

# PHARMACOGENETICS AND GENOMICS

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## Enantiospecific effects of cytochrome P450 2C9 amino acid variants on ibuprofen pharmacokinetics and on the inhibition of cyclooxygenases 1 and 2

**Objective:** According to in vitro data, the polymorphic cytochrome P450 enzyme 2C9 (CYP2C9) may be the major *S*-ibuprofen hydroxylase. In humans, there are 2 variants of CYP2C9 with a high population frequency. We studied their impact on ibuprofen pharmacokinetics and on the inhibition of cyclooxygenases 1 and 2.

**Methods:** Kinetics of an oral dose of 600 mg racemic ibuprofen were studied in 21 healthy volunteers with all combinations of the CYP2C9 variants \*2 (arginine144cysteine) and \*3 (isoleucine359leucine). Blood concentrations of racemic ibuprofen and of *S*-(+)-ibuprofen and *R*-(-)-ibuprofen were measured by HPLC, and thromboxane B<sub>2</sub> and prostaglandin E<sub>2</sub> were measured with use of an enzyme immunoassay. Data were evaluated with a population pharmacokinetic model that integrated pharmacogenetic information.

**Results:** The pharmacokinetics of racemic and of *S*-ibuprofen depended on the CYP2C9 isoleucine359leucine amino acid polymorphism: population mean *S*-ibuprofen clearances were 3.25 L/h (95% confidence interval [CI], 2.84 to 3.73), 2.38 L/h (95% CI, 2.09 to 2.73), and 1.52 L/h (95% CI, 1.33 to 1.74) in carriers of the CYP2C9 genotypes \*1/\*1, \*1/\*3, and \*3/\*3, respectively. The CYP2C9 variant \*2 exhibited no significant effect. Ex vivo formation of thromboxane B<sub>2</sub>, reflecting cyclooxygenase type 1 inhibition, depended significantly on the CYP2C9 polymorphism. The maximal inhibition of thromboxane B<sub>2</sub> formation and the area under the effect-time curve were larger in carriers of the slow CYP2C9 genotypes \*1/\*3, \*2/\*3, and \*3/\*3 than in \*1/\*1 carriers; the same trend was observed for prostaglandin E<sub>2</sub>, reflecting cyclooxygenase type 2 inhibition.

**Conclusions:** The reduced *S*-ibuprofen total clearance accompanied by increased pharmacodynamic activity may have medical impact in patients receiving ibuprofen. (Clin Pharmacol Ther 2002;72:62-75.)

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Ibuprofen is one of the most frequently used nonsteroidal anti-inflammatory drugs (NSAID) in the world. It is available as an over-the-counter remedy in many countries. Ibuprofen is a nonselective inhibitor of cyclooxygenases 1 and 2 (COX-1 and COX-2), with a short duration of action.<sup>1</sup> Most preparations contain the racemic mixture of *R*-(-)- and *S*-(+)-ibuprofen. The dextrorotatory *S*-ibuprofen is a more potent inhibitor of COX-1 and COX-2,<sup>1,2</sup> and *S*-ibuprofen has been given a marketing authorization only recently. After administration of racemic ibuprofen, a mean of about 60% of *R*-ibuprofen is inverted to the *S*-enantiomer in the hu-

man body, but there is no measurable inversion in the other direction.<sup>2-6</sup> The biochemical mechanism and the enzymes that catalyze this inversion were discovered not long ago.<sup>7</sup>

Both enantiomers of ibuprofen are eliminated after oxidative biotransformation and glucuronidation, and less than 1% is eliminated unchanged in urine.<sup>1</sup> Data from biochemical studies have indicated that the genetically polymorphic cytochrome P450 enzyme 2C9 (CYP2C9) may be the major *S*-ibuprofen hydroxylase, whereas *R*-ibuprofen appeared to be metabolized by CYP2C8.<sup>8,9</sup> CYP2C9 contributes to the metabolism of many drugs, such as phenytoin, losartan, torsemide (INN, torasemide), vitamin K antagonists such as *S*-warfarin and acenocoumarol, oral antidiabetic drugs such as tolbutamide, glipizide, glyburide (INN, glibenclamide), and nateglinide, and NSAIDs such as celecoxib and diclofenac.<sup>10-21</sup> However, to our knowledge, there is no in vitro data and no data from clinical studies on the impact of the CYP2C9 amino acid variants cysteine144 and leucine359 on ibuprofen biotransformation and pharmacokinetics in humans.

Three alleles of CYP2C9 are relatively frequent in white populations and exhibit different activities: the wild-type allele CYP2C9\*1 is characterized by an arginine at codon 144 and an isoleucine at codon 359; in the CYP2C9\*2 variant, the arginine 144 is replaced with cysteine; and in the variant CYP2C9\*3, the isoleucine 359 is replaced with leucine. The fourth possible haplotype from these 2 single nucleotide polymorphisms—namely, the combination of cysteine144 and leucine359 on 1 chromosome—has not yet been found in humans. According to in vitro data and to human pharmacokinetic studies, the activity of the enzyme encoded by CYP2C9\*2 is only little less than that of the enzyme encoded by the wild-type allele CYP2C9\*1, whereas CYP2C9\*3 leads to an enzymatic activity that is between 10% and 30% of that of the wild type and depends on the substrate studied.<sup>10,14,17,22</sup>

Although ibuprofen is considered to be a relatively well-tolerated nonsteroidal antirheumatic drug, it is not free of adverse events, such as major upper intestinal bleeding or deterioration of kidney function. These adverse events could be explained by genetic polymorphisms in biotransformation or transport proteins that may lead to high tissue concentrations of NSAIDs in some subjects. In addition, genetic polymorphism may be one explanation for nonresponse. We investigated the impact of the CYP2C9 polymorphism on ibuprofen pharmacokinetics in 21 healthy volunteers with all allelic combinations of the 2 CYP2C9 variants Arg144Cys (\*2) and Ile359Leu (\*3). In addition, we

measured the inhibition of the constitutively expressed COX-1 on the basis of the ex vivo generation of thromboxane B<sub>2</sub> and inhibition of the inducible COX-2 on the basis of the ex vivo generation of prostaglandin E<sub>2</sub>. Prostanoid formation is an established surrogate marker of NSAID activity in humans<sup>23-25</sup> and would also reflect any effect of active metabolites of ibuprofen.

## METHODS

### Subjects

From about 800 genotyped healthy volunteers, 21 male individuals with all possible combinations of the CYP2C9 alleles \*1, \*2, and \*3 were asked to participate in the study, resulting in 6 groups with the genotypes CYP2C9\*1/\*1 (n = 4), CYP2C9\*1/\*2 (n = 4), CYP2C9\*1/\*3 (n = 4), CYP2C9\*2/\*2 (n = 3), CYP2C9\*2/\*3 (n = 3), and CYP2C9\*3/\*3 (n = 3). The sample size was large enough to detect the clinical relevant difference of more than 100% between 2 study groups with a power of 90%. All participants were nonsmokers, and all abstained from caffeine- and alcohol-containing beverages and from grapefruit during the course of the study. The prestudy health check consisted of a physical examination and laboratory tests that included blood cell count and hepatic function tests, urine analyses, and electrocardiograms. All volunteers gave written informed consent. The study protocol was approved by the Ethics Committee of the Charité University Medical Center of Humboldt University of Berlin.

Each subject received an oral dose of 600 mg immediate-release racemic ibuprofen (Ibuprofen ST, Stada, Germany), and plasma samples were taken at 0, 0.5, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 8, 10, 24, 28, 34, and 48 hours after administration. A standard lunch was served after a fasting period of 12 hours before and 4 hours after drug intake. The intake of tap water was allowed during the whole study.

### Genotyping procedure

DNA was extracted from 5 mL blood that contained ethylenediaminetetraacetic acid (EDTA) as the anticoagulant with use of a standard phenol-chloroform extraction method. DNA samples were dissolved in 10 mmol/L Tris and 1 mmol/L EDTA, pH 8.0, and stored at 4°C. Polymerase chain reaction–restriction fragment length polymorphism was used to detect the different alleles of CYP2C9 as described earlier.<sup>26</sup> The CYP2C9\*2 allelic polymerase chain reaction resulted in a 372-bp amplicon that was digested by the restriction endonuclease *Sau96I* to 179-, 119-, and 74-bp fragments in presence of the wild-type allele and to 253

**Table I.** Pharmacokinetic-pharmacodynamic model selection criteria

<i>Model</i>	<i>No. of kinetic parameters</i>	<i>Common variance of clearance</i>	<i>Objective function</i>	<i>Other comments</i>
First-order absorption; monoexponential disposition; 1 population clearance	3	0.062	1027	Simple model that does not take genotype information into account
Subgroup specific clearances for alleles 1 and 3	4	0.051	1023	Corresponds to completely codominant mode of inheritance
Subgroup specific clearances for alleles 1, 2, and 3	5	0.043	1020	
Subgroup specific clearances for all 6 genotypes	8	0.032	1014	Corresponds to an additional dominant component in the mode of inheritance
Absorption according to an inverse Gaussian distribution function; subgroup-specific clearances for alleles 1, 2, and 3	6	0.043	970*	Modeling of peak concentrations substantially improved

\*Does not fulfill criteria for statistical comparison with the 4 functions given above by use of the log-likelihood ratio test.

and 119-bp fragments in the case of allele *2C9\*2*. The *CYP2C9\*3* allelic polymerase chain reaction resulted in a 130-bp amplicon that was cut into 104 and 26 bp by digestion with restriction endonuclease *StyI* in the case of allele *2C9\*3*, whereas the wild-type allele remained uncut. To control for reproducibility of *CYP2C9* genotyping, all *CYP2C9* genotype analyses were performed twice and a 100% concordance between both analyses was observed.

#### Concentration analyses: Racemic ibuprofen

Ibuprofen was analyzed by use of a method that was essentially the same as one published previously.<sup>27</sup> In brief, 0.25 mL plasma was acidified with 0.1 mL of 0.1N hydrochloric acid and then extracted into 1.5 mL of *n*-hexane/*t*-butyl methyl ether (80/20, vol/vol) for 15 minutes. The organic solvent was then removed, and the sample was reconstituted in HPLC solvent. The solvent consisted of phosphate buffer (0.02 mol/L; pH 3) and acetonitrile (solvent A: buffer/acetonitrile, 95/5; solvent B: buffer/acetonitrile, 45/55). Separation of the peaks of interest (ibuprofen, 2-hydroxyibuprofen, and the internal standard flurbiprofen) was achieved on a LiChrospher 100 RP18 column (Merck, Darmstadt, Germany; 125 mm × 4 mm, 5 μm particle size). A gradient was run at 1.5 mL/min, starting with 50% solvent B and progressing linearly to 100% solvent B within 5 minutes. Retention times were 1.98, 8.90, and 9.70 minutes for 2-hydroxyibuprofen, ibuprofen, and flurbiprofen, respectively. The column effluent was

monitored at 220 nm with an ultraviolet detector. The calibration range was from 0.5 mg/L to 40 mg/L. The limit of quantification was 0.5 mg/L, and the interassay coefficient of variation for ibuprofen was 15% at a concentration of 1 mg/L and 9% at a concentration of 20 mg/L. The interassay coefficient of variation for 2-hydroxyibuprofen was 22% at a concentration of 1 mg/L and 11% at a concentration of 20 mg/L.

#### *R*- and *S*-ibuprofen

The enantiomers were separated on a LiChroCart 250-4 WhelkO1(S,S) column from Merck and detected by ultraviolet absorption at 230 nm. The eluent was *n*-hexane/2-propanol/acetic acid (98/2/0.05 vol/vol/vol), delivered at 0.5 mL/min at a temperature of 25°C. Retention times were 10.1, 11.0, 14.5, and 16.7 minutes for *R*-(-)-ibuprofen, *S*-(+)-ibuprofen, and both flurbiprofen enantiomers, respectively. Sample preparation was the same as that as described earlier with an extraction solvent that consisted of *n*-hexane/diethyl ether (95/5, vol/vol). The calibration range was from 0.5 to 25 mg/L for both enantiomers. The limit of quantification for *R*-ibuprofen was 0.5 mg/L, and the interassay coefficients of variation were 6.8% and 7.8% at concentrations of 2.5 mg/L and 15 mg/L, respectively. With *S*-ibuprofen, the limit of quantification was 0.5 mg/L and the interassay coefficient of variation was 14% and 10% at concentrations of 2.5 mg/L and 15 mg/L, respectively.

**Table II.** Population kinetic parameters for racemic ibuprofen and *R*- and *S*-ibuprofen

Parameter	Racemic ibuprofen		<i>R</i> -ibuprofen		<i>S</i> -ibuprofen	
	Population mean (SD)†	Interindividual variability as 95% CI	Population mean (SD)†	Interindividual variability as 95% CI	Population mean (SD)†	Interindividual variability as 95% CI
Volume of distribution (L)	10.9 (0.67)	10.1-11.7	10.7 (0.80)	9.80-11.1	9.72 (0.60)	8.88-10.6
MAT (h) ‡	0.94 (0.12)	0.81-1.09	1.04 (0.09)	0.93-1.16	1.28 (0.24)	1.13-1.48
Normalized variance of MAT (CVA) ‡	0.57 (0.83)	—	0.62 (0.09)	—	0.79 (0.16)	—
Clearance for						
<i>CYP2C9</i> *1/*1 (L/h)	3.77 (0.56)	3.39-4.04	4.44 (0.60)	4.00-4.92	3.25 (0.69)§	2.84-3.73
<i>CYP2C9</i> *1/*2 (L/h)	3.32 (0.85)	3.04-3.36	4.14 (0.55)	3.73-4.59	3.19 (0.72)§	2.79-3.66
<i>CYP2C9</i> *2/*2 (L/h)	2.94 (0.43)	2.70-3.21	3.84 (0.49)	3.47-4.26	3.13 (0.74)§	2.74-3.59
<i>CYP2C9</i> *1/*3 (L/h)	3.03 (0.83)	2.78-3.32	4.36 (0.60)	3.94-4.84	2.38 (0.57)§	2.09-2.73
<i>CYP2C9</i> *2/*3 (L/h)	2.65 (0.38)	2.44-2.90	4.07 (0.55)	3.67-4.51	2.32 (0.60)§	2.03-2.67
<i>CYP2C9</i> *3/*3 (L/h)	2.37 (0.33)	2.17-2.59	4.29 (0.61)	3.87-4.76	1.52 (0.42)§	1.33-1.74

CI, Confidence interval; MAT, mean absorption time; CVA, normalized variance of MAT.

†Parameters are given as population mean values together with the precision of parameter estimates (as standard deviation). The interindividual variability of the parameters is given as 95% confidence limits. With the nonlinear mixed-effect modeling (by use of NONMEM), precision of parameter estimates and population variability can be estimated separately.

‡The absorption of ibuprofen was described with the inverse gaussian function, as published by Weiss,<sup>32</sup> which describes the input of the drug from the gut into the systemic circulation by a function of a MAT and the variance of the MAT. CVA therefore described the shape of the absorption time function. For CVA, no interindividual variability was estimated to avoid overparameterization of the model.

§To obtain the real clearances of *S*-ibuprofen, the data given here have to be multiplied by 1.6 to account for the fact that, on average, 60% of *R*-ibuprofen is converted into *S*-ibuprofen.

### Thromboxane B<sub>2</sub> and prostaglandin E<sub>2</sub> concentration analysis

Samples of whole blood without anticoagulant were drawn at the same intervals as the samples for drug concentration analyses within the first 10-hour period of the study. To measure NSAID-mediated inhibition of thromboxane B<sub>2</sub> generation, each blood sample was incubated at 37°C for 1 hour before separation of the serum by centrifugation. Serum was then kept at -70°C until assayed for thromboxane B<sub>2</sub>. This assay predominantly measures the activity of the constitutively expressed COX-1. Thromboxane B<sub>2</sub> and prostaglandin E<sub>2</sub> were quantified with methods described elsewhere by use of commercial enzyme immunoassays from Biotrend (Cologne, Germany).<sup>28-30</sup> The limit of quantification was 7.98 ng/L and the interassay coefficient of variation ranged from 3.6% to 7.6% for the thromboxane concentrations between 44 and 3000 ng/L.

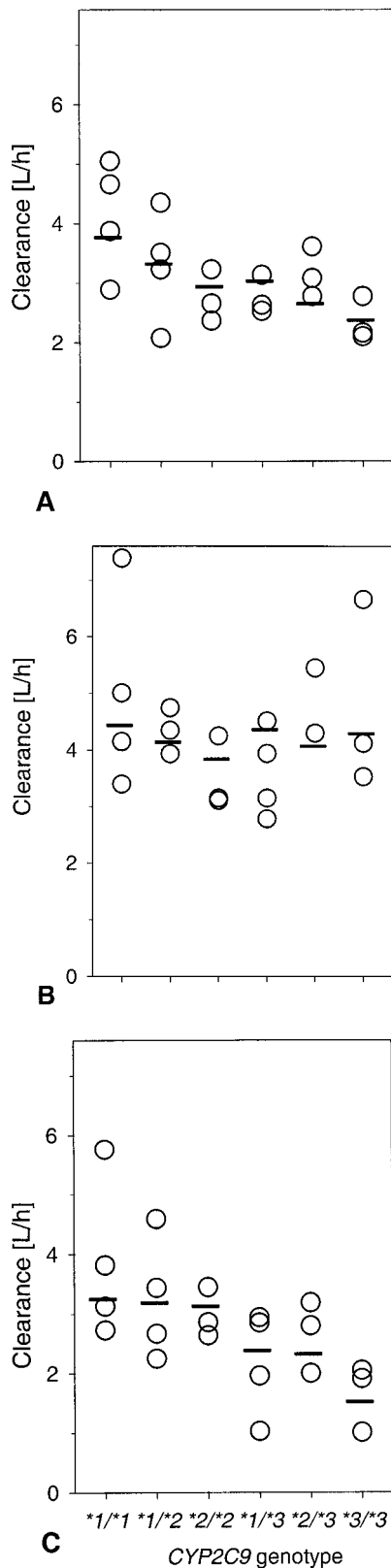
To measure ibuprofen-mediated inhibition of the formation of prostaglandin E<sub>2</sub>, heparinized blood was treated with 10 µg/mL *Escherichia coli* serotype lipopolysaccharide (type 026:B6) and was incubated at 37°C for 24 hours. Plasma was separated by centrifugation and kept at -70°C until assayed for prostaglandin E<sub>2</sub> with an enzyme-linked immunoassay. This assay predominantly reflects the activity of the inducible COX-2. The limit of quantification was 36.2 ng/L and

the interassay coefficient of variation ranged from 3.0% to 5.1% for concentrations between 111.3 and 1902 ng/L.

### Pharmacokinetic data analysis with use of a population pharmacokinetic approach

The pharmacokinetic and pharmacodynamic analyses were based on nonlinear mixed-effects modeling with use of the program NONMEM, version V, level 1.1.<sup>31</sup> This statistical approach allowed us to use all data simultaneously and to take into account interindividual and residual (random) variability as specified in the model. The individual pharmacokinetic parameters elimination half-life, volume of distribution, and clearance were obtained from population estimates according to the Bayes' theorem by use of the POSTHOC option of NONMEM. The first-order conditional estimate calculation variant was chosen as recommended for rich data situations, and a proportional error model was used. All analyses were performed with untransformed data, and analyses were performed separately with concentrations of both enantiomers and with the concentrations of the racemate.

Different models have been evaluated by use of the usual comparisons of model predictions with measured data and with statistical criteria. Our modeling approach started with the fitting of a 1-compartment model to the data (first-order input and 1-exponential

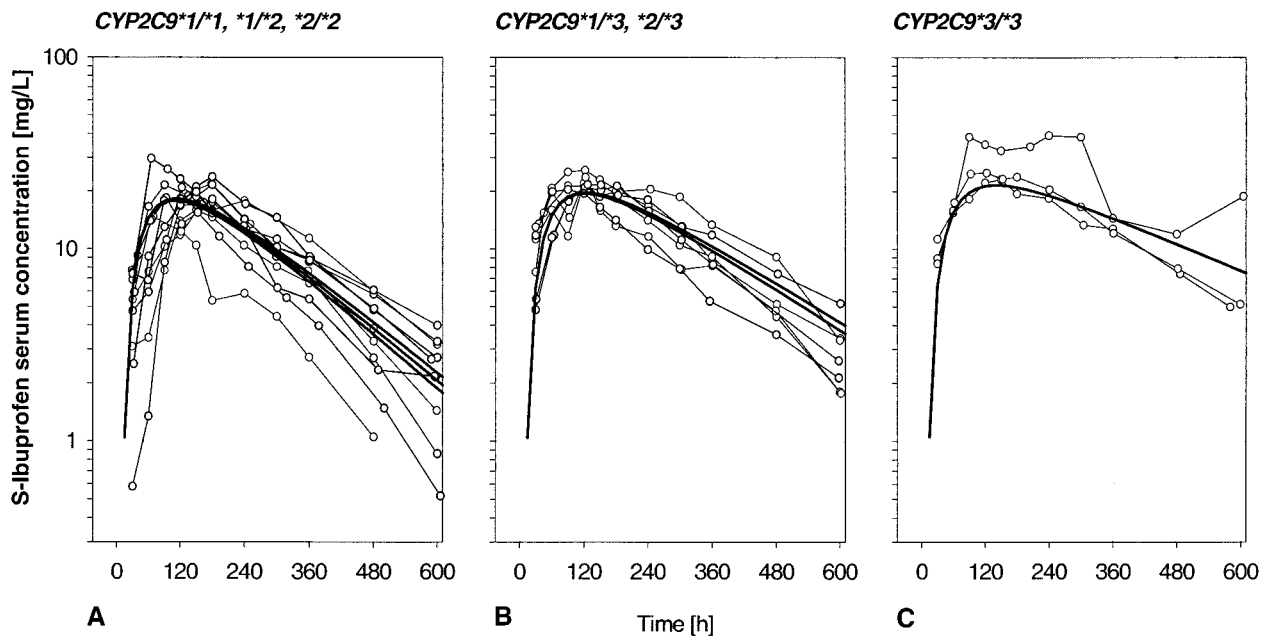


disposition), as implemented in the NONMEM subroutine ADVAN 2. The statistical analysis of the individual estimates of clearances revealed the following ranking among *CYP2C9* genotypes: \*1/\*1, \*1/\*2, \*2/\*2, \*1/\*3, \*2/\*3, and \*3/\*3. Partial clearances for each allele were therefore included in the pharmacokinetic modeling approach; for example, the clearance of a carrier of the *CYP2C9* genotype \*1/\*3 was calculated as the sum of the 2 partial clearances of the *2C9\*1* and *2C9\*3* alleles. Because the reduction of activity in carriers of allele *2C9\*2* was apparently only a minor one, we did not at first differentiate between the alleles *2C9\*1* and *2C9\*2*. Later, the allele *2C9\*2*-specific information was also included. In addition to the linear combination, which corresponds in terms of genetics to a completely codominant mode of inheritance, a parameterization was used, which accounts for a more, but not exclusively, dominant mode of inheritance. We implemented this by fitting additional factors for clearance, accounting for an interaction between alleles \*1/\*2, \*1/\*3, and \*2/\*3. A summary of the different models used is presented in Table I. The likelihood ratio test, with  $\alpha = .05$ , was used for a formal comparison of the different models. All genotype-specific clearance parameters were included as factors of the clearance for allele *2C9\*1* to allow the statistical comparison between the extended (full) and the more simple (reduced) models. The other factors could therefore be either fixed to unity or estimated. In addition, the description of the absorption concentration–time course could be significantly improved: The input time distribution was described by an inverse Gaussian distribution with the parameters of mean absorption time (MAT) and normalized variance (CVA) as described by Weiss.<sup>32</sup> It should be noted that normalized variance of MAT (CVA) is not a variance parameter that describes population variability of MAT in this context but a parameter that describes the shape of the input function.

#### Pharmacodynamic analysis

The individual concentration data for thromboxane B<sub>2</sub> and prostaglandin E<sub>2</sub> were normalized to the highest concentration measured before occurrence of the trough in thromboxane B<sub>2</sub> or prostaglandin E<sub>2</sub> concentrations caused by inhibition (normalized thromboxane B<sub>2</sub> or

**Fig 1.** Oral clearance of racemic (A), R-ibuprofen (B), and S-ibuprofen (C), depending on *CYP2C9* genotypes. Circles show the individual pharmacokinetic parameters, and lines show the population pharmacokinetic estimates for the respective group.



**Fig 2.** Measured concentration–time courses and concentrations predicted by the population parameters for S-(+)-ibuprofen. Measured values are represented by *open circles* connected by *straight lines*. Population mean curves are represented by *bold lines*. **A**, Data for carriers of *CYP2C9*\*1/\*1, \*1/\*2, and \*2/\*2. Population mean curves for *CYP2C9* genotypes 2*C9*\*1/\*1, 2*C9*\*1/\*2, and 2*C9*\*2/\*2 are almost identical and are represented by the *lower, middle, and upper curves*, respectively. **B**, Data for carriers of *CYP2C9*\*1/\*3 and \*2/\*3. Population mean curves for *CYP2C9* genotypes 2*C9*\*1/\*3 and 2*C9*\*2/\*3 are represented by the *lower and upper curves*, respectively. **C**, Measured concentrations and population mean curves for *CYP2C9*\*3/\*3.

prostaglandin E<sub>2</sub> concentration equals measured thromboxane B<sub>2</sub> or prostaglandin E<sub>2</sub> concentration divided by the individually measured maximal concentration). Therefore the minimal or zero effect of NSAIDs was normalized to 1 and the maximal effect was normalized to 0. To analyze and illustrate data in the form of typical concentration–effect curves (eg, in the last figure of this presentation), the difference of unity and the normalized effect was used, and consequently the maximal effect E<sub>max</sub> was then normalized to 1 (cyclooxygenase 1 or 2 inhibition equals 1 minus normalized thromboxane B<sub>2</sub> or prostaglandin E<sub>2</sub> concentration). The relation between serum concentration and prostanoid formation was described by the so-called simple E<sub>max</sub> function:

$$\text{Effect} = E_{\max} \cdot C_{\text{ibuprofen}} / (C_{\text{ibuprofen}} + EC_{50})$$

in which E<sub>max</sub> represents the maximal effect, C<sub>ibuprofen</sub> is the plasma concentration of ibuprofen, and EC<sub>50</sub> is the ibuprofen concentration exhibiting the half-maximum effect. For thromboxane B<sub>2</sub>, the concentration–response relationship was better described by an exponential E<sub>max</sub> function with the exponent  $\gamma$ :

$$\text{Effect} = E_{\max} \cdot C_{\text{ibuprofen}}^{\gamma} / (C_{\text{ibuprofen}}^{\gamma} + EC_{50}^{\gamma})$$

In addition to an EC<sub>50</sub> population mean parameter common to all subjects, *CYP2C9* genotype–dependent EC<sub>50</sub> population parameters had to be applied to adequately describe the pharmacokinetic–pharmacodynamic relation in prostaglandin E<sub>2</sub> formation.

#### Pharmacokinetic and pharmacodynamic data analysis with use of a noncompartmental approach

In addition to the population pharmacokinetic modeling approach, the data were analyzed by nonparametric statistical methods with use of WinNonlin (version 1.5, Pharsight Corp, Mountain View, Calif). Values for area under the plasma concentration–time curve (AUC) were calculated by use of the linear trapezoidal rule with extrapolation to infinity for the pharmacokinetic data but without extrapolation beyond the last measured concentration for the pharmacodynamic data, prostaglandin E<sub>2</sub>, and thromboxane B<sub>2</sub>. Maximal (C<sub>max</sub>) and minimal (C<sub>min</sub>) concentrations were the respective data as measured. Time of 50% inhibition of prostaglandin

**Table III.** Summary of nonparametric analysis of ibuprofen pharmacokinetics

<i>CYP2C9</i> genotype	AUC			
	Racemate <sup>†</sup> (g · h/L)	<i>R</i> -ibuprofen (g · h/L)	<i>S</i> -ibuprofen (g · h/L)	Hydroxyibuprofen <sup>‡</sup> (mg · h/L)
*1/*1	0.16 (0.12-0.23)	0.06 (0.04-0.09)	0.10 (0.05-0.13)	5.7 (4.4-21.5)
*1/*2	0.20 (0.15-0.25)	0.08 (0.06-0.13)	0.11 (0.07-0.13)	13.5 (5.7-20.0)
*2/*2	0.23 (0.21-0.23)	0.10 (0.10-0.13)	0.11 (0.09-0.13)	10.8 (6.6-13.5)
*1/*3	0.26 (0.22-0.27)	0.09 (0.07-0.12)	0.15 (0.11-0.19)	10.5 (5.1-12.9)
*2/*3	0.22 (0.18-0.25)	0.06 (0.05-0.07)	0.11 (0.09-0.17)	18.7 (9.2-25.5)
*3/*3	0.29 (0.24-0.33)	0.07 (0.05-0.10)	0.17 (0.17-0.37)	5.0 (4.9-10.5)

AUC, Area under the concentration–time curve; C<sub>max</sub>, maximum concentration.

All parameters are given as medians and ranges.

<sup>†</sup>Racemate, *R*-ibuprofen, *S*-ibuprofen, and hydroxyibuprofen correspond to pharmacokinetic parameters that reflect racemic ibuprofen (nonenantioselective HPLC), *R*-ibuprofen, *S*-ibuprofen, and 2-hydroxyibuprofen.

<sup>‡</sup>The enantiomers of hydroxyibuprofen were not separated.

E<sub>2</sub> and thromboxane B<sub>2</sub> synthesis was obtained from the concentration–time plots by means of linear interpolation between the measured concentrations. The Kruskal-Wallis test was used to test the statistical significance of differences among the *CYP2C9* genotypes, and the Jonckheere-Terpstra trend test was used as implemented in the SPSS software (version 10; SPSS Inc, Chicago, Ill) to test for gene–dose dependent trends. For the Jonckheere-Terpstra test the a priori defined trend was in the following order of *CYP2C9* genotypes: \*1/\*1, \*1/\*2, \*2/\*2, \*1/\*3, \*2/\*3, and \*3/\*3.

## RESULTS

The population pharmacokinetic parameters for the racemic ibuprofen and for the *S*(+)- and *R*(-)- enantiomers are given in Table II. *CYP2C9* allele-specific partial clearances of racemic ibuprofen were 1.89 L/h (allele 2*C9*\*1), 1.47 L/h (allele 2*C9*\*2), and 1.19 L/h (allele 2*C9*\*3); the resulting clearances for the 6 genotypes of *CYP2C9* are given in the Table II and their dependence on *CYP2C9* genotype is illustrated in Fig 1.

The population mean clearance of carriers of the *CYP2C9*\*3/\*3 genotype was 2.37 L/h (95% confidence interval [95% CI], 2.17 to 2.59 L/h). It was about 50% lower than that of carriers of the genotype *CYP2C9*\*1/\*1 in whom clearance was 3.77 L/h (95% CI, 3.39 to 4.04 L/h). The 95% CI of these 2 genotypes did not overlap (Table II).

The *CYP2C9* genotype–related effect on ibuprofen clearance was entirely restricted to the *S*-enantiomer. Fig 2 illustrates the concentration–time curves of *S*-ibuprofen. Apparently, the disposition phase is adequately described by a 1-compartmental model. As illustrated in Fig 1, the population mean clearances of racemic ibuprofen and of *S*-ibuprofen decreased among

genotypes in the following order: *CYP2C9*\*1/\*1 > \*1/\*2 > \*2/\*2 > \*1/\*3 > \*2/\*3 > \*3/\*3. The partial *S*-ibuprofen clearances calculated for the *CYP2C9* alleles \*1, \*2, and \*3 were 1.63, 1.57, and 0.76 L/h, respectively. The mean *S*-ibuprofen clearance of carriers of the *CYP2C9* allele \*3 was only 50% of the clearance of carriers of allele \*1. The clearances in carriers of the 3 possible combinations of the 2*C9*\*1 and 2*C9*\*3 alleles appeared to follow a completely codominant mode of inheritance. The clearance of the heterozygotes (2.38 L/h; 95% CI, 2.09 to 2.73 L/h) was between that of the homozygous carriers of 2 wild-type alleles (3.25 L/h; 95% CI, 2.84 to 3.73 L/h) and that of carriers of two 2*C9*\*3 alleles (1.52 L/h; 95% CI, 1.33 to 1.74 L/h). In contrast, the clearance values of the *R*-enantiomer were almost identical in carriers of \*3/\*3 and in carriers of \*1/\*1 (Table II).

Because the effect of the *CYP2C9* variant \*2 was apparently negligible compared with that of the \*3 variant, we pooled all carriers of alleles \*1 and \*2 in one group and compared that group with heterozygous and homozygous carriers of the \*3 allele. The population pharmacokinetic analysis of the clearance difference between these 3 groups (\*1/\*1 plus \*1/\*2 plus \*2/\*2 versus \*1/\*3 plus \*2/\*3 versus \*3/\*3) revealed a highly significant effect. The different pharmacokinetic–pharmacogenetic models used were compared by use of the usual criteria, such as comparison of observed and predicted data and formal tests. The population variance of clearance was 0.064 L<sup>2</sup>/h<sup>2</sup> when 1 clearance was used for all subjects, but it was only 0.043 L<sup>2</sup>/h<sup>2</sup> when the 3 allele-specific partial clearances were used. When 6 separate *CYP2C9* genotype-specific clearances were used, population variance was even lower (0.032 L<sup>2</sup>/h<sup>2</sup>; Table I). The model that used 3 allele-specific partial clearances was statistically su-

Racemate (mg/L)	$C_{max}$		
	<i>R</i> -ibuprofen (mg/L)	<i>S</i> -ibuprofen (mg/L)	Hydroxyibuprofen (mg/L)
33.8 (28.9-36.4)	17.0 (12.7-18.3)	16.8 (14.7-21.9)	1.34 (0.86-2.20)
39.9 (36.3-47.3)	16.5 (16.3-18.4)	20.0 (17.5-22.1)	1.71 (1.19-2.01)
47.9 (44.2-50.3)	23.4 (17.1-33.6)	23.8 (19.8-29.6)	1.54 (1.29-2.61)
44.1 (37.4-49.4)	23.0 (19.1-25.3)	22.9 (21.4-25.8)	1.73 (0.66-2.13)
37.2 (36.5-42.2)	16.0 (14.5-17.0)	21.3 (20.4-21.7)	1.39 (1.24-1.95)
45.8 (38.7-46.2)	19.0 (14.1-20.4)	25.0 (23.1-39.1)	0.85 (0.77-0.89)

rior to the model with 1 clearance parameter for the entire sample, as verified by the likelihood ratio test of the objective function values ( $P < .05$ ).<sup>31</sup> Further improvement was achieved when the model of complete codominant inheritance was abandoned. However, 3 additional parameters had to be included to model the specific effects of all 6 genotypes. Formal testing of the objective function values with the likelihood ratio test revealed that these further attempts to improve the model were not justified.

Elimination half-lives of racemic ibuprofen were significantly different between the *CYP2C9*\*1/\*1 and *CYP2C9*\*3/\*3 genotypes (2.04 hours and 3.09 hours, respectively). This was again entirely attributable to differences in the half-life of *S*-ibuprofen (2.08 hours versus 4.28 hours), whereas the half-life of *R*-ibuprofen was the same in both *CYP2C9* genotype groups (1.65 hours).

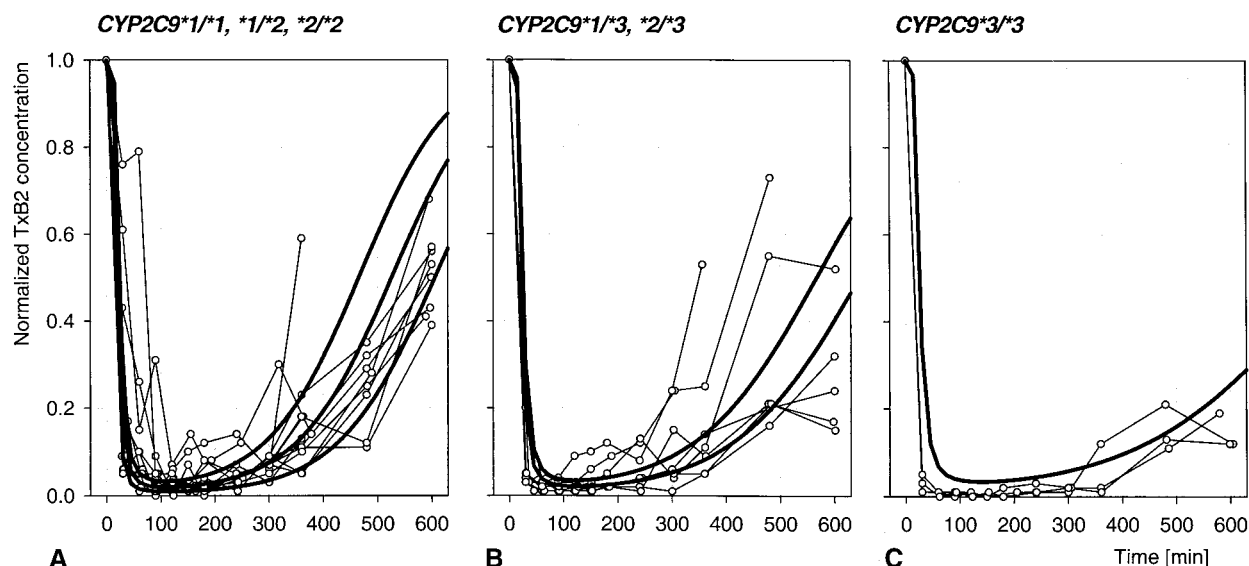
The results obtained with population pharmacokinetic modeling were confirmed by use of a noncompartmental model method and nonparametric statistical analysis. As shown in Table III, the AUC and the measured maximal plasma concentrations ( $C_{max}$ ) of racemic ibuprofen and of *S*-ibuprofen were higher in carriers of the *CYP2C9* slow genotypes. The nonparametric Jonckheere-Terpstra trend test showed this trend to be statistically significant ( $P = .008$  and  $P = .025$  for racemic ibuprofen AUC and  $C_{max}$ , respectively, and  $P = .046$  and  $P = .005$  for *S*-ibuprofen AUC and  $C_{max}$ , respectively). The Kruskal-Wallis test, which does not use any prerequisite for the order of genotypes, also showed these associations between genotype and the AUC and  $C_{max}$  parameters to be statistically significant. The AUC values of racemic ibuprofen and *S*-ibuprofen were almost twice as high in carriers of the *CYP2C9*\*3/\*3 genotype compared with carriers of the *CYP2C9*\*1/\*1 genotype.

Plasma concentrations of 2-hydroxyibuprofen were about 10% of those of the parent drug. The median  $C_{max}$  of 2-hydroxyibuprofen was only 0.85 mg/L (range, 0.77 to 0.89 mg/L), compared with a median concentration of 1.34 mg/L (range, 0.86 to 2.20 mg/L) in carriers of the rapid genotype *CYP2C9*\*1/\*1. The expected trend between genotype and 2-hydroxyibuprofen was not statistically significant (Table III) but, nevertheless, the median plasma concentrations of the 2-hydroxymetabolite were lower in carriers of the \*3/\*3 genotype than in carriers of the other genotypes. This is compatible with the assumption that 2-hydroxyibuprofen is generated by *CYP2C9*.

The possible impact of the *CYP2C9*-related pharmacokinetic differences on the effects of ibuprofen was evaluated by measurement of prostaglandin  $E_2$  and thromboxane  $B_2$  ex vivo. As illustrated in Fig 3, the prolonged half-life of ibuprofen in carriers of the \*1/\*3, \*2/\*3, and \*3/\*3 genotypes resulted in a prolonged inhibition of thromboxane  $B_2$  formation. It appeared that prostanoid formation did not recover to the initial baseline values, even after 24 hours, in carriers of the *CYP2C9* genotype \*3/\*3 (Fig 3).

These pharmacodynamic parameters are summarized in Tables IV and V. Table IV gives the  $EC_{50}$  data. An exponential model was required for thromboxane  $B_2$  to adequately fit the concentration–response relationship. In addition, the AUC obtained from the population pharmacokinetic-pharmacodynamic analysis is provided. The AUC values of thromboxane  $B_2$  and prostaglandin  $B_2$  provide a measurement of the ibuprofen concentration effect over time.

In thromboxane  $B_2$  formation, the population parameter  $EC_{50}$ , which describes the pharmacodynamic effect, reached population mean values of 3.9, 3.0, 4.3, 2.2, 3.5, and 4.7 mg/L in the *CYP2C9* genotypes \*1/\*1, \*1/\*2, \*2/\*2, \*1/\*3, \*2/\*3, and \*3/\*3, respectively; the



**Fig 3.** Measured concentration–time courses of thromboxane  $B_2$  and population group mean estimates differentiated for the *CYP2C9*\*3 variant (Ile359Leu). This assay predominantly reflects the inhibition of cyclooxygenase 1. Measured values are represented by *open circles* connected by *straight lines*. Population mean curves are represented by *bold lines*. **A**, Data for carriers of *CYP2C9*\*1/\*1, \*1/\*2, and \*2/\*2. *CYP2C9* genotypes \*2/\*2, \*1/\*2, and \*1/\*1 are represented by the *lower, middle, and upper curves*, respectively. **B**, Data for carriers of *CYP2C9*\*1/\*3 and \*2/\*3. The *lower curve* represents the *CYP2C9* genotype \*2/\*3, and the *upper curve* represents the genotype \*1/\*3. **C**, Measured concentrations and population mean effect-time curve of carriers of *CYP2C9*\*3/\*3. The areas above the population mean time–effect curves reflect the inhibitory effect of ibuprofen; these integrals were 429, 501, 541, 602, 637, and 775 relative inhibition units  $\cdot$  min for *CYP2C9* genotypes \*1/\*1, \*1/\*2, \*2/\*2, \*1/\*3, \*2/\*3, and \*3/\*3, respectively.

common standard deviation was 1.50 mg/L. The population estimate of  $\gamma$  was 2.21, with a precision of 0.58. Because data were normalized to 1, the  $E_{\max}$  parameter was fixed to unity. The mean AUC values of thromboxane  $B_2$ , integrated from 0 to 10 hours, were 429, 501, 541, 602, 637, and 775 relative inhibition units  $\cdot$  min in the *CYP2C9* genotypes \*1/\*1, \*1/\*2, \*2/\*2, \*1/\*3, \*2/\*3 and \*3/\*3, respectively (Fig 3). These AUC values corresponded to the areas above the *bold population mean effect curves* shown in Fig 3, and these areas can be interpreted in the same manner as other well-known effect-time parameters (eg, those in physics).

As an additional confirmation, a completely nonparametric statistical analysis was performed. The AUC values of thromboxane  $B_2$  were calculated from the measured data, without normalization with the linear trapezoidal rule. Table V shows that decreasing ibuprofen metabolizing capacity is associated with a lower AUC of thromboxane  $B_2$ ; this trend was statistically significant in the nonparametric Jonckheere-Terpstra trend test ( $P = .046$ ). In addition, the minimal levels of

the prostanoids were compared as an indicator of the maximum strength of inhibition of cyclooxygenases 1, and the duration of 50% inhibition of thromboxane  $B_2$  formation was calculated. Minimal median concentrations of thromboxane  $B_2$  were 1.00 mg/L (range, 0.43 to 3.00 mg/L) in carriers of the *CYP2C9* genotype \*1/\*1 compared with 0.33 mg/L (range, 0.28 to 1.55 mg/L) in carriers of *CYP2C9*\*2/\*3 and 0.31 mg/L (range, 0.28 to 0.75 mg/L) in carriers of *CYP2C9*\*3/\*3. The trend test verified this to be statistically significant ( $P = .05$ ). The duration of the 50% inhibition of cyclooxygenases 1 was longer in carriers of the slow metabolic genotypes (Table V).

In prostaglandin  $E_2$  formation, the population parameter  $EC_{50}$  reached population mean values of 5.4, 4.9, 3.2, 4.4, 2.8, and 1.1 mg/L in the *CYP2C9* genotypes \*1/\*1, \*1/\*2, \*2/\*2, \*1/\*3, \*2/\*3, and \*3/\*3, respectively; the common population standard deviation was 0.77 mg/L. The  $E_{\max}$  parameter was fixed to unity. The population mean AUC values of prostaglandin  $E_2$ , integrated from 0 to 10 hours, increased in the *CYP2C9*

**Table IV.** Pharmacodynamic parameter estimates from population pharmacokinetic/pharmacodynamic analysis

<i>CYP2C9</i> genotype	Thromboxane B <sub>2</sub> (COX-1)			Prostaglandin E <sub>2</sub> (COX-2)	
	EC <sub>50</sub> (mg/L)	γ	AUC (relative inhibition units · min)	EC <sub>50</sub> (mg/L)	AUC (relative inhibition units · min)
*1/*1	3.37 (3.27-4.97)	2.10 (1.94-2.12)	524 (290-561)	5.45 (4.80-5.63)	408 (244-492)
*1/*2	3.08 (2.39-4.17)	2.12 (2.11-2.13)	577 (390-856)	4.18 (2.49-7.34)	464 (367-631)
*2/*2	2.62 (2.59-2.83)	1.86 (1.79-1.95)	692 (587-702)	3.28 (2.98-5.53)	487 (460-578)
*1/*3	3.62 (3.06-7.70)	2.15 (2.12-2.27)	711 (539-898)	3.21 (0.91-4.74)	628 (456-1999)
*2/*3	2.97 (2.69-4.84)	2.09 (1.96-2.10)	708 (426-890)	2.26 (1.33-2.63)	733 (581-817)
*3/*3	3.38 (3.33-5.70)	2.09 (2.09-2.17)	836 (829-946)	0.65 (0.12-1.82)	1058 (835-2014)

Data are given as medians and ranges.  
EC<sub>50</sub>, Ibuprofen concentration for half-maximum inhibition; γ, corresponds to the exponent (Hill coefficient) in the concentration–effect model; AUC, reflects the cumulative inhibitory effect from 0 to 10 hours after administration of ibuprofen.

**Table V.** Summary of nonparametric analysis of ibuprofen pharmacodynamics

<i>CYP2C9</i> genotype	Thromboxane B <sub>2</sub>			Prostaglandin E <sub>2</sub>		
	Dur <sub>50</sub> (h)	AUC (μg · h/L)†	C <sub>min</sub> (μg/L)	Dur <sub>50</sub> (h)	AUC (μg · h/L)†	C <sub>min</sub> (μg/L)
*1/*1	9.1 (5.3-9.5)	0.20 (0.13-0.30)	1.00 (0.43-3.00)	6.1 (4.6-8.5)	0.32 (0.10-0.56)	6.86 (3.60-11.1)
*1/*2	5.9 (3.4-9.5)	0.31 (0.06-0.68)	0.98 (0.73-6.34)	8.4 (5.3-14.5)	0.27 (0.06-0.42)	4.40 (2.64-11.0)
*2/*2	7.3 (6.8-9.2)	0.09 (0.07-0.29)	0.66 (0.50-1.54)	6 (3.1-6.7)	0.26 (0.10-0.38)	5.49 (3.54-9.69)
*1/*3	8.0 (6.5-7.4)	0.16 (0.13-0.19)	0.93 (0.38-1.21)	5 (4.2-5.1)	0.18 (0.07-0.37)	5.44 (4.56-5.79)
*2/*3	13.2 (5.1-24)	0.06 (0.06-0.19)	0.33 (0.28-1.55)	14 (8.2-15.5)	0.06 (0.06-0.23)	3.07 (2.98-5.86)
*3/*3	11.5 (10.0-13.2)	0.09 (0.09-0.14)	0.31 (0.28-0.75)	20 (19-24)†	0.23 (0.13-0.40)	5.86 (5.40-27.3)

All parameters are given as medians and ranges.  
Dur<sub>50</sub>, Time interval during which the respective cyclooxygenase was inhibited by 50% (this was estimated nonparametrically with use of linear interpolation if necessary); C<sub>min</sub>, minimum concentration.  
Thromboxane B<sub>2</sub> C<sub>min</sub> and AUC were significantly dependent on *CYP2C9* genotype (*P* = .04; Jonckheere-Terpstra trend test).  
†Partial AUC from 0 to 10 hours calculated by use of the linear trapezoidal rule and marking the area below the prostanoid concentration–time curve (in contrast to the parametrically calculated AUC values given in Table IV, which correspond to the area above the effect–time curve).

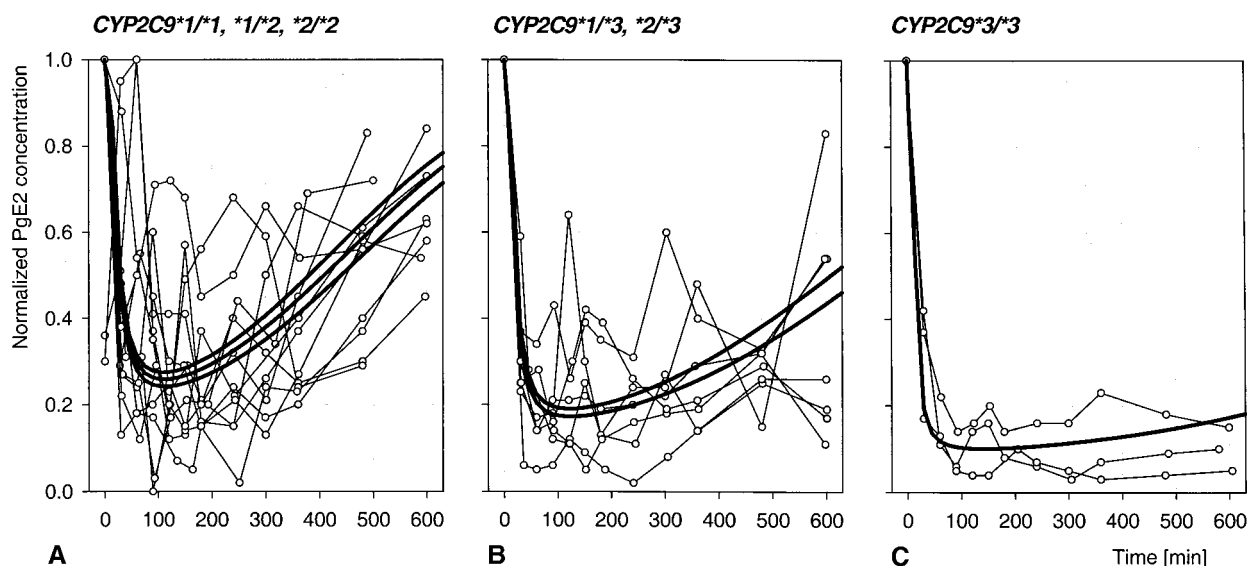
genotypes \*1/\*1, \*1/\*2, \*2/\*2, \*1/\*3, \*2/\*3, and \*3/\*3, with mean values of 356, 393, 438, 359, 669, and 1258 relative inhibition units · hours, respectively. These AUC values correspond to the areas above the *bold population mean effect curves* shown in Fig 4. Medians of the minimal measured concentrations of prostaglandin E<sub>2</sub> and the AUC(0-10h) data did not depend significantly on the *CYP2C9* genotypes (Table V). However, the duration of 50% inhibition was significantly longer in carriers of the *CYP2C9* genotypes \*2/\*3 and \*3/\*3 (14 hours [range, 8.2 to 15.5 hours] and 20 hours [range, 19 to 24 hours]), respectively, compared with the wild-type genotype *CYP2C9*\*1/\*1 (Table V).

The ibuprofen concentration–response curves on thromboxane B<sub>2</sub> formation and prostaglandin E<sub>2</sub> formation showed similar potency of ibuprofen for inhibition of COX-1 and COX-2 but a more steep concentration–response curve for COX-1 (Fig 5). Although the concentration–response curves for thromboxane B<sub>2</sub>

did not depend on the *CYP2C9* genotype, the corresponding curves for prostaglandin E<sub>2</sub> did. The “Discussion” section addresses possible mechanisms for this unexpected finding.

## DISCUSSION

In vitro studies led us to anticipate that the inherited amino acid polymorphisms in the human cytochrome P450 enzyme 2C9 would have an effect on ibuprofen clearance. This hypothesis was confirmed by the clinical pharmacokinetic and pharmacodynamic data from our study. The clearance of racemic ibuprofen differed by about 50% and the clearance of the dextrorotatory *S*-enantiomer differed by 100% between carriers of the rapid *CYP2C9* genotype \*1/\*1 and the slow *CYP2C9* genotype \*3/\*3. The impact of the *CYP2C9* polymorphisms on ibuprofen kinetics apparently was enantio-specific for *S*-ibuprofen, and the effect of the *CYP2C9* polymorphism on the pharmacodynamic effects of ibu-



**Fig 4.** Measured concentration–time courses of prostaglandin E<sub>2</sub> and population group mean estimates differentiated for the *CYP2C9*\*3 variant (Ile359Leu). This assay reflects inhibition of cyclooxygenase 2. Measured values are represented by *open circles* connected by *straight lines*. Population mean curves are represented by the *bold lines*. **A**, The *lower, middle, and upper* curves represent the *CYP2C9* genotypes \*2/\*2, \*1/\*2, and \*1/\*1, respectively. **B**, The *lower* curve represents the *CYP2C9* genotype \*2/\*3 and the *upper* curve represents the genotype \*1/\*3. **C**, *CYP2C9*\*3/\*3.

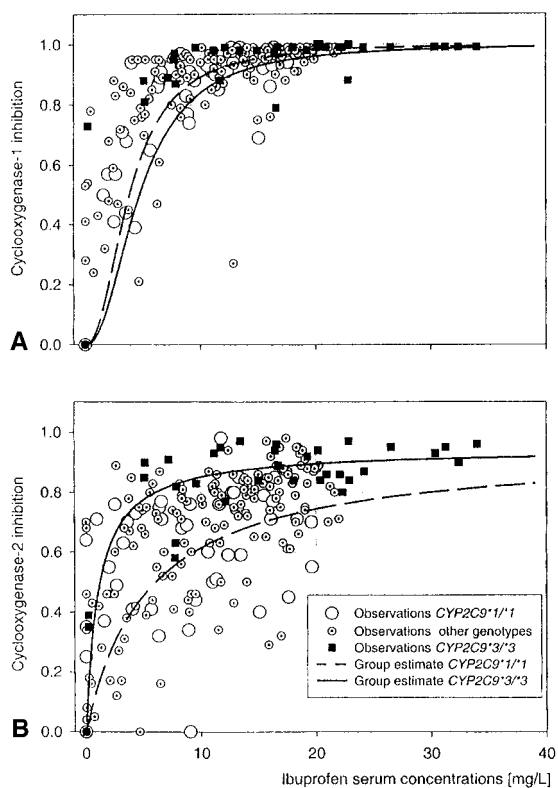
profen was marked, particularly for inhibition of cyclooxygenase 1 (Fig 3).

Population pharmacokinetic methods have only rarely been applied for the pharmacokinetic analysis of pharmacogenetic data. One of the advantages of the use of NONMEM pharmacokinetic-pharmacodynamic modeling is that all data can be taken into account simultaneously. This is particularly useful when certain types of data are limited, as the data in our study were because of the rareness of some allele combinations (such as the *CYP2C9*\*2/\*3 and *CYP2C9*\*3/\*3 genotypes), in the population. Joint processing of all the available information for every participant allows population pharmacokinetics to provide access to more reliable estimates, even for carriers of rare genotypes for whom data input is small.

In vitro investigations with use of human liver microsomes or isolated transgenically expressed cytochrome P450 enzymes have already identified a number of nonsteroidal anti-inflammatory drugs as substrates of cytochrome P450 2C9. These *CYP2C9* substrates include ibuprofen,<sup>9</sup> indomethacin (INN, indometacin),<sup>33</sup> flurbiprofen,<sup>34,35</sup> naproxen,<sup>36,37</sup> diclofenac,<sup>38</sup> piroxicam,<sup>39</sup> lornoxicam,<sup>40</sup> mefenamic acid, meloxicam,<sup>41</sup> and celecoxib.<sup>42-44</sup> The prescribing information of cele-

coxib (Celebrex, Pfizer Inc, New York, NY) even recommends that genetic polymorphisms in *CYP2C9* be considered in therapy. Many of the data cited above have been obtained and confirmed by use of in vitro studies only, but the importance of clinical confirmation in humans cannot be overestimated and is illustrated by diclofenac: Although diclofenac is a well-established substrate of *CYP2C9*, several studies, including our own (Kirchheiner J, Meineke I, Steinbach N, Meisel C, Roots I, Brockmüller J, unpublished data), have been unable to reveal any significant effects of the *CYP2C9* polymorphisms on diclofenac pharmacokinetics.<sup>45-48</sup>

An interesting feature in pharmacokinetic studies of ibuprofen is the conversion of the *R*-enantiomer to the *S*-enantiomer. If this conversion is not accounted for, the total clearances of *S*-ibuprofen are underestimated. It is known that a mean fraction of 60% of *R*-ibuprofen is converted to *S*-ibuprofen. Lötsch et al<sup>49</sup> recently showed how to integrate this information in the pharmacokinetic analysis of racemic ibuprofen. This approach is equivalent to the use of a correction factor of 1.6 for *S*-ibuprofen clearances under the prerequisite of linear pharmacokinetic processes. The numbers given in Tables II and III and in the text of this article are not



**Fig 5. A,** Concentration–response relationship for inhibition of cyclooxygenase 1 as measured by inhibition of the formation of thromboxane B<sub>2</sub>. **B,** Concentration–response relationship for inhibition of cyclooxygenase 2 measured as inhibition of the formation of prostaglandin E<sub>2</sub>. Solid squares represent data from subjects with *CYP2C9*\*3/\*3 genotype; open circles represent data from *CYP2C9*\*1/\*1; circles with dots represent data from the other genotypes. The solid lines depict the population mean dose–response curves for *CYP2C9* genotype \*1/\*1, and the broken lines show the corresponding curves for *CYP2C9*\*3/\*3.

corrected by this factor. Individual conversion factors would be obtained only by the administration of the pure *R*-ibuprofen enantiomer. According to earlier studies, however, population variability of the racemic interconversion is relatively small<sup>49</sup> and therefore should not cause a systematic error in our analysis of the *CYP2C9* genotype effects.

When ibuprofen serum concentrations were correlated with prostaglandin E<sub>2</sub> ex vivo data, the concentration–response curves were shown to be genotype dependent: the same *S*-ibuprofen concentrations exhibited a stronger inhibitory effect in carriers of the *CYP2C9* genotype \*3/\*3 than in carriers of the

*CYP2C9* genotype \*1/\*1. Apparently, there is a *CYP2C9* genotype–related effect on pharmacodynamics in addition to the effect on plasma concentrations (Figs 3 and 4). There are several possible explanations for this phenomenon. First, the *CYP2C9* that is expressed in leukocytes could have been active during the ex vivo incubation, which would have resulted in differences in the concentrations of active *S*-ibuprofen between carriers of *CYP2C9*\*1/\*1 and carriers of \*3/\*3 that were even greater than those in plasma when blood samples were taken. According to recent data, however, *CYP2C9* expression in blood cells appears to be low or even undetectable.<sup>50</sup> Second, *CYP2C9* alone may play a role in the regulation and biotransformation of prostanooids. This is at least suggested by experimental data<sup>51</sup> and is not completely unexpected, considering that NSAIDs as competitors in prostanooid metabolism must have structural features in common with prostanooids. Third, this result may have occurred just by chance and, unless confirmed by additional data, it should not be overemphasized.

Ibuprofen is generally considered to be a relatively safe member of NSAID family of drugs. Nevertheless, it is not free of typical adverse effects such as gastric bleeding, deterioration of kidney function, or worsening of heart failure as a result of sodium retention. One might anticipate from our data that carriers of the slow *CYP2C9* genotypes are particularly susceptible to these side effects. Earlier studies<sup>24</sup> have shown that ibuprofen is an unselective inhibitor of both cyclooxygenases COX-1 and COX-2. This is confirmed by our data on the ibuprofen concentration–response relationship, shown in Fig 5. The minimal thromboxane B<sub>2</sub> concentration (reflecting, for instance, a possible effect of ibuprofen on thrombocyte aggregation) was only 0.31 μg/L (range, 0.28 to 0.75 μg/L) in carriers of the *CYP2C9*\*3/\*3 genotype compared with 1.04 μg/L (range, 0.70 to 3.00 μg/L) in carriers of *CYP2C9*\*1/\*1. The maximal inhibition tended to correlate inversely with the enzymatic activity due to the *CYP2C9* genotypes. For thromboxane B<sub>2</sub> (COX-1), this trend was observed for all genotypes, and it was confirmed in the evaluation of C<sub>min</sub> and of AUC (Table V).

Because only surrogate parameters were measured in our study, the question of whether or not the *CYP2C9* polymorphism has any real medical impact cannot be answered conclusively. Nevertheless, the results presented are consistent with the hypothesis that NSAID-induced side effects, such as acute upper intestinal bleeding or renal failure, are probably related to the *CYP2C9* genotype. This hypothesis can be verified only in epidemiologic studies (eg, case-control studies that

include patients who are admitted to hospitals with acute upper intestinal bleeding).

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