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**Role of CYP2C9 and its variants (CYP2C9\*3 and CYP2C9\*13) in the metabolism of lornoxicam in human**

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**Running Title :** CYP2C9 variants and lornoxicam metabolism

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**Non-Standard Abbreviation:** CYP, cytochrome P450; NSAID, nonsteroidal anti-inflammatory drug; AUC, area under the plasma concentration-time curve; CL/F, oral clearance; HPLC, high performance liquid chromatography;

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### Abstract

CYP2C9 is an important member of the cytochrome P450 enzyme superfamily with some twelve *CYP2C9* alleles (\*1-\*12) being previously reported. Recently we identified a new *CYP2C9* allele with a Leu90Pro mutation in a Chinese poor metabolizer of lornoxicam (Si et al., 2004). The new allele, designated *CYP2C9\*13*, was found to occur in approximately 2% of the Chinese population. To examine enzymatic activity of the *CYP2C9\*13* allele, kinetic parameters for lornoxicam 5'-hydroxylation were determined in COS-7 cells transiently transfected with pcDNA3.1 plasmids carrying wild-type *CYP2C9\*1*, variant *CYP2C9\*3* and *CYP2C9\*13* cDNA. The protein levels of cDNA-expressed *CYP2C9\*3* and \*13 in postmitochondrial supernatant (S9) from transfected cells were lower than from wild-type *CYP2C9\*1*. Mean values of  $K_m$  and  $V_{max}$  for *CYP2C9\*1*, \*3 and \*13 were 1.24, 1.61 and 2.79  $\mu\text{M}$  and 0.83, 0.28 and 0.22 pmol/min/pmol, respectively. Intrinsic clearance values ( $V_{max}/K_m$ ) for variant *CYP2C9\*3* and *CYP2C9\*13* on the basis of *CYP2C9* protein levels were separately decreased to 28% and 12% compared with wild type. In a subsequent clinical study, the AUC of lornoxicam was increased by 1.9-fold and its CL/F decreased by 44% in three *CYP2C9\*1/\*13* subjects, compared with *CYP2C9\*1/\*1* individuals. This suggests that the *CYP2C9\*13* allele is associated with decreased enzymatic activity both in vitro and in vivo.

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### Introduction

CYP2C9 constitutes approximately 20% of the cytochrome P450 protein content of human liver microsomes and is responsible for the metabolism of many clinically important drugs. These include drugs with a narrow therapeutic index such as warfarin and phenytoin and other routinely prescribed drugs such as acenocoumarol, tolbutamide, losartan, glipizide and some non-steroidal anti-inflammatory drugs (Lee et al., 2002).

The *CYP2C9* gene is highly polymorphic. At least thirteen *CYP2C9* alleles have been identified to date and most of them are associated with reduced CYP2C9 activity. Among them, *CYP2C9*\*3 with an Ile359Leu mutation, has been most widely studied. In vitro studies show it has significantly impaired catalytic activity to various CYP2C9 substrates relative to the wild type (Takanashi et al., 2000). In vivo investigations show that individuals heterozygous and homozygous for *CYP2C9*\*3 have reduced intrinsic clearance of warfarin, phenytoin and glipizide and are more at risk of clinical toxicity from these drugs (Kidd et al., 1999; Aithal et al., 1999; Ninomiya et al., 2000). Other CYP2C9 alleles such as *CYP2C9*\*2 (Arg144Cys), *CYP2C9*\*4 (Ile359Thr), *CYP2C9*\*5 (Asp360Glu), *CYP2C9*\*6 (null allele), *CYP2C9*\*11 (Arg335Trp) and *CYP2C9*\*12 (Pro489Ser) also show decreased enzymatic activity in vitro and in vivo (Aithal et al., 1999; Kichheiner et al., 2002; Crespi et al., 1997; Imai et al., 2000; Dickmann et al., 2001; Kidd et al., 2001; Blaisdell et al., 2004; Allabi et al., 2004).

Recently a new *CYP2C9* allele designated *CYP2C9*\*13 has been identified in a poor Chinese metabolizer of lornoxicam. It possesses a T269C transversion in exon 2 of *CYP2C9* that leads to a Leu90Pro substitution. Frequency analysis shows that approximately 2% of the

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Chinese populations carry the allele (Si et al., 2004). Genotyping of this poor lornoxicam metabolizer revealed a *CYP2C9\*3/\*13* genotype with the two mutations located on separate alleles. His lornoxicam half-life of about 105 h was markedly longer than that of other *CYP2C9\*1/\*3* and *CYP2C9\*1/\*1* carriers (half-lives of 5.8-8.1 and 3.2-6.3 h, respectively; Zhang et al., 2005) suggesting the *CYP2C9\*13* allele has a larger effect on CYP2C9 metabolic capability than other alleles.

CYP2C9 has been shown to be the primary enzyme responsible for the biotransformation of the NSAID lornoxicam to its major metabolite, 5'-hydroxylornoxicam, in human liver microsomes (Bonnabry et al., 1996; Kohl et al., 2000). Recently, it was reported that lornoxicam 5'-hydroxylation by the *CYP2C9\*3* allele was markedly reduced compared to wild type both in vitro and in vivo (Iida et al., 2004; Zhang et al., 2005). Thus lornoxicam is an ideal substrate for the study of CYP2C9 enzyme activity. The purpose of this study was to compare enzymatic activity of *CYP2C9\*1*, *CYP2C9\*3* and *CYP2C9\*13* towards lornoxicam both in vitro in appropriately transfected COS-7 cells and in vivo in subjects with *CYP2C9\*1/\*3*, *CYP2C9\*1/\*13* and *CYP2C9\*1/\*1* genotypes.

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### Materials and methods

**Materials.** Lornoxicam was purchased from Shangdi Xinshiji medical academy (Beijing, China.). 5'-Hydroxylornoxicam was provided by the Laboratory of Microorganisms, Shenyang Pharmaceutical University (Shenyang, China). Dulbecco's modified Eagle's medium, pcDNA3.1(+) and Lipofectamine<sup>TM</sup> 2000 were purchased from Invitrogen (Carlsbad, CA). Fetal bovine serum was purchased from Tianjin, H&Y Bio Co. Ltd (Tianjing, China). *KpnI*, *XhoI* and *DpnI* enzymes were purchased from New England Bio Labs (Beverly, MA). *Pfu* DNA polymerase was purchased from Bio Basic Inc (Toronto, Canada). A pREP9 plasmid containing human *CYP2C9\*1* cDNA and *E. coli* Top 10 were provided by the Department of Pathophysiology and the Laboratory of Medical Molecular Biology, School of Medicine, Zhejiang University (Zhejiang, China). Rabbit anti-human cytochrome P450 2C9 antibody was purchased from Serotec Ltd (Oxford, UK). Alkaline phosphatase-labeled anti-rabbit IgG, 5-bromo-4-chloro-3-indolyl phosphate/nitro blue tetrazolium (BCIP/NBT) and bovine serum albumin were purchased from Beijing Dingguo Biotechnology Development Center (Beijing, China). NADPH was purchased from Roche Molecular Biochemicals (Basel, Switzerland). COS-7 cells were kindly donated by the Vaccination Center, Jilin University (Changchun, China). Human recombinant NADPH-P450 reductase (CPR) was purchased from MBL International Corporation (Woburn, MA). All other reagents were of analytical grade.

**Construction of expression plasmids.** *CYP2C9\*1* cDNA in pREP9 plasmid was subcloned into pcDNA3.1(+) by digestion with *KpnI* and *XhoI* enzyme. Site-directed mutagenesis to introduce the A→C transition at position 1075 (*CYP2C9\*3*) and T→C transition at position 269 (*CYP2C9\*13*) was performed using pcDNA3.1(+) plasmids

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carrying *CYP2C9\*1* cDNA as the template for polymerase chain reaction (PCR) amplification by *Pfu* DNA polymerase. The specific base transition was introduced into the amplification products by a pair of completely complementary primers containing substituted base. The mutagenic primers for *CYP2C9\*3* and *\*13* were 5'CGA GGT CCA GAG ATA CCT TGA CCT TCT CCC CAC 3' and 5'GGA AGC CCT GAT TGA TCC TGG AGA GGA GTT TTC TG3', respectively (mutations underlined). After incubation with *DpnI* enzyme, the origin templates were digested but the new amplified PCR products containing substituted base remained and were transformed to *E. coli* Top 10. Clones containing the desired nucleotide change were identified by sequencing carried out by Shanghai Sangon Biological Engineering Technology & Service Co. Ltd (Shanghai, China).

### **Transfection of COS-7 cells and preparation of postmitochondrial supernatant (S9).**

COS-7 cells were seeded into 10-cm culture flasks in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum, 100 U/ml penicillin and 100 µg/ml streptomycin. When cells were 90-95% confluent, the culture medium was replaced with DMEM without penicillin and streptomycin, and the *CYP2C9* expression plasmids (24 µg/flask), purified with QIAGEN plasmid mini kit (QIAGEN Inc.,Valencia, CA), were transfected into COS-7 cells using 60 µl/flask of Lipofectamine<sup>TM</sup> 2000 as per the manufacturer's instructions. Forty-eight hours after transfection, cells were scraped from the culture flask and washed twice with D-Hank's balanced salt solution. The pellets were resuspended in 20 mM potassium phosphate buffer pH 7.4 containing 0.2 mM EDTA, 1 mM dithiothreitol and 20% glycerol, and sonicated with twelve 5s pulses at 23% power of a SONICS Vibra-Cell sonicator (USA). The homogenate was centrifuged at 9000 g, 4°C for 20 min

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and the postmitochondrial supernatant (S9 fraction) collected for assay or storage at  $-70^{\circ}\text{C}$ . Protein concentrations in S9 were determined by the Bradford method (Bradford, 1976) using bovine serum albumin as standard.

**Quantification of CYP2C9 protein by Western-blotting.** S9 fraction (50  $\mu\text{g}$ ) and human liver microsomes (10  $\mu\text{g}$ ) were separated on 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis and transferred to a polyvinylidene difluoride membrane (Millipore Corp., Billerica, MA). The membrane was incubated with rabbit anti-human cytochrome p450 2C9 antibody as the primary antibody and then with alkaline phosphatase-labeled anti-rabbit IgG as the secondary antibody. Bands were visualized by incubation with BCIP/NBT and quantified by microsomes from insect cells expressing human CYP2C9 (Invitrogen, Carlsbad, CA) as a standard with ImageJ software (National Institutes of Health, USA).

**In vitro lornoxicam 5'-hydroxylation of recombinant CYP2C9 protein.** Lornoxicam 5'-hydroxylation activity of the recombinant CYP2C9 protein was determined as described previously (Kohl et al., 2000) with minor modifications. S9 fraction containing CYP2C9s was incubated with 100 mM Tris buffer (pH 7.5), 200  $\mu\text{M}$  NADPH and lornoxicam at  $37^{\circ}\text{C}$  for 1 hour in the presence or absence of CPR. The reaction was stopped by addition of 500  $\mu\text{l}$  methanol and stored overnight at  $-20^{\circ}\text{C}$  to allow complete protein precipitation. After centrifugation for 30 min at 12000 rpm, the supernatants were reduced to 100 $\mu\text{l}$  by warming at  $65^{\circ}\text{C}$  and subjected to HPLC assay. HPLC was carried out on a SB-300A C<sub>18</sub>, 10 $\mu\text{m}$  column (4.6 $\times$ 200mm, Agilent Tech., USA) using a mobile phase of 0.1M NaH<sub>2</sub>PO<sub>4</sub>, pH 6.0 : acetonitrile (7: 3), at a flow rate of 1 ml/min. Detection was by UV absorption at 371 nm.



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Under these conditions, retention times of 5'-hydroxylornoxicam and lornoxicam were 6.9 and 11.8 min, respectively. A six-point standard curve was used to quantify 5'-hydroxylornoxicam.

**In vivo lornoxicam metabolism.** The study was approved by the Independent Ethics Committee of the People's Hospital of Liaoning Province (Shenyang, China). Genotyping of *CYP2C9\*3* and *CYP2C9\*13* was carried out as described previously (Zhang et al., 2005; Si et al., 2004). Thirteen *CYP2C9\*1* homozygotes, seven *CYP2C9\*3* heterozygotes and three *CYP2C9\*13* heterozygotes participated in the phenotyping study. All subjects were in good health and were required to refrain from all medication and alcohol prior to the pharmacokinetic study. In vivo lornoxicam metabolism was performed according to the method described previously (Zhang et al., 2005). In brief, after a single oral dose of 8 mg lornoxicam, blood samples were collected before dosing, and at 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 10, 13, and 24 h post dose. Plasma concentrations of lornoxicam were determined using a validated LC/MS/MS method reported elsewhere (Zeng et al., 2004).

**Data analysis.** Michaelis–Menten analysis was performed by non-linear regression curve fitting using the computer program Prism v4.0 (Graphpad Software, San Diego, CA). Pharmacokinetic parameters were calculated using standard noncompartmental methods. Student's *t* test was used for intergroup comparison. A value of  $P < 0.05$  was considered to be statistically significant.

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### Results

A representative immunoblot of postmitochondrial supernatant (S9) prepared from COS-7 cells expressing CYP2C9\*1, CYP2C9\*3 and CYP2C9\*13 proteins is presented in Fig. 1. All constructs yielded immunodetectable CYP2C9 protein as did human liver microsomes. The expressed protein level of CYP2C9\*1 was 7.51 pmol/mg of S9 protein, and the expression levels of variant CYP2C9\* 3 and \*13 were 69.9% and 35.5%, respectively of that of CYP2C9\*1.

The effect of exogenous CPR on lornoxicam 5'-hydroxylation was studied by incubation with recombinant CYP2C9\*1. S9 fraction containing 3.75 pmol of CYP2C9\*1 was mixed with varying amounts of CPR and 10  $\mu$ M of lornoxicam. When CPR was 0, 5, 12.5, 25  $\mu$ M, the mean value of  $V_{\max}$  for CYP2C9\*1 was 0.66, 0.74, 0.68 and 0.68 pmol/minpmol, respectively. There was no significant alteration in catalytic efficiency of CYP2C9\*1 with the increasing CPR concentration, indicating that endogenous reductase is enough for lornoxicam metabolism in COS-7 expression system. Thus exogenous CPR was not utilized in the following kinetic study.

Michaelis-Menten kinetics of lornoxicam for wild type and mutant CYP2C9 is shown in Fig. 2. Corresponding kinetic parameters are summarized in Table 1. Both CYP2C9\*3 and CYP2C9\*13 on the basis of protein levels of S9 fraction exhibited lower intrinsic clearance of lornoxicam ( $P < .01$ ) than wild type CYP2C9\*1 resulting from higher values of  $K_m$  (1.3-fold and 2.3-fold, respectively) and lower values of  $V_{\max}$  (76% and 90%, respectively). When the enzymatic activities were normalized to CYP2C9 protein levels, intrinsic clearance values ( $V_{\max}/K_m$ ) for variant CYP2C9\*3 and CYP2C9\*13 were also separately decreased to 28% and

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12% compared with wild type. Clearly the activity of CYP2C9\*13 is lower than that of CYP2C9\*3.

In the in vivo study, the CYP2C9 genotype significantly affected the pharmacokinetics of lornoxicam (Fig. 3 and Table 2). The AUC of lornoxicam increased 1.9-fold and CL/F was decreased by 44% in *CYP2C9\*1/\*13* individuals compared with *CYP2C9\*1/\*1* individuals. Similar changes were found in *CYP2C9\*1/\*3* individuals.

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### Discussion

To investigate the catalytic activity of the *CYP2C9\*13* allele *in vitro*, we established a COS cell expression system. This has been widely applied for functional characterization of CYP alleles containing CYP2C9, CYP2D6, CYP2B6 and CYP2E1 (Veronese et al., 1993; Marcucci et al., 2002; Jinno et al., 2003; Hu et al., 1997). However, due to the low levels of expression in COS-7 cells, we failed to quantify CYP2C9 holoenzyme contents by CO-different spectroscopy. Thus CYP2C9 proteins were quantified by immunoblotting with microsomes from insect cells expressing human CYP2C9 as standard. This kind of method quantifying P450 was also used for the functional characterization of CYP2D6, CYP2B6 and CYP2E1 allelic variants in COS expression system (Marcucci et al., 2002, Jinno et al., 2003, Hanioka et al., 2003). Our results show that COS-7 cells can efficiently express active CYP2C9 protein. The protein levels of cDNA-expressed CYP2C9\*3 and \*13 in postmitochondrial supernatant (S9) from COS-7 cells were lower than in wild-type CYP2C9\*1. The reduced protein levels in the CYP2C9 variants may contribute to lower transcription, translation efficiency and protein stability. Otherwise, although polyclonal antibody was used, the immunoreactivity of mutant CYP2C9s may be altered by mutagenization. Therefore, the enzymatic activities were assayed in two ways, on the basis of S9 protein level and CYP2C9 protein level. These data obtained using the COS expression system need to be confirmed by baculovirus system, which are better suited to obtain the quantity of P450 by spectral analysis. These studies are currently under investigation in our laboratory.

In this study, the presence of the *CYP2C9\*3* allele impairs both intrinsic clearance and

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systemic clearance of lornoxicam. A recent report shows that *CYP2C9\*1/\*3* individuals have a 55% decrease in CL/F and a 1.9-fold increase in AUC of lornoxicam compared to *CYP2C9\*1/\*1* individuals (Zhang et al., 2005). The magnitude of these changes in pharmacokinetic parameters is consistent with our in vivo results. Iida et al. (2004) reported that *CYP2C9\*3* expressed in baculovirus-infected insect cells significantly decreased lornoxicam 5'-hydroxylation relative to wild type with a 2.3-fold increase in  $K_m$  ( $p < 0.05$ ) and 76% decrease in  $V_{max}$ . In our COS-7 cells, the  $K_m$  showed a 1.3-fold increase ( $p > 0.05$ ) and the  $V_{max}$  a 66% decrease on the basis of CYP2C9 protein level relative to wild type. The reason for the discrepancy is due to the difference in the heterologous cell expression system. Since the addition of exogenous reductase did not significantly affect the lornoxicam 5'-hydroxylation by CYP2C9\*1, it indicates that the endogenous reductase in COS-7 cells is not limiting for lornoxicam metabolism.

In addition to the *CYP2C9\*3* variant, the catalytic activity of a recently identified *CYP2C9\*13* variant that contains a Leu90Pro substitution was investigated in this study. Compared with wild type *CYP2C9\*1*, the *CYP2C9\*13* variant also has lower intrinsic clearance for lornoxicam 5'-hydroxylation due to a 2.3-fold increase in  $K_m$  and 73% decrease in  $V_{max}$  on the basis of CYP2C9 protein level. The results are consistent with our in vivo observation that individuals with *CYP2C9\*1/\*13* genotype have an impaired clearance of lornoxicam compared to individuals with *CYP2C9\*1/\*1* genotype. Interestingly, in our study, individuals with *CYP2C9\*1/\*3* and *CYP2C9\*1/\*13* genotypes reveal the same extent of reduction in oral clearance of lornoxicam despite the fact that, in vitro, *CYP2C9\*13* is associated with a lower intrinsic clearance of lornoxicam than *CYP2C9\*3*.

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Given the small number of *CYP2C9\*1/\*13* subjects studied (n=3), and in the absence of any individuals homozygous for the *CYP2C9\*13* allele, we recognize that further in-vivo studies are required in order to draw firm conclusions about the role of the *CYP2C9\*13* allele. According to a crystal structure of CYP2C9 published by Williams et al. (2003) and Wester et al., (2004), Leu90 is located in the B-B' loop, which is not the heme-binding region and far from the binding pocket of substrate. Thus the reason for the increase in  $K_m$  for lornoxicam 5'-hydroxylation is not clear. Homology modeling based on the crystal structure of human CYP2C9 is ongoing in our laboratory (Wester et al., 2004).

Our results show that the activities of both *CYP2C9\*3* and *CYP2C9\*13* towards lornoxicam in vitro are compatible with their activities in vivo and there is a reasonable correlation between in vitro activity and in vivo metabolic clearance of lornoxicam. Recently, it was reported that individuals carrying the *CYP2C9\*3* allele are at risk of experiencing drug toxicity, especially of drugs with a narrow therapeutic index such as warfarin and phenytoin (Kidd et al., 1999; Aithal et al., 1999; Ninomiya et al., 2000). By extrapolation, one may speculate that carriers of the *CYP2C9\*13* allele would experience greater risk from these drugs. Therefore genotyping for *CYP2C9\*13* may be important to allow individualization of dosing for CYP2C9 substrate drugs.

In conclusion, the Leu90Pro substitution of *CYP2C9\*13* markedly decreases the intrinsic clearance of lornoxicam in vitro and in vivo. The reduction in activity due to *CYP2C9\*13* is greater than that due to *CYP2C9\*3* in vitro. Whether carriers of the *CYP2C9\*13* allele may be at greater risk of toxicity from *CYP2C9* substrate drugs with narrow therapeutic index remains to be confirmed by further in vivo studies.

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### **Footnotes**

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Fig. 1. CYP2C9 protein levels in postmitochondrial supernatant (S9) from COS-7 cells expressing wild type and variant CYP2C9s. A. Immunoblot analysis of recombinant human CYP2C9 protein. S9 fraction (50  $\mu$ g) isolated from COS-7 cells transfected with the wild type and variant *CYP2C9* cDNA was utilized for immunoblotting using anti-human CYP2C9 antibody. Human liver microsome (10  $\mu$ g) was used as positive control. B. Protein levels of CYP2C9 were quantified by densitometric analysis. The results are expressed as a percentage of the level of CYP2C9\*1. Each bar represents the mean  $\pm$  S.E.M. of three independent experiments.

Fig. 2. Michaelis-Menten kinetics of lornoxicam by postmitochondrial supernatant (S9) from COS-7 cells expressing wild type and variant CYP2C9s. S9 fraction corresponding to 500  $\mu$ g of protein was incubated with different concentration of lornoxicam in the absence of CPR. Experimental conditions are described under *Materials and Methods*. Each point represents the mean of three independent experiments. ■, CYP2C9\*1; ▲, CYP2C9\*3; ▼, CYP2C9\*13.

Fig. 3. Plasma concentration-time curves of lornoxicam in healthy subjects with genotype *CYP2C9*\*1/\*1(◆)(n = 13), *CYP2C9*\*1/\*3(□) (n = 7), and *CYP2C9*\*1/\*13 (△)(n = 3) after a single oral dose of 8 mg lornoxicam

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TABLE 1

*Kinetic parameters for lornoxicam 5'-hydroxylation from COS-7 cells expressing wild type and variant CYP2C9s*

Each value represents the mean  $\pm$  S.E.M. of three independent experiments.

Variant	$K_m$	$V_{max}$		$V_{max}/K_m$	
		Protein	P450	Protein	P450
	$\mu M$	<i>pmol/min/mg</i>	<i>pmol/min/pmol</i>	$\mu l/min/pmol$	$\mu l/min/pmol$
CYP2C9*1	1.24 $\pm$ 0.09	6.23 $\pm$ 0.14	0.83 $\pm$ 0.02	5.10 $\pm$ 0.11	0.68 $\pm$ 0.05
CYP2C9*3	1.61 $\pm$ 0.30	1.47 $\pm$ 0.23**	0.28 $\pm$ 0.01**	0.90 $\pm$ 0.14**	0.19 $\pm$ 0.03**
CYP2C9*13	2.79 $\pm$ 0.26**	0.61 $\pm$ 0.06**	0.22 $\pm$ 0.01**	0.23 $\pm$ 0.02**	0.08 $\pm$ 0.01**

\*\* $P < 0.01$  versus CYP2C9\*1

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TABLE 2

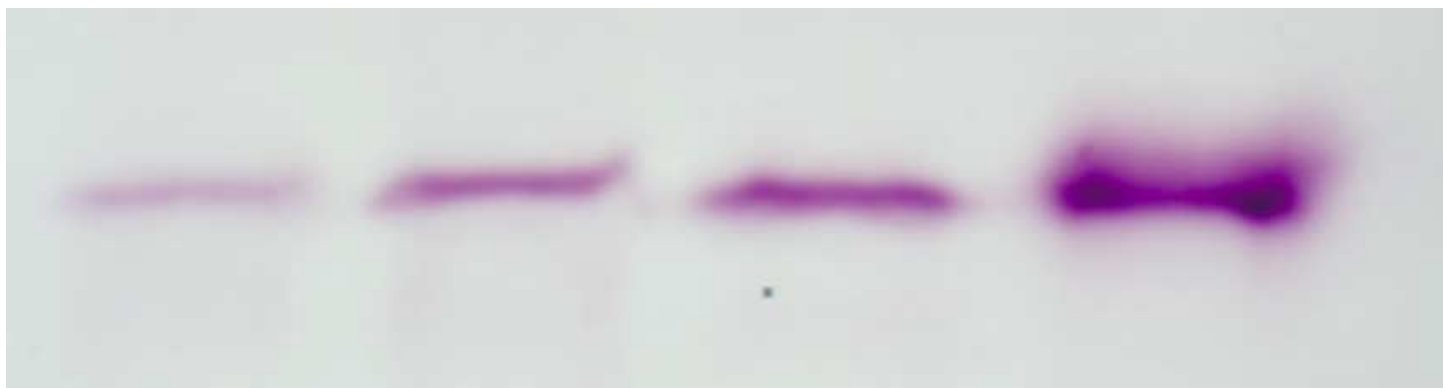
*Pharmacokinetic parameters of lornoxicam in healthy subjects with CYP2C9\*1/\*1, CYP2C9\*1/\*3 and CYP2C9\*1/\*13 genotype*

Data are given as mean and 95% confidence interval

	<i>CYP2C9*1/*1</i> (n = 13)	<i>CYP2C9*1/*3</i> (n = 7)	<i>CYP2C9*1/*13</i> (n = 3)
AUC <sub>0-24</sub> (μg ml <sup>-1</sup> h)	7.07 (3.84, 12.38)	13.12 (10.63, 15.78)**	13.02 (11.75, 14.23) **
AUC <sub>0-∞</sub> (μg ml <sup>-1</sup> h)	7.58 (3.88, 15.81)	14.98 (11.85, 18.42) **	14.82 (13.35, 17.30) **
C <sub>max</sub> (ng ml <sup>-1</sup> )	1281 (869, 1741)	1621 (1414, 2105) *	1828 (1258, 2414) *
t <sub>max</sub> (h)	2.2 (1.0, 4.0)	2.1 (1.5, 2.5)	1.8 (1.0, 2.5)
t <sub>1/2</sub> (h)	5.01 (2.95, 13.19)	7.98 (6.21, 9.95) *	7.97 (6.11, 10.08)
CL/F (ml min <sup>-1</sup> )	20.5 (8.4, 34.4)	9.1 (7.2, 11.3) **	9.1 (7.7, 10.0) *

\*  $P < 0.05$ , \*\* $P < 0.01$  versus *CYP2C9\*1/\*1*

**Figure 1A**



**2C9\*13**

**2C9\*3**

**2C9\*1**

**liver microsomes**

Figure 1B

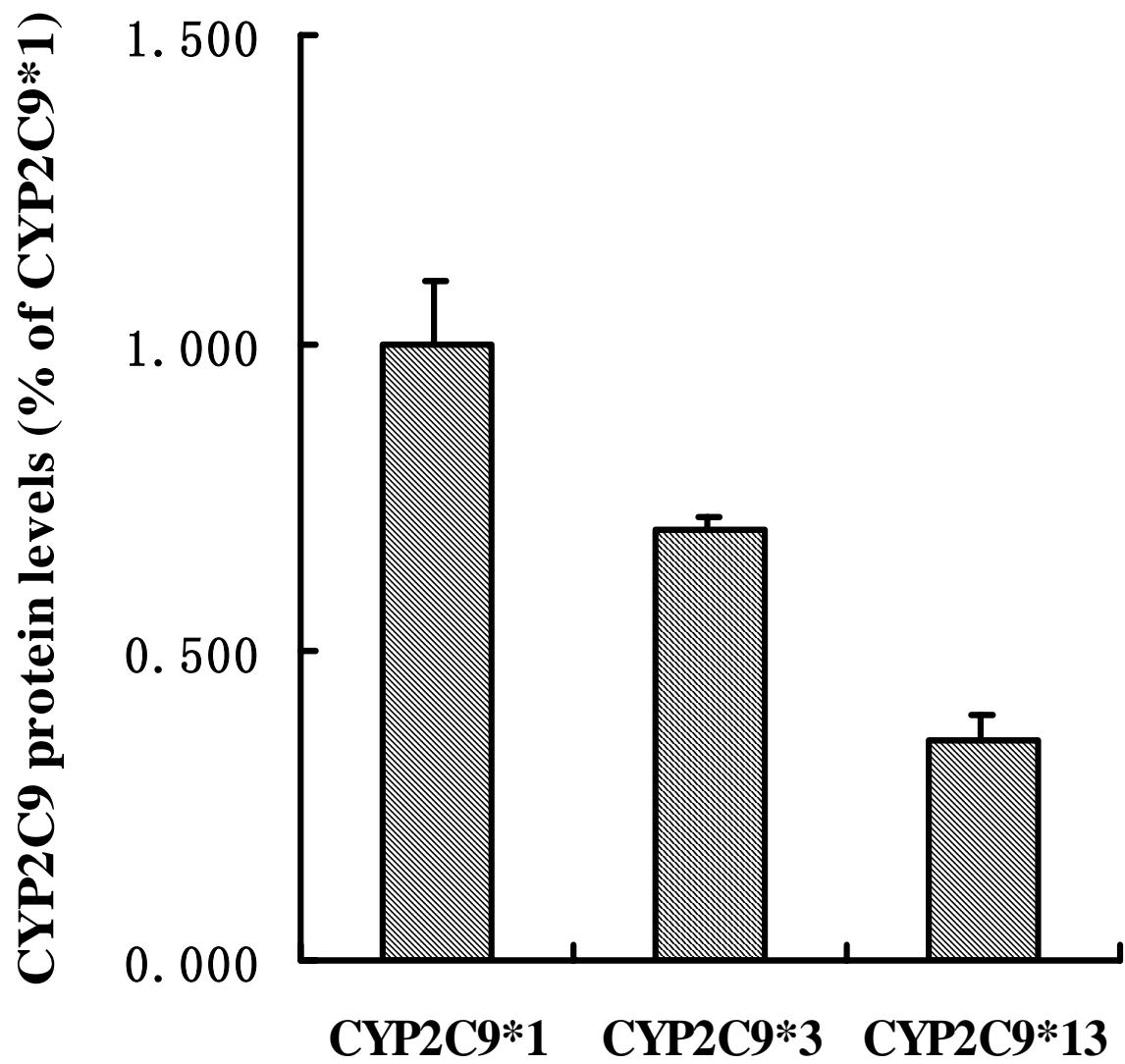




Figure 2

