

Pharmacokinetics of lansoprazole in Chinese healthy subjects in relation to *CYP2C19* genotypes

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KEY WORDS lansoprazole; pharmacokinetics; human *CYP2C19* protein; genotype

ABSTRACT

AIM: To study the kinetic characteristics of lansoprazole in healthy Chinese subjects in relation to *CYP2C19* genotype status for the individualized dose regimen of lansoprazole. **METHODS:** Nine homozygous extensive metabolizers (homo EMs) and 9 poor metabolizers (PMs) were recruited for the study from a total of 70 healthy Chinese volunteers, whose *CYP2C19* genotype status was determined by the PCR-RFLP techniques. After a single oral dose of 30 mg lansoprazole capsule, plasma concentrations of lansoprazole were determined with HPLC method. **RESULTS:** In Chinese subjects, the allele frequencies of the *CYP2C19m1* and *CYP2C19m2* mutation were 0.35 and 0.07, respectively. The concentration-time curves in the two groups were best fitted to a one-compartment model. In the homo EMs and the PMs groups, the main kinetic parameters were as follows: T_{\max} (2.44±0.85) and (2.33±0.94) h, C_{\max} (1.10±0.34) and (1.73±0.56) mg/L, Cl/F (16.55±6.38) and (3.58±1) L/h, $T_{1/2ke}$ (1.96±0.51) and (4.21±0.53) h, AUC were (3.23±1.08) and (11.05±3.23) mg·h·L⁻¹. A significant difference in AUC, $T_{1/2ke}$, Cl/F , C_{\max} values existed between the two groups ($P<0.01$). **CONCLUSION:** *CYP2C19* genotype is the major factor to influence the interindividual kinetic variability of lansoprazole. Individualized dose regimen of lansoprazole, based on identification of genotype, can be of great benefit for the reasonable use of this drug.

INTRODUCTION

Lansoprazole is a proton pump inhibitor (PPI) that effectively inhibit gastric acid secretion by irreversibly binding to the Proton pump (H^+/K^+ -ATPase) in gastric parietal cells^[1]. This drug is being increasingly used in the treatment of acid-related diseases^[2]. The hydroxylation of lansoprazole (OH-lansoprazole formation) by a polymorphic S-mephenytoin 4'-hydroxylase [cytochrome P450 2C19 (*CYP2C19*)] is the main metabolic route^[3]. The genetic polymorphism of *CYP2C19* should

be of a clinical concern in the treatment of acid-related diseases with lansoprazole^[4].

The frequency of poor metabolizer phenotype of *CYP2C19* shows a considerable inter-ethnic difference: approximately 3 %-5 % of Caucasians have been identified as poor metabolisers (PMs) of S-mephenytoin, whereas the frequencies of PMs in Japanese (19 %-23 %), Chinese (15 %), and Koreans (13 %) are much higher than those in Caucasians^[5-7]. de Morais *et al*, have reported that the primary defect in PMs was a single base pair mutation in exon 5 of *CYP2C19* (m1)^[8]. A second mutation in exon 4 of *CYP2C19* (m2) appears to be present only in Asians^[9]. According to the genotyping analysis of *CYP2C19*, PMs consist of three genotypes (ie, m1/m1, m2/m2, or m1/m2), while extensive metabolisers (EMs) include homozygous EMs (ie, wt/

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wt), and heterozygous EMs (ie, wt/m1 or wt/m2)^[6].

An interindividual difference in the activity of *CYP2C19* has been reported in relation to the metabolic disposition of lansoprazole^[4]. The acid-inhibitory effect of lansoprazole has also recently been reported to be affected by *CYP2C19* genotype status^[10]. Because in Chinese subjects data are not currently available on the metabolism of lansoprazole in relation to *CYP2C19* pharmacogenetics, an attempt should be made to investigate the kinetic characteristics of lansoprazole in Chinese subjects who have different *CYP2C19* genotype status and to supply the basis of pharmacokinetics for the individualized dose regimen of lansoprazole, based on identification of genotypes.

MATERIALS AND METHODS

Subjects Blood samples were obtained from 70 unrelated healthy Chinese Han subjects after being given written informed consent. *CYP2C19* genotyping of each sample was performed. Homo EMs were homozygous for the wild-type (wt) alleles in both exon 5 and 4 (wt/wt) and classified as the homo EMs group. PMs were heterozygous for both the m1 mutation and m2 mutation (m1/m2) or homozygous for the m1 mutation without the m2 mutation (m1/m1), and for the m2 mutation without the m1 mutation (m2/m2), and classified as the PMs group.

Eighteen subjects (9 homo EMs and 9 PMs) were selected to participate in this study. None of the subjects had a history of significant medical illness or hypersensitivity to any drug. They did not consume extensive amounts of alcohol and were non-smokers. None of them had taken any drugs for at least 1 week before the study and did not take any other drugs during the study. Their normal health status was judged on the basis of a physical examination with screening

blood chemistries, including a complete blood count and liver function test, urinalysis, and electrocardiogram performed before the study. Demographic and clinical characteristics of subjects enrolled in the study is shown in Tab 1. The study protocol were approved by the Ethic Committee of School of Medicine, Zhengzhou University.

Study protocol Each subject received a single oral dose of lansoprazole 30 mg as the entericoated capsule (Takepron, Takeda Pharmaceutical Co, Osaka, Japan) with 200 mL water at 8:00 AM after an overnight fast. Standard meals (lunch and supper) were prepared at 12:00 PM and 18:00 PM. Venous blood samples (4 mL each) were drawn into the heparinized tubes, immediately before the drug administration and at 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, and 24 h after dosing. Within 15 min of blood collection, plasma was separated by centrifugation at 3000×g for 10 min. Plasma samples were frozen and maintained at -20 °C until analysis.

***CYP2C19* genotyping** Genotyping identifying *CYP2C19* wild-type gene and the two mutated alleles, *CYP2C19m1* in exon 5 and *CYP2C19m2* in exon 4, were performed by a polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method, as originally described by de Morais *et al*^[9,11], with minor modifications as reported by Goldstein and Blaisdell^[12].

Assay of lansoprazole in plasma Plasma concentrations of lansoprazole were determined by the validated HPLC method developed by Karol *et al*^[13]. In brief, to each 0.5 mL volume of plasma sample, 10 µL of the internal standard (Ethyl p-hydroxybenzoate purchased from Peking Chemical Regents Co, purity 99.8 %) solution (12.5 mg/L in methylene chloride) was added and mixed. Then 5 mL of diethyl ether anhydrous-methylene chloride (6:4, v:v) was added. Extraction

Tab 1. Demographic and clinical characteristics of subjects enrolled in study. Mean±SD. (range).

Study group	<i>CYP2C19</i> genotype (subject number)	Age (years)	Height (cm)	Body weight (kg)	Male/female ratio
Homo EMs	wt/wt (n=9)	21.0±0.91 (19-25)	170.2±6.6 (157-179)	62.7±12 (47-80)	8/1
PMs	m1/m1 (n=6) m1/m2 (n=3)	21.3±1.7 (19-25)	169.8±5.8 (160-180)	60.8±8.4 (49-79)	8/1
All subjects	(n=18)	21.2±1.3 (19-25)	170.1±6.0 (157-179)	61.7±10.1 (47-79)	8/1

wt, Wild type; m1, *CYP2C19m1*; m2, *CYP2C19m2*; Homo EMs, homozygous extensive metabolisers; PMs, poor metabolisers.

was conducted by shaking for 5 minutes and then by centrifugation at $4000\times g$ for 10 min. An aliquot of 4 mL of the organic extract was transferred to a glass tube and evaporated to dryness under a gentle stream of nitrogen at $40\text{ }^{\circ}\text{C}$ and residue dissolved in 200 μL mobile phase (adjusted to pH 7.5 with phosphoric acid), of which 80 μL aliquot was injected into the HPLC system. Chromatographic analyses were performed using a HPLC system consisting of an Agilent 1100 solvent delivery pump, Agilent 1100 diode array detector, and a chromatograph (Agilent 1100 Chemstation, Agilent Technology Co, Ltd). The separation was performed at $40\text{ }^{\circ}\text{C}$ by using a ZORBAX SB-C18 column (5 μm , 150 mm \times 2.1 mm). The mobile phase consisted of acetonitrile-water-octylamine (400:600:1, v:v:v; adjusted to pH 7.0 with phosphoric acid) and was pumped at a flow rate of 0.5 mL/min. Effluent was monitored at a wavelength of 285 nm and corresponding peak areas were recorded. The limit of quantitation for lansoprazole was 25 ng/mL plasma. The intraday and interday coefficients of variation of lansoprazole were less than 8.1 % and 9.1 % over the concentration range from 25 to 4 mg/L. Short-term stability showed that lansoprazole (Sigma Chemical Co, USA; purity 99.99 %) is stable in plasma for at least 16 h at room temperature, while long-term stability studies showed that lansoprazole is stable in plasma for at least 64 d when stored at $-20\text{ }^{\circ}\text{C}$.

Pharmacokinetic analysis Individual lansoprazole plasma-concentration data were analyzed by compartmental analysis using the 3P97 (practical pharmacokinetic program) edited by Mathematical Board of the Chinese Pharmacological Society. The maximum lansoprazole concentration (C_{max}) and the corresponding peak times (T_{max}) were determined from the respective observed plasma concentration-time data. The elimination rate constant (ke) was obtained from the least-square fitted terminal log-linear portion of the plasma concentration-time profile. The elimination half-life ($T_{1/2ke}$) was calculated as $0.693/ke$. The area under the curve to the last measurable concentration (AUC_{0-t}) was calculated by the linear trapezoidal rule. The area under the curve extrapolated to infinity ($\text{AUC}_{0-\infty}$) was calculated as $\text{AUC}_{0-t} + C_t/ke$, where C_t is the last measurable concentration. The apparent oral clearance (Cl_{oral}) of lansoprazole was calculated as $Cl_{\text{oral}} = \text{Dose}/\text{AUC}_{0-\infty}$.

Statistical analysis The values were expressed as mean \pm SD. Differences in pharmacokinetic data between the homo EM and PM groups were evaluated statistically by independent-samples *t*-test. $P < 0.05$ was

considered statistically significant.

RESULTS

Genotyping for CYP2C19 Five different allelic band patterns were observed (Fig 1). Of the 70 individuals analyzed, 22 were homozygous for the wt allele in both exon 5 and exon 4 (wt/wt; 31.4 %), 30 were heterozygous for the *CYP2C19m1* mutation (wt/m1) without the *CYP2C19m2* mutation (42.9 %), 7 were heterozygous for the *CYP2C19m2* (wt/m2) without the *CYP2C19m1* mutation (10.0 %), 3 were heterozygous for the *CYP2C19m1* and the *CYP2C19m2* mutation (m1/m2; 4.3 %), and 8 were homozygous for the *CYP2C19m1* mutation (m1/m1) without the *CYP2C19m2* mutation (11.4 %). No subject in this study was found to be homozygous for the *CYP2C19m2* mutation. Thus the allele frequencies of the m1 and m2 mutations in our subjects were 0.35 and 0.07, respectively.

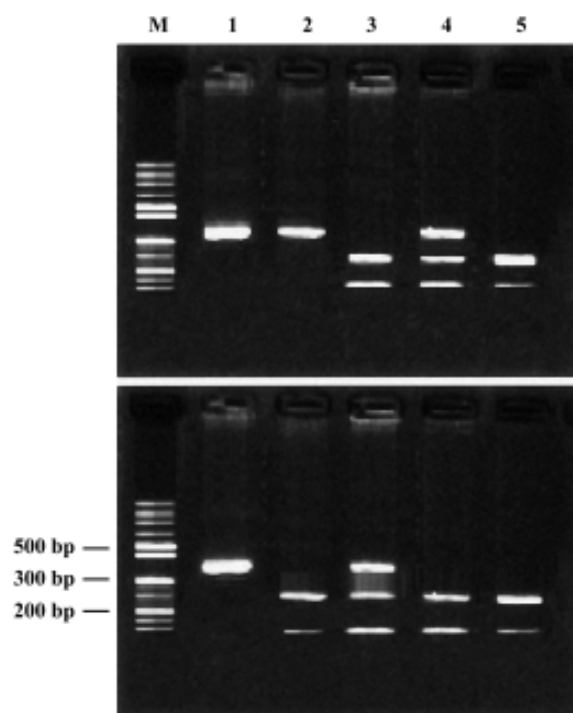


Fig 1. PCR-RFLP analysis with *Sma* I and *Bam*HI for *CYP2C19m1* and *CYP2C19m2* polymorphism. Lane 1: wt/wt, Lane 2: wt/m1, Lane 3: wt/m2, Lane 4: m1/m1, Lane 5: m1/m2, M: 50 bp DNA Ladder.

Pharmacokinetics of lansoprazole The typical chromatograms of lansoprazole in plasma were illustrated in Fig 2. The mean plasma concentration-time

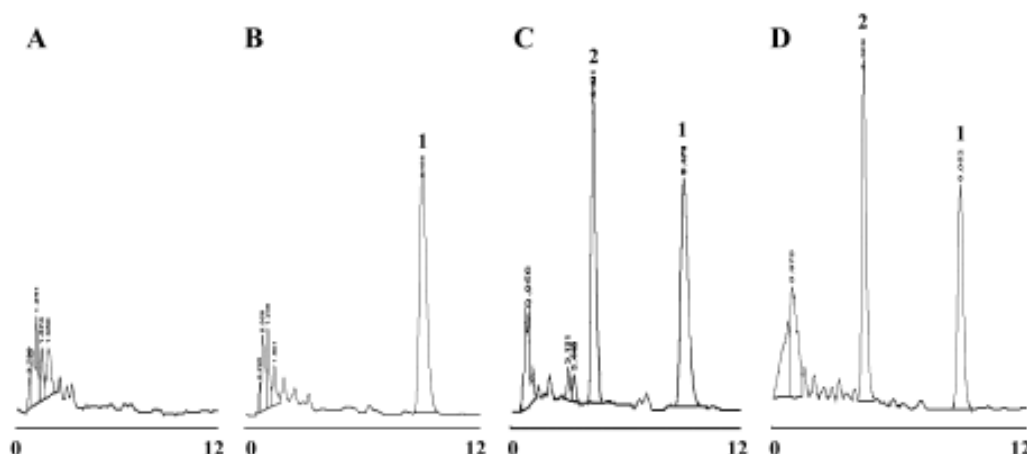


Fig 2. Chromatograms of lansoprazole and Ethyl p-hydroxybenzoate (internal standard, IS) in human plasma. A. Blank plasma sample; B. Plasma sample spiked with IS; C. Plasma sample spiked with lansoprazole 2 mg/L and IS; D. Plasma sample 2 h after oral 30 mg lansoprazole. Peak 1, 2 refer to IS and lansoprazole.

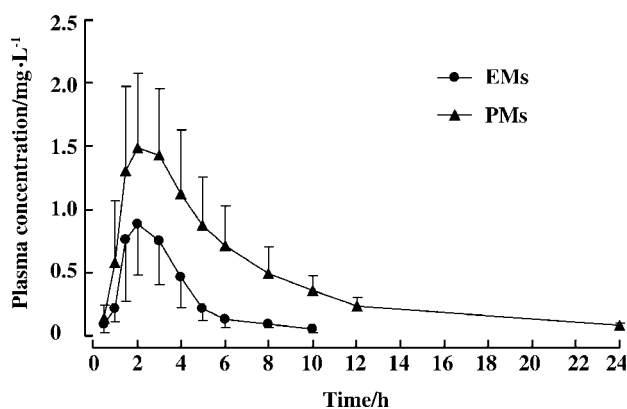


Fig 3. Plasma concentration-time curves of lansoprazole after an oral dose of 30 mg in nine homozygous extensive metabolisers (EMs) and nine poor metabolisers (PMs). $n=9$. Mean \pm SD.

profiles of lansoprazole (Fig 3) in the homo EMs and the PMs groups were both fitted to a one-compartment model. The mean plasma concentration of lansoprazole was much greater in the PMs group than that in the homo EMs group. Plasma concentrations of lansoprazole were measurable up to the last time point (24 h after dosing) in all of the PMs, whereas the concentration was undetectable from 12 h after the dosing in all of the homo EMs.

There was a significant intergenotypic difference ($P<0.01$) between the two groups in the mean pharmacokinetic parameters of lansoprazole except for the T_{max} , $T_{1/2ka}$, K_a (Tab 2). The mean C_{max} and AUC in the homo EMs were approximately 64 % and 29 % lower than that in the PMs and mean Cl_{oral} in the homo EMs was

Tab 2. Pharmacokinetic data of lansoprazole in homozygous extensive metabolisers (Homo EMs) and poor metabolisers (PMs) of CYP2C19. $n=9$. Mean \pm SD. $^cP<0.01$ vs the homo EMs group.

Parameter	Homo EMs	PMs
C_{max} (mg/L)	1.10 \pm 0.34	1.73 \pm 0.56 ^c
T_{max} (h)	2.44 \pm 0.85	2.33 \pm 0.94
$T_{1/2ke}$ (h)	1.96 \pm 0.51	4.21 \pm 0.53 ^c
$T_{1/2ka}$ (h)	0.88 \pm 0.34	0.93 \pm 0.77
V/F (L)	47.56 \pm 21.61	23.59 \pm 7.59 ^c
Cl/F (L/h)	16.55 \pm 6.38	3.58 \pm 1.00 ^c
K_a (h ⁻¹)	0.92 \pm 0.41	1.27 \pm 1.12
K_e (h ⁻¹)	0.38 \pm 0.10	0.17 \pm 0.02 ^c
AUC_0^t (mg·L ⁻¹ ·h ⁻¹) ^a	3.00 \pm 1.07	10.37 \pm 3.12 ^c
AUC_0^∞ (mg·L ⁻¹ ·h ⁻¹)	3.23 \pm 1.08	11.05 \pm 3.23 ^c

AUC_0^t (mg·L⁻¹·h⁻¹)^a: $t=10$ h for Homo EMs; $t=24$ h for PMs.

4.6 times greater than that in the PMs, whereas the mean $T_{1/2ke}$ was 2.1 times longer in the latter than in the former group (Fig 3).

No clinically undesirable effects that could possibly be attributed to the administration of lansoprazole were observed throughout the study period. All subjects completed the study according to the protocol.

DISCUSSION

Previous studies have revealed a wide inter-individual variability in the plasma concentrations of lansoprazole when the same dose was administered to

different individuals^[14]. The findings obtained in this study strongly indicated that CYP2C19-related pharmacogenetics was the major factor causing the wide interindividual variability in the plasma concentrations of lansoprazole in healthy Chinese subjects. We observed a significant differences in the metabolic disposition of lansoprazole between two groups exhibiting different genotypes with respect to CYP2C19.

In the homo EMs group, lansoprazole reached the maximal plasma concentration within 2.5 h post dose. The drug metabolized earlier, mainly by CYP2C19 and eliminated rapidly from the systemic circulation. A single oral administration of 30 mg lansoprazole for the homo EMs group was, therefore, not sufficient to achieve effective plasma lansoprazole concentrations of gastric acid suppression. Moreover, after the rapid elimination of lansoprazole, the new proton pump would be assumed to be resynthesized in gastric parietal cells. Therefore the increase in intragastric pH after a single oral dose of 30 mg of lansoprazole would be insufficient for the homo EMs subjects. The dosing of 30 mg of lansoprazole 4 times daily (total daily dose of 120 mg of lansoprazole) has been reported to be sufficient for inhibiting acid secretion during both the daytime and the nocturnal time in Japanese homo EMs^[15].

On the other hand, because the hydroxylation of lansoprazole (ie, OH-lansoprazole formation), which is the main metabolic route of lansoprazole, was dampened in the PMs group, the duration of the exposure to the high plasma concentration of lansoprazole was longer in the PMs group than that in the homo EMs group. Thus the proton pump in the parietal cells is assumed to be more inactivated by the longer exposure to a higher concentration of lansoprazole, resulting in a stronger or longer sustained acid inhibition (ie, a higher intragastric pH) in the PMs group. Therefore the less dose or longer interval of administer for lansoprazole in the PMs would be sufficient for inhibiting acid secretion.

In summary, we showed that the metabolism of lansoprazole in healthy Chinese subjects was dependent on genotype of *CYP2C19*. *CYP2C19* genotyping appears to be useful for the optimal treatment of acid-related upper gastrointestinal disorders. We should develop a new strategy for the optimal prescription of a proton pump inhibitor such as lansoprazole, where the drug-dosing scheme is determined on the basis of the individual's genotype of genetically determined drug-metabolizing enzyme, such as CYP2C19.

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