (P<0.0001) than those in the non-fasting group. No significant difference in $t_{1/2}$ and λ_z was found between the two groups.

PD of cinacalcet in healthy subjects

Changes in iPTH and serum calcium concentrations during the study are shown in *Figure 4*. These changing values varied widely between the subjects. iPTH and serum calcium declined significantly from the baseline at four hours post-administration. Ninety-six hours post-administration, iPTH and serum calcium returned to the baseline in both the fasting and non-fasting group. The specific concentrations of iPTH and serum calcium at different points in time are shown in *Table 6*.

Association of CASR and VDR genotypes with PD of cinacalcet in the fasting group

No significant differences were found between SNPs of VDR and CASR concerning the changing level of iPTH

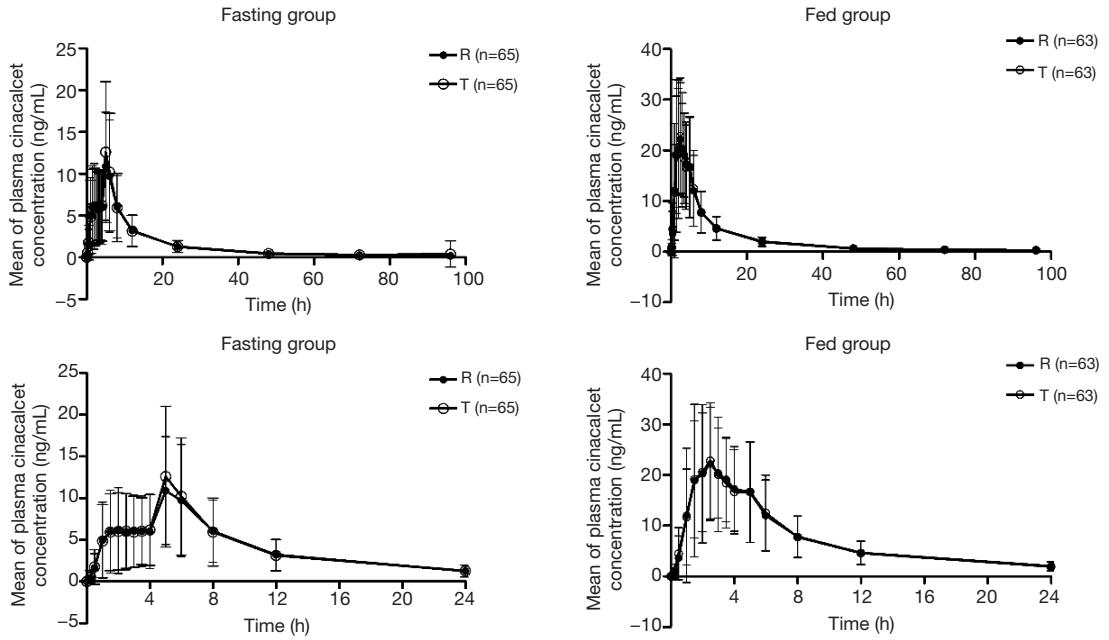

Figure 2 Arithmetic mean (SD) cinacalcet plasma concentration-time profiles for the test and reference preparations in the fasting and non-fasting groups. Dose: 50 mg; R: reference administration; T: test administration.

Table 4 Summary of pharmacokinetic parameters of cinacalcet following administration of a single oral dose (50 mg) of the test and reference preparations in healthy Chinese subjects (mean ± SD)

PK parameters of cinacalcet	Fasting group (n=65)		Fed group (n=63)	
	Test	Reference	Test	Reference
AUC_{0-t} (ng·h/mL)	138.9±73.0	134.1±70.09	230.0±99.34	231.5±100.2
$AUC_{0-\infty}$ (ng·h/mL)	150.5±80.11	145.3±75.88	248.6±108.3	247.9±109.2
C_{max} (ng/mL)	14.26±8.176	13.20±7.149	28.08±12.73	28.24±15.16
T_{max} (h)	4.154±1.761	4.231±1.761	2.786±1.230	2.675±1.100
$t_{1/2}$ (h)	37.42±20.47	34.34±15.68	39.46±20.56	35.98±12.95
λ_z (1/h)	0.024±0.013	0.026±0.016	0.023±0.012	0.022±0.009
CL/F (L/h)	436.9±268.3	439.2±265.2	251.0±159.7	251.7±164.4
Vd/F (L)	21,099±13,983	20,595±12,205	13,208±8,071	11,883±5,257

in 65 healthy subjects after receiving test or reference cinacalcet tablets under fasting conditions (*Figure 5*). For VDR rs7975232, the mean changing level of iPTH was lower in subjects with AA genotype (homozygote minor allele) than those with AC (heterozygote allele) and CC (homozygote major allele) genotypes. CASR rs10190, rs1802757, and rs2221266 showed a similar trend. However, no statistical differences were found due to the volatile nature of data between individuals.

Association of CYPs genotypes with PK of cinacalcet in the fasting group

A significant difference was found between the SNP of CYP3A4 rs4646437 and the PK parameters (C_{max} and AUC_{0-t}) in 65 healthy subjects after receiving test or reference cinacalcet tablets under fasting conditions (*Figure 6*). The average C_{max} and AUC_{0-t} of subjects carrying T alleles of rs4646437 were higher than those with major

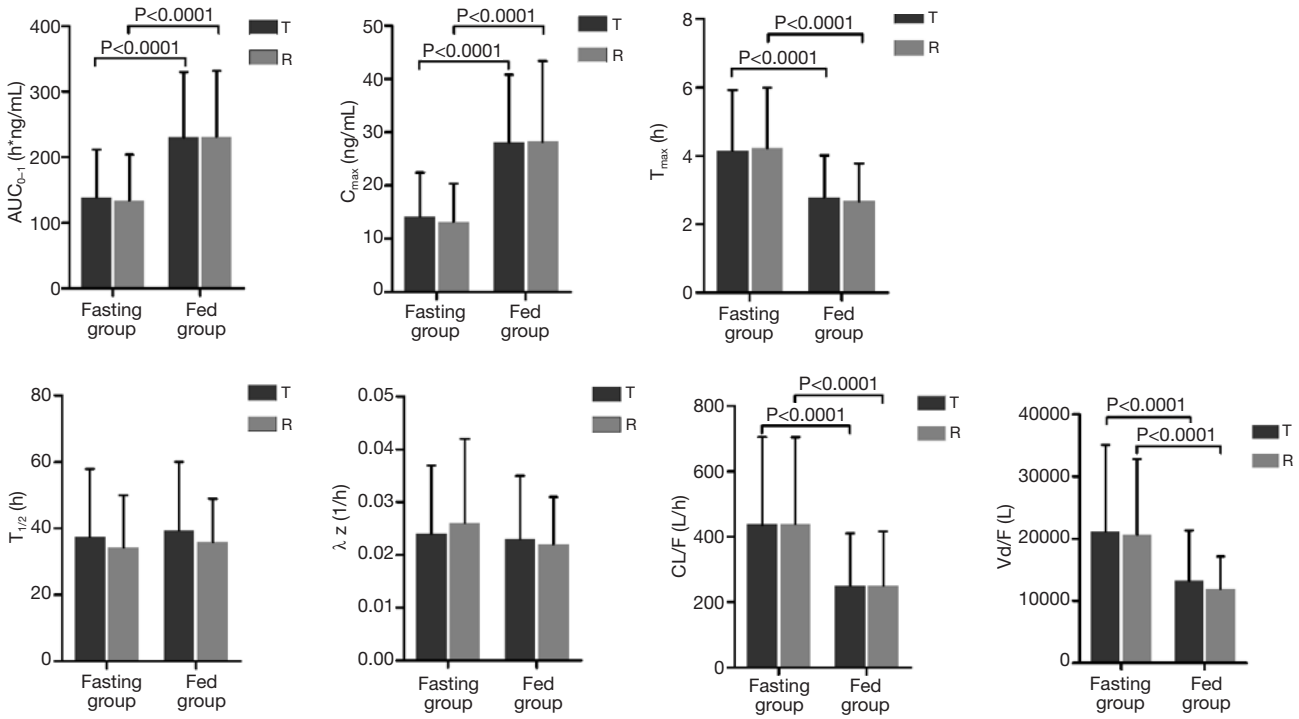

Figure 3 Comparison diagram of PK parameters of cinacalcet between fasting and non-fasting group. Dose: 50 mg; R: reference administration; T: test administration. PK, pharmacokinetics.

Table 5 Statistical comparison of pharmacokinetic parameters for cinacalcet following administration of a single oral dose of the test and reference preparations under both fasting and fed conditions in healthy Chinese subjects

PK parameters	Geometric mean		Ratios (T/R), %	90% CI
	Test	Reference		
Fasting group (n=65)				
C_{max} (ng/mL)	12.296	11.189	109.90	98.36–122.79%
AUC_{0-t} (ng·h/mL)	121.412	115.272	105.33	98.04–113.15%
$AUC_{0-\infty}$ (ng·h/mL)	136.127	125.083	108.83	98.90–119.75%
Fed group (n=63)				
C_{max} (ng/mL)	25.014	24.831	100.74	92.65–109.54%
AUC_{0-t} (ng·h/mL)	208.513	210.431	99.09	94.79–103.58%
$AUC_{0-\infty}$ (ng·h/mL)	225.159	224.573	100.26	96.00–104.71%

homozygote alleles (CC).

Discussion

This clinical trial was conducted to compare the bioequivalence between test and reference preparations of cinacalcet hydrochloride tablets and to evaluate the safety of cinacalcet. The results demonstrated that the reference and test preparations were bioequivalent under both fasting and non-fasting conditions. Safety evaluations revealed that both the test and reference preparations were well tolerated by subjects and were safe to use.

Common AEs of cinacalcet observed in dialysis patients include hypocalcemia, nausea, vomiting, diarrhea,

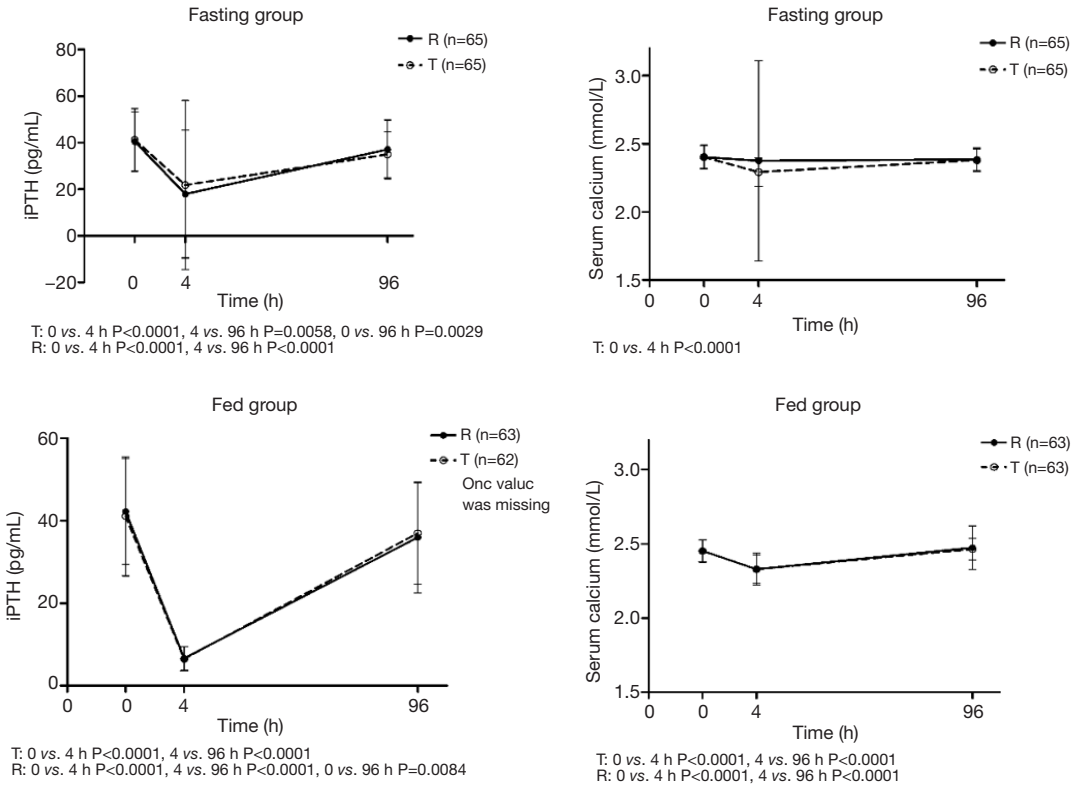

Figure 4 Mean (SD) change of iPTH and serum calcium concentration in the fasting and non-fasting groups after receiving test and reference administration. Dose: 50 mg; R: reference administration; T: test administration.

Table 6 Pharmacodynamic profiles for cinacalcet following administration of a single oral dose (50 mg) of the test and reference preparations under both fasting and fed conditions in healthy Chinese subjects (mean ± SD)

PD profiles of cinacalcet	Fasting group (n=65)		Fed group (n=63)	
	Test	Reference	Test	Reference
iPTH (pg/mL)				
0 h	41.21±13.44	40.54±12.72	41.00±14.34	42.23±12.89
4 h	21.81±36.39	17.95±27.39	6.520±2.926	6.670±2.784
96 h	34.92±9.840	37.13±12.67	36.95±12.18	35.93±13.51
Serum calcium (mmol/L)				
0 h	2.401±0.08400	2.402±0.08605	2.452±0.07576	2.453±0.7265
4 h	2.291±0.1035	2.375±0.7356	2.329±0.1083	2.329±0.09498
96 h	2.379±0.08427	2.385±0.08290	2.464±0.07482	2.475±0.1474

abdominal distention, myalgia, dizziness, hypertension, asthenia, anorexia, prolongation of QT interval, and non-cardiac chest pain (7,35,36). There are limited reports of cinacalcet AEs occurring in healthy subjects. The most frequently occurring AE observed in our study was hypocalcemia, followed by changes in plasma iPTH (37,38). Reports describing the prognosis of patients administered cinacalcet are currently lacking (39). Additionally, all AEs

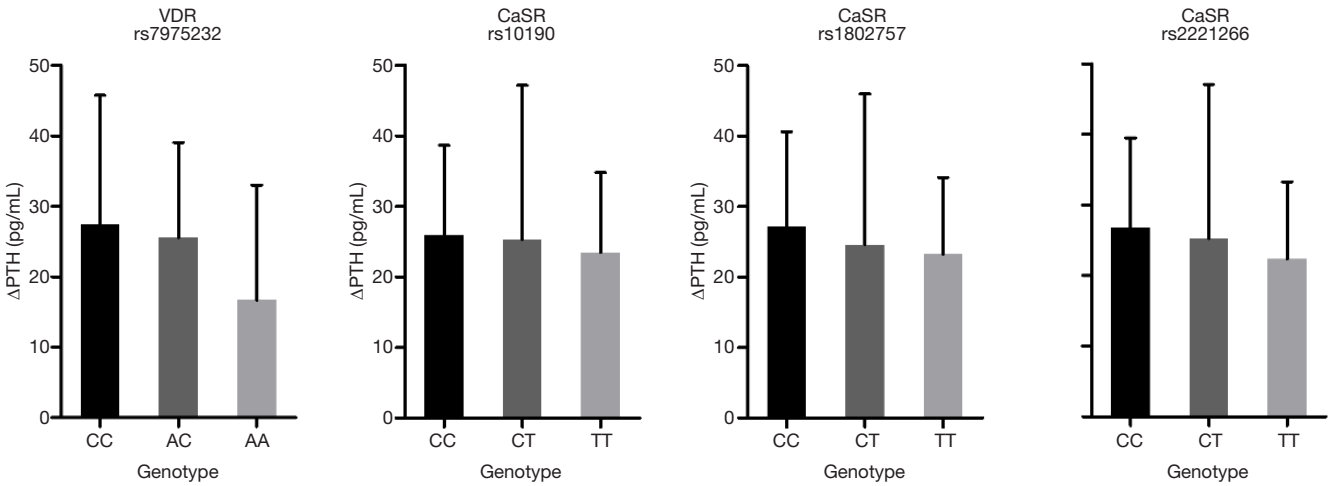

Figure 5 No statistically significant associations were found between SNPs of VDR and CASR and the changing level of iPTH in 65 healthy subjects after receiving 50 mg of cinacalcet under fasting conditions. ΔPTH =$iPTH_{Baseline}$ – $iPTH_{4\,h}$ (pg/mL); ΔCa = $Ca_{Baseline}$ – $Ca_{4\,h}$ (mmol/L), the values were obtained by calculating the average of two cycles after subjects receiving test and reference administration).

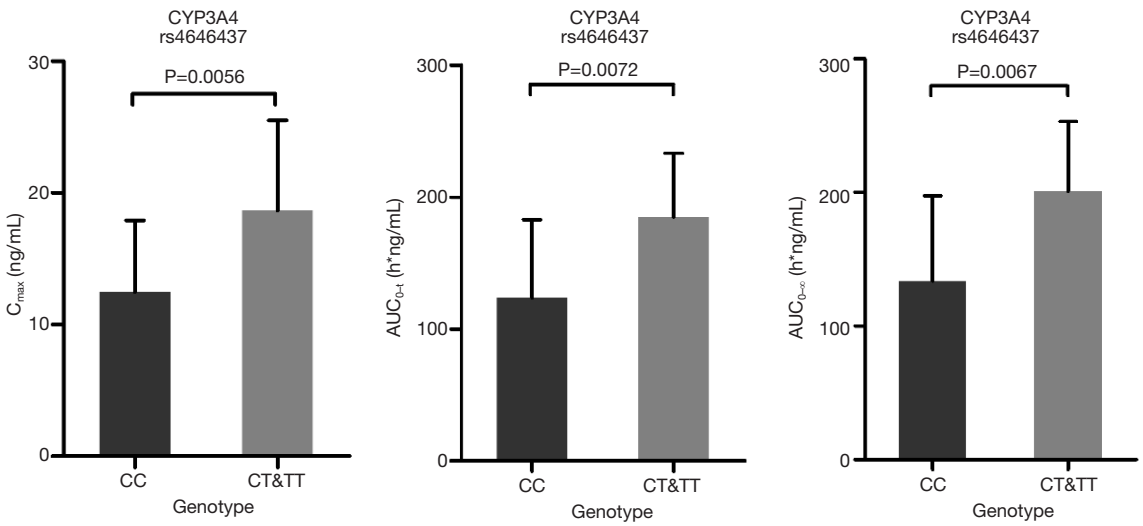

Figure 6 Significant associations were found between CYP3A4 rs4646437 and C_{max} and AUC in 65 healthy subjects after receiving 50 mg of cinacalcet under fasting conditions. The values were obtained by calculating the average of two cycles after subjects receiving test and reference administration. Because only one subject was with TT genotype, so the subjects with CT and TT genotypes were classified as the same group for statistics.

with an incidence of more than 5% occurred in the fasting group, including decreased fibrinogen (5.9%), occult blood in the urine (5.9%), white blood cells in the urine (6.1%) and leukocyte esterase in the urine (6.1%). All AEs were mild, and no SAEs occurred, which shows both the test and reference cinacalcet tablets were well tolerated and safe.

Changes in iPTH and serum calcium differed significantly among subjects after receiving either the test or reference preparations under fasting conditions, while subjects in the non-fasting group showed fewer individual differences. Also, the C_{max} and AUC of subjects in the non-fasting group were significantly higher (P<0.0001) than in

the fasting group, suggesting that dietary factors affected the PK and PD of cinacalcet. Taking cinacalcet with meals or immediately after eating may improve its absorption.

It has been reported that CASR rs1802757 and rs10190 are significantly associated with individual variation in cinacalcet response (16). Studies have also previously found that CASR rs2221266 may be associated with differences in PTH levels and that the VDR gene polymorphism rs7975232 is associated with PTH and calcium regulation (25,26). However, the current study found no significant associations between SNPs of VDR and CASR and changes in iPTH and calcium levels (34). A study by Jeong *et al.* recruited SHPT patients who had been taking cinacalcet for more than three months, and patients whose iPTH levels were elevated even after three months of cinacalcet treatment were defined as non-responders. However, all subjects in the current study were healthy, and each participant exhibited a decline in iPTH at least once after a single dose. Therefore, diverse factors may affect the significant associations between SNPs and cinacalcet PD, and SNPs of CASR and VDR may influence cinacalcet PD less under non-pathological conditions.

CYP3A4 is the dominant enzyme that metabolizes cinacalcet, others being CYP1A2 and CYP2D6 (7,16). No literature has yet reported whether these CYP enzymes affect the PK of cinacalcet (40). In the current study, we found that the C_{max} and AUC of subjects with T alleles of CYP3A4 rs4646437 were significantly higher than those with the CC genotype ($P<0.01$), indicating that the SNP of CYP3A4 rs4646437 may affect cinacalcet PK under fasting conditions.

Conclusions

CYP3A4 rs4646437 may influence the PK of cinacalcet. Significant associations were found in the current study between CYP3A4 rs4646437 and PK parameters (C_{max} and AUC) of cinacalcet ($P<0.01$). Subjects carrying T alleles of rs4646437 appeared to metabolize cinacalcet poorly, and the reference and the test cinacalcet preparations were found to be bioequivalent in healthy Chinese subjects under both fasting and non-fasting conditions. An evaluation of drug safety showed that cinacalcet was well-tolerated by patients and that it was safe to use. Also, it was found that dietary factors had a significant effect on the PK and PD of cinacalcet in that cinacalcet exposure was greater in the non-fasting group. Therefore, cinacalcet may be better absorbed when taken with or immediately after eating.

Acknowledgments

The authors would like to thank all subjects for their participation in this study. We also would like to thank the clinical investigators, nurses, and study coordinators for their contributions to this study.

Funding: This project was supported by grants from the National Natural Sciences Foundation of China (81673515, 81870436, 81503160), Natural Science Foundation of Jiangsu Province (BK20161591), Six Talent Peaks Project in Jiangsu Province (2014-YY-001), Jiangsu Provincial Medical Youth Talent (QNRC2016215), Suzhou Science and Education Youth Project (KJXW2016067), Suzhou Industrial Technology Innovation (SYSD2016046), National Key Research & Development Plan of the Ministry of Science and Technology of the People's Republic of China (2018YFC1314900, 2018YFC1314901), the 2016 Industry Prospecting and Common Key Technology Key Projects of Jiangsu Province Science and Technology Department (BE2016002-4), the 2017 Projects of Jiangsu Provincial Department of Finance (2150510), and the 2016 Projects of Nanjing Science Bureau (201608003).

Footnote

Reporting Checklist: The authors have completed the CONSORT reporting checklist. Available at http://dx.doi.org/10.21037/atm-20-1329

Peer Review File: Available at http://dx.doi.org/10.21037/atm-20-1329

Data Sharing Statement: Available at http://dx.doi.org/10.21037/atm-20-1329

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at http://dx.doi.org/10.21037/atm-20-1329). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by ethics board of the First Affiliated Hospital of Nanjing Medical University (No. 2016-MD-208) and informed consent was taken from

all the patients.

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(English Language Editors: B. Madden and J. Chapnick)

Cite this article as: Liu YJ, Sun LN, Cheng ZP, Qian Y, Ma ZQ, Zhang XH, Zhang HW, Xie LJ, Yu L, Yuan ZQY, Liu Y, Wang YQ. Pharmacogenetic and safety analysis of cinacalcet hydrochloride in healthy Chinese subjects. Ann Transl Med 2020;8(21):1385. doi: 10.21037/atm-20-1329

