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Lornoxicam pharmacokinetics in relation to cytochrome P450 2C9 genotype

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Aims
To investigate the pharmacokinetics of lornoxicam and the relationship with CYP2C9 polymorphism in healthy Chinese subjects.

Methods
A single oral dose of 8 mg lornoxicam was administered to 18 healthy Chinese male subjects. Plasma was sampled for 24 h post dose, and plasma concentrations of lornoxicam were measured using a validated LC/MS/MS method. CYP2C9 genotype was determined by polymerase chain reaction-based restriction fragment length polymorphism or by direct sequencing of the coding region of the CYP2C9 gene.

Results
Of the18 subjects, one subject was found to be a very poor metabolizer of lornoxicam with a long $t_{1/2}$ of 106 h, a low CL/F of 0.71 ml min^{-1}, and a high $AUC_{0-\infty}$ of 187.6 μg ml^{-1} h. Genotyping studies revealed that this subject was heterozygous for *CYP2C9*3* and a new variant *CYP2C9* allele. Of the other 17 subjects, 13 were **1/*1* carriers, three were **1/*3* carriers, and one was a **1/*2* carrier. Mean $AUC_{0-\infty}$ values (95% confidence intervals) of lornoxicam were 9.25 (6.55, 11.95) *vs.* 4.75 (3.55, 5.95) μg ml^{-1} h in **1* heterozygotes *vs.* **1* homozygotes, and mean CL/F values were 14.8 (10.2, 19.4) *vs.* 32.9 (24.5, 41.3) ml min^{-1}, respectively ($P < 0.05$ for both AUC and CL/F).

Conclusions
The results show that the pharmacokinetics of lornoxicam are dependent on CYP2C9 polymorphism. In particular, the presence of the *CYP2C9*3* allele impairs the oral clearance of lornoxicam.

Introduction

Lornoxicam is a nonsteroidal anti-inflammatory drug that decreases prostaglandin synthesis by inhibiting cyclooxygenase. It has analgesic, antipyretic and anti-inflammatory effects. Unlike other oxicams, lornoxicam has a short elimination half-life of 3–5 h [1]. 5′-Hydroxylation is the main metabolic pathway, which accounts for up to 95% of total intrinsic lornoxicam clearance, and cytochrome P450 2C9 (CYP2C9) has been proven to be the primary enzyme involved in the formation of 5′-hydroxy lornoxicam *in vitro* [2].

CYP2C9 is an important enzyme in the metabolism of many drugs including phenytoin, tolbutamide, *S*-warfarin and a large number of anti-inflammatory drugs. It shows large interindividual variability due to the polymorphisms that affect the enzyme activity.

*CYP2C9*2* (Arg144Cys) and **3* (Ile359Leu), which have allele frequencies of 0.08–0.125 and 0.03–0.085, respectively, in Caucasians, have been recognized in humans as main *CYP2C9* variants and have reduced catalytic activity compared with the wild type (*CYP2C9*1*) [3]. Several newly discovered *CYP2C9* alleles, which affect the activity of CYP2C9, include the *CYP2C9*4* (Ile359Thr), *CYP2C9*5* (Asp360Glu) and null *CYP2C9*6* (ΔA818) alleles [4–6]. Previous studies have shown that lornoxicam has a large inter-individual variability in pharmacokinetics [7–11]. It has been reported recently that lornoxicam 5′-hydroxylation via CYP2C9 is markedly decreased by *CYP2C9*3 in vitro*, but studies *in vivo* were not conducted [12].

In this study, the pharmacokinetics of lornoxicam in Chinese subjects was investigated within a bioequivalence study and the impact of *CYP2C9* alleles on the pharmacokinetics of lornoxicam was assessed.

Methods

Subjects

Eighteen healthy male subjects (20–24-year-old Chinese within ±10% range of their ideal body weight) were enrolled in the study. Before enrolment, each subject was considered to be in good health through medical history, physical examination, electrocardiograms (ECGs), and routine laboratory tests. No medication was used for at least 2 weeks before the study and alcohol was forbidden within 72 h prior to drug administration.

Ethics

The study was approved by the Independent Ethics Committee of the People's Hospital of Liaoning Province and was in full compliance with the principles of the Declaration of Helsinki (current revision) and Good Clinical Practice guidelines. Written informed consent was obtained from each subject before the study.

Study design

An open randomized two-way crossover study with a wash-out period of 7 days was conducted. After an overnight fast, each subject received 8 mg lornoxicam either as the test or the reference tablet (Xafon; Nycomed Austria GmbH, Linz, Austria) in a fasted state. Standard meals were provided at 2, 5, and 10 h after drug administration. Venous blood (5 ml) was collected into heparinized tubes just before dosing, and at 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 10, 13, and 24 h post dose. Separated plasma was stored frozen (–20 °C) until assay. From each subject, a 1-ml blood sample for DNA extraction was collected into a tube with EDTA as anticoagulant.

Lornoxicam assay

Plasma concentrations of lornoxicam were determined using a validated LC/MS/MS method reported elsewhere [13]. The lower limit of quantification was 2.0 ng ml^{-1}. Concentrations were linear ($r > 0.998$) over the range of 2.0–1600 ng ml^{-1}. The intra- and interrun precision values for the concentrations of 5, 100 and 1000 ng ml^{-1} were all <7.0%, and the accuracy ranged from 98.1% to 98.5% of the nominal value.

Genotyping procedure

Genotyping for CYP2C9 was performed after the pharmacokinetic study. Detection of the *CYP2C9*2* and **6* alleles was performed using a polymerase chain reaction-based restriction fragment length polymorphism (PCR-RFLP) analysis [5, 6]. For *CYP2C9*3*, **4*, and **5* alleles in exon 7, the exon was amplified by PCR and subsequently sequenced using the ABI Prism 310 Genetic Analyser and BigDye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems Inc., Foster City, CA, USA). For one of the subjects, *CYP2C9* gene (exons 1–9) was amplified by PCR and subsequently sequenced. The detailed analysis of genotyping was described elsewhere [14].

Data analysis

Only the data of the reference product are presented in this paper. Pharmacokinetic parameters were calculated using standard noncompartmental methods. Student's *t*-test was used to assess the statistical differences between the genotyped groups.

Results

As it was a bioequivalence study, plasma concentrations of lornoxicam at 168 h (before dosing in period 2) were measured. They were all under the lower limit of quantification except for subject 14. The concentration of lornoxicam at 168 h for this subject was 395 ng ml^{-1}. Pharmacokinetic analysis indicated that this subject had a remarkably different kinetic profile with a long $t_{1/2}$ of 106 h, a low CL/F of 0.71 ml min^{-1}, and a high $AUC_{0-\infty}$ of 187.6 μg ml^{-1} h. The concentration–time curve for this subject is shown in Figure 1A.

Genotyping studies revealed that subject no. 14 did not carry any other known variant *CYP2C9* alleles except for *CYP2C9*3*. Meanwhile, an unreported mutation that leads to a Leu90Pro substitution was identified. Of the other 17 subjects, 13 were *CYP2C9*1/*1* carriers, three were **1/*3* carriers, and one was a **1/*2* carrier.

According to CYP2C9 genotype, all the subjects were divided into three groups, *CYP2C9*1* homozygotes,

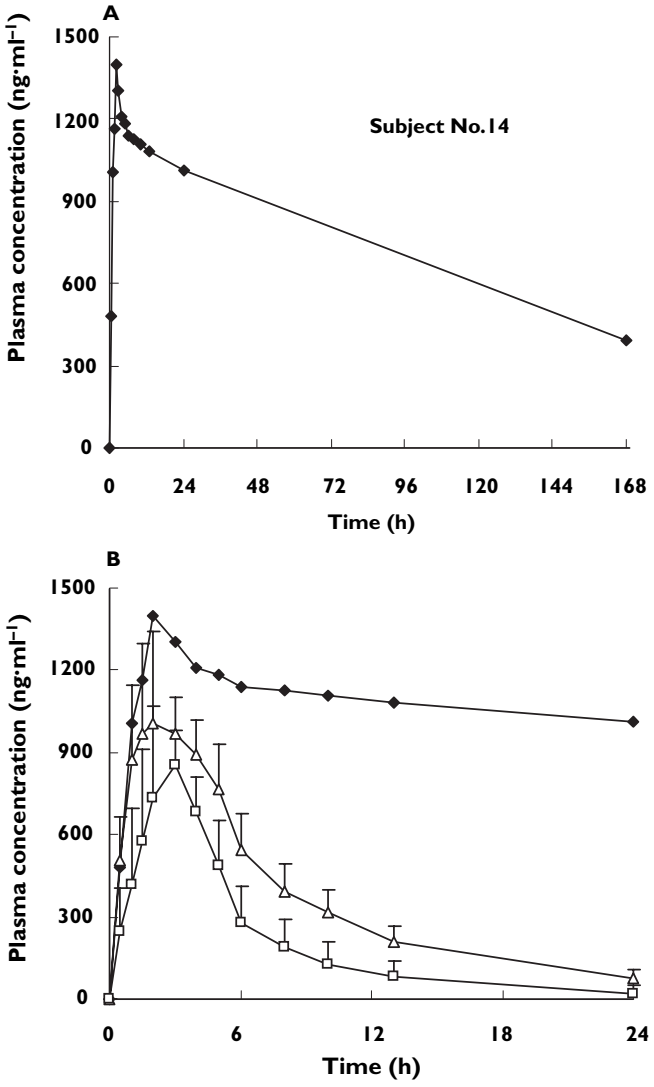

Figure 1
Plasma concentration–time curves of lornoxicam in *CYP2C9*1* homozygotes (□) ($n = 13$), *CYP2C9*1* heterozygotes (△) ($n = 4$), and subject 14 (◆) after a single oral dose of 8 mg lornoxicam

1 heterozygotes (individuals with *1/*3* or *1/*2* genotypes), and subject 14. The calculated parameters are presented in Table 1. The mean plasma concentration–time curves are shown in Figure 1B. $AUC_{0-\infty}$ of lornoxicam was significantly greater in *1* heterozygotes than in *1* homozygotes, with the *1* heterozygotes demonstrating a two-fold increase ($P < 0.01$), and CL/F was significantly lower (55.0%) in *1* heterozygotes than in *1* homozygotes ($P < 0.05$).

Discussion

In the present study, genotyping was carried out after a very poor metabolizer of lornoxicam was found in a bioequivalence study. Genotyping for *CYP2C9*3* was performed first, because it has been reported to lead to low activity of CYP2C9. The results indicated that this subject was a *CYP2C9*3* carrier and was presumed to be *CYP2C9*1/*3*. In addition, three other *CYP2C9*1/*3* carriers and one *1/*2* carrier were found in the other 17 subjects, although the frequencies of *CYP2C9*2* and *3* are reported to be low in Asian populations [15]. The oral clearance of lornoxicam in these subjects was much higher than that in the poor metabolizer. These results indicate that, in addition to the presence of the *3* allele, other factors must also have played a role in the poor metabolizer. A new as yet unnamed variant *CYP2C9* allele was then found after sequencing the whole *CYP2C9* gene in this subject [14]. Further studies are in progress to elucidate its enzyme properties.

In this study, we have also found that there are significant differences in lornoxicam elimination between *CYP2C9*1* heterozygotes and *1* homozygotes. This is similar to tolbutamide, which is used frequently as a phenotypic probe of CYP2C9 activity *in vivo*. It has been reported that the AUC of tolbutamide was

Table 1
Pharmacokinetic parameters of lornoxicam in different groups (mean and 95% confidence interval)

	A (13 subjects)	B (4 subjects)	C (1 subject)
$AUC_{0,last}$ (μg ml^{-1} h)†	4.61 (3.51, 5.71)	8.52 (6.47, 10.57)**	127.0
$AUC_{0-\infty}$ (μg ml^{-1} h)	4.75 (3.55, 5.95)	9.25 (6.55, 11.95)**	187.6
C_{max} (ng ml^{-1})	927 (798, 1056)	1144 (956, 1331)	1402
t_{max} (h)	2.31 (2.06, 2.54)	2.13 (1.15, 3.05)	2.00
$t_{1/2}$ (h)	4.32 (3.75, 4.89)	6.54 (4.52, 8.56)**	106
CL/F (ml min^{-1})	32.9 (24.5, 41.3)	14.8 (10.2, 19.4)*	0.71

†*The last time point was 24 h for group A and group B, but 168 h for group C.* *P < *0.05;* **P < *0.01: compared with group A. Group A: CYP2C9*1 homozygotes. Group B: CYP2C9*1 heterozygotes. Group C: subject no. 14.*

increased by 1.9-fold and its CL/F was decreased by 52% in *CYP2C9*1/*3* individuals, compared with **1/*1* individuals [16]. The same changes were also found in the pharmacokinetics of flurbiprofen, glyburide and glimepiride [17, 18]. Due to the known interindividual variability in CYP2C9-mediated metabolism, there is a clinical need for a reliable phenotypic probe for CYP2C9 in humans. An ideal phenotypic probe of CYP450 activity should pose minimal risk to the patient, particularly if studies in large populations are being considered. The risk of hypoglycaemia is a major drawback to the use of tolbutamide as a CYP2C9 probe, while lornoxicam has proven to be a safe compound with relatively few side-effects. For this reason, lornoxicam might be superior to tolbutamide as a CYP2C9 probe.

In conclusion, we have found that the pharmcokinetics of lornoxicam are highly dependent on CYP2C9 polymorphism. The presence of the *CYP2C9*3* allele particularly impairs its elimination and should be clinically relevant to poor metabolizers, such as subject 14. These subjects have a high tendency to the accumulation of lornoxicam. Therefore, lornoxicam is not recommended for these people for safety reasons.

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