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Effect of *CYP2C9*3* Allele on the Pharmacokinetics of Naproxen in Korean Subjects

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The genetically polymorphic CYP2C9 metabolizes many non-steroidal anti-inflammatory agents, including naproxen. This study examined the effects of a *CYP2C9* genetic polymorphism on the pharmacokinetics of naproxen in Korean subjects. Twenty healthy male subjects carrying a *CYP2C9*1/*1* (n=14) or *CYP2C9*1/*3* (n=6) polymorphism were enrolled. After a single-dose of 275 mg naproxen Na, blood samples were collected at various times over a 72 h period and the plasma naproxen concentration was measured. The plasma concentration of naproxen was determined by HPLC analysis with UV detection, and the pharmacokinetic parameters were calculated. The mean plasma concentration-time profiles of naproxen in the *CYP2C9*1/*3* and *CYP2C9*1/*1* individuals were similar. There were no significant differences in the pharmacokinetics of naproxen between *CYP2C9*1/*1* and *CYP2C9*1/*3* genotypes. The $AUC_{0-\infty}$ (p = 0.759) and oral clearance (p = 0.823) of naproxen were also similar in individuals with *CYP2C9*1/*3* and *CYP2C9*1/*1*. Overall, a genetic polymorphism of *CYP2C9* does not significantly affect the pharmacokinetics of naproxen. Therefore, naproxen does not require a dose adjustment for individuals with the *CYP2C9*1/*3* genotype.

Key words : Naproxen, Pharmacokinetics, CYP2C9, Genotype, Allele

Selected by Editors

INTRODUCTION

Cytochrome P_{450} 2C9 (CYP2C9) is one of the main enzymes for the oxidative metabolism of a wide variety of endogenous substances and drugs, including oral hypoglycemic agents, angiotensin II receptor blockers, anticoagulants, antidepressants, diuretics and non-steroidal anti-inflammatory drugs (NSAIDs), such as naproxen (Rendic and Carlo, 1997; Miner and Birkett, 1998). The *CYP2C9* allele shows significant differences in enzyme expression and activity according to the genotype. Therefore, some patients administered drugs affecting CYP2C9 substrates with the usual dose may show unpredictable and adverse side-effects (Xie et al., 2002).

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CYP2C9, a CYP2C9-encoding gene, is highly polymorphic with more than 32 mutant alleles (*CYP2C9*1* to *CYP2C9*32*) in its coding regions thus far (ref. www.cypalleles.ki.se/cyp2c9.htm). Among them, *CYP2C9*2* (C430T/Arg144Cys) and **3* (A1075C/ Ile359Leu) have been recognized as the main variants with lower catalytic activity than the wild type (*CYP2C9*1*). *CYP2C9*2* has a 10-19% allele frequency in Caucasians while it is almost absent in East Asian populations, such as Koreans, Chinese and Japanese (Xie et al., 2002). The allele frequency of *CYP2C9*3* was reported to be 1.1-6.1% in Koreans (Yoon et al., 2001; Bae et al., 2005; Myrand et al., 2008), 1.1-6.8% in Japanese, and 1.7-4.9% in Chinese populations, and only the heterozygous mutant type of the *CYP2C9*3* allele was found in East Asians (Xie et al., 2002).

Naproxen is a member of the arylacetic acid class of NSAIDs that has anti-inflammatory, antipyretic and analgesic effects through the reduction of prostaglandin production by inhibiting cyclooxygenase (DeArmond et al., 1995; Dollery, 1999). Naproxen is commonly used in clinical practice to treat rheumatoid arthritis, ankylosing

spondylitis, osteoarthritis, migraine headache, infectious diseases and dysmenorrhea (Dollery, 1999). It undergoes a single oxidative biotransformation to form 6-desmethyl naproxen as an inactive metabolite (Thomson and Collins, 1973). The enzymes involved in naproxen desmethylation are CYP2C9 and CYP1A2 but there are differences in the relative contribution of each isoform to the formation of 6-desmethyl naproxen (Rodrigues et al., 1996; Miners et al., 1996). CYP2C9 has an approximately 3 times higher activity than CYP1A2. CYP2C9 is involved in 6-desmethyl naproxen formation *in vitro* resulting in the maximum velocity, approximately 6 times higher than that of CYP1A2 (Rodrigues et al., 1996; Miners et al., 1996). Overall, it is expected that CYP2C9 would be approximately 4 times higher contribution than CYP1A2. Moreover, it appears that CYP2C9 plays an important role in naproxen desmethylation, which has also been reported for other NSAIDs (Tracy et al., 1997).

Based on the above reports, it was predicted that the different *CYP2C9* genotypes would show significant differences in the pharmacokinetics of naproxen. Therefore, this study examined the effects of a difference in *CYP2C9* genotype on the pharmacokinetics of naproxen in selected subjects based on previous results, which reported the genotype and allele frequency of *CYP2C9* in a population of 358 Koreans (Bae et al., 2005).

MATERIALS AND METHODS

Subjects

Twenty unrelated healthy male subjects with either *CYP2C9*1/*1* (n=14) or *CYP2C9*1/*3* (n=6) were enrolled in the pharmacokinetic study of naproxen from a total 358 healthy Korean volunteers who had been screened for the *CYP2C9* genotype in a previous study (Bae et al., 2005). The mean age of the subjects in the *CYP2C9*1/**1 and *CYP2C9*1/**3 groups was 22.5 ± 1.7 and 22.2 ± 1.9 years, respectively. The mean body weight in the *CYP2C9*1/*1* and *CYP2C9*1/**3 groups was 66.4 ± 6.7 and 70.7 ± 6.3 kg, respectively. All subjects were in good health, as determined by their medical history, physical examination, electrocardiograms and routine laboratory tests. The subjects were not permitted to consume any medication, alcohol or any caffeine containing beverage throughout the study period.

Study protocol

This study was approved by the Sungkyunkwan University Institutional Review Board (SKKU IRB). All procedures were carried out in accordance with the recommendations of the Declaration of Helsinki on biomedical research involving human subjects, and written informed consent to participate in the study was obtained from all subjects. After the same meal, all subjects were fasting for 10 h before and for 4 h after administering the test drug. A single oral dose (275 mg) of naproxen Na tablet (Anaprox®, Chong Kun Dang, Seoul, Korea) was administered with 200 mL of water to each subject. Blood samples (10 mL) were collected before and at 0.5, 0.75, 1, 1.5, 2, 3, 4, 6, 10, 24, 48 and 72 h after dosing.

Pharmacokinetic study

The plasma concentration of naproxen was measured by high performance liquid chromatography (HPLC) with ultra violet (UV) detection using a slight modification of previously reported methods (Andersen and Hansen, 1992; Lee et al., 2000). Briefly, 250 μL of each plasma sample was mixed with 50 μL of flurbiprofen (400 μg/mL), as an internal standard. Subsequently, 400 μL of acetonitrile and 100 μL of 50% *ortho*-phosphoric acid were added to the mixture. After centrifuging the mixture, a 45 μL aliquot of the supernatant was injected into the HPLC autosampler and analyzed. The mobile phase consisted of 20 nM potassium phosphate dibasic anhydrous and acetonitrile at a volume ratio of 60 to 40 (pH 3.0 with *ortho*-phosphoric acid). Chromatography was carried out on a Shiseido Capcell Pak C_{18} UG120 5 μm (4.6 × 150 nm). The flow rate of naproxen was 2 mL/min. Quantification was carried out using a UV detector at 238 nm. A good linear relationship was obtained in the range of 5 to 100 μg/mL with a detection limit of 0.5 μg/mL. The inter- and intra- assay coefficients of variation for reproducibility ranged from 1.2 to 1.7%.

Data analysis

The individual values for the pharmacokinetic parameters (C_{max}, T_{max}, CL/F, $t_{1/2}$, $AUC_{0-\infty}$) of naproxen were determined by standard non-compartmental analysis using BA calc 2002 (KFDA, Seoul, Korea) (Kiang et al., 1989). The naproxen $AUC_{0-\infty}$ was calculated using the log-linear trapezoidal method. The half-life ($t_{1/2}$) was calculated from $\ln_2/k_e$. The oral clearance was determined by dividing the naproxen dose by the calculated $AUC_{0-\infty}$.

Statistical analysis

All the data are expressed as the mean ± standard deviation (SD). All parameters between the *CYP2C9* genotypes were compared using a one-way analysis of variance (ANOVA) (SigmaStat ver 2.03). A p-value < 0.05 was considered significant.

RESULTS

Table I lists the characteristics of the 20 subjects. The subjects were all males; 14 subjects had the *CYP2C9*1/*1* genotype and 6 subjects carried 1 mutant **3* allele

Table I. Subjects' characteristics

Genotype	*CYP2C9*1/*1*	*CYP2C9*1/*3*
N	14	6
Age (years)	22.5 ± 1.7	22.2 ± 1.9
Weight (kg)	66.4 ± 6.7	70.7 ± 6.3
Height (cm)	173.8 ± 6.0	175.0 ± 4.3

The data is expressed as the mean ± S.D.

(*CYP2C9*1/*3*).

Fig. 1 shows the plasma concentration-time profiles of naproxen between the *CYP2C9*1/*1* and *CYP2C9*1/*3* genotypes. There were no significant differences in any of the pharmacokinetic parameters of naproxen between the genotypes (Table II, Fig. 2). The $AUC_{0-\infty}$ (750.68 ± 134.45 μg/mL·h *vs.* 769.93 ± 102.50 μg/mL·hr, $p = 0.759$) and oral clearance (0.383 ± 0.069 L/h *vs.* 0.377 ± 0.040 L/hr, $p = 0.823$) were similar in the *CYP2C9*1/*3* and *CYP2C9*1/*1* groups. The plasma half-life of naproxen was also similar in the *CYP2C9*1/*3* and *CYP2C9*1/*1* groups (17.86 ± 3.14 h *vs.* 17.64 ± 2.33 h, $p = 0.881$). In addition, the *CYP2C9*1/*3*

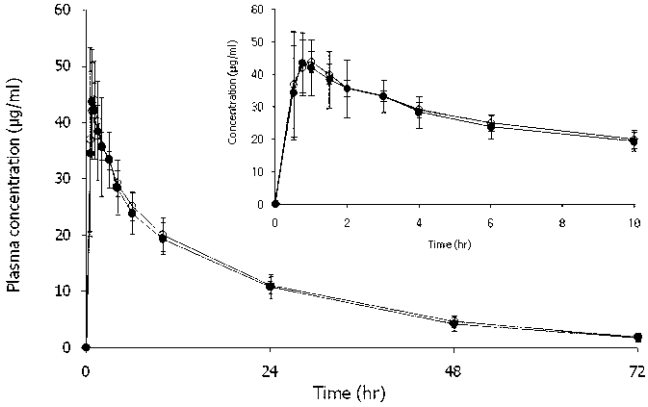

Fig. 1. The mean plasma concentration-time curve of naproxen in 14 and 6 subjects with *CYP2C9*1/*1* (*closed circles*) and *CYP2C9*1/*3* (*open circles*), respectively

Table II. Pharmacokinetic parameters of naproxen on *CYP2C9* genotypes

PK parameters	Genotypes		*P* value
	*CYP2C9*1/*1* (n=14)	*CYP2C9*1/*3* (n=6)	
$AUC_{0-\infty}$ (μg/mL·h)	750.68 ± 134.45	769.93 ± 102.50	0.759
CL/F (L/h)	0.383 ± 0.069	0.377 ± 0.040	0.823
C_{max} (μg/mL)	46.73 ± 6.25	45.75 ± 3.90	0.726
T_{max} (hr)	0.84 ± 0.65	0.71 ± 0.19	0.637
$T_{1/2}$ (hr)	17.86 ± 3.14	17.64 ± 2.33	0.881

The data is expressed as the mean ± S.D.

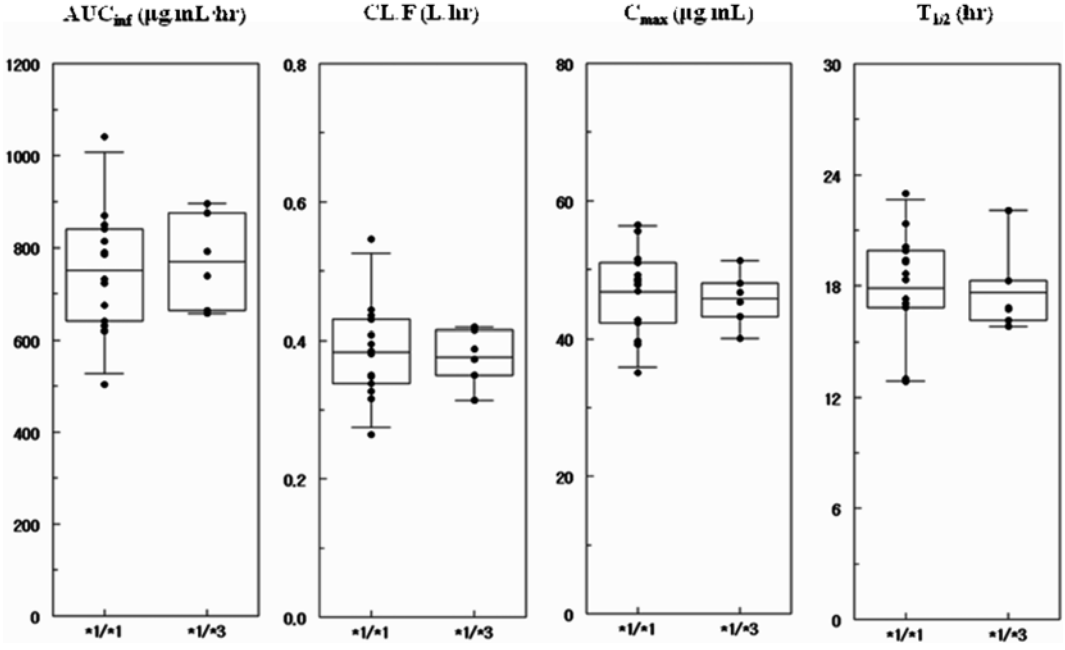

Fig. 2. The individual pharmacokinetic parameters of naproxen in 14 and 6 subjects with *CYP2C9*1/*1* and *CYP2C9*1/*3*, respectively

and *CYP2C9*1/*1* groups showed a similar C_{max} (46.73 ± 6.25 μg/mL *vs*. 45.75 ± 3.90 μg/mL, $p = 0.726$) and T_{max} (0.84 ± 0.65 hr *vs*. 0.71 ± 0.19 hr, $p = 0.637$).

DISCUSSION

The *CYP2C9* genotype may be a clinically relevant risk factor because most non-steroidal anti-inflammatory drugs (NSAIDs) are metabolized by CYP2C9 *in vitro*. Many *in vivo* studies have reported that the heterozygous type of the *CYP2C9*3* allele affects the pharmacokinetic parameters of NSAIDs (Lee et al., 2003; Liu et al., 2006; perini et al., 2005). In addition, Sandberg et al. (2002) examined the livers from a larger set of organ donors, and demonstrated that the rate of celecoxib hydroxylation was lower (~50%) in the livers with the *CYP2C9*1/*3* genotype than that of *CYP2C9*1/*1*. This is the first report describing the effects of a genetic polymorphism of *CYP2C9* on the pharmacokinetics of naproxen in Korean subjects.

A commonly used NSAID, naproxen, is also metabolized by CYP2C9 to the inactive metabolite 6-desmethyl naproxen. Therefore, theoretically, a *CYP2C9* genetic polymorphism should affect the pharmacokinetics of naproxen. However, there were no significant differences in the pharmacokinetics of naproxen between the *CYP2C9*1/*1* and *CYP2C9*1/*3* genotypes. Although the impact of the heterozygote for *CYP2C9*3* is somewhat lower than that of the homozygote for *CYP2C9*3* (Kidd et al., 1999), there are many reports showing that the *CYP2C9*1/*3* genotype has a significant effect on the pharmacokinetic parameters compared with *CYP2C9*1/*1* (Vianna-Jorge et al., 2004; Liu et al., 2006; Suzuki et al., 2006; Chen et al., 2006; Becker et al., 2008). Similar observations have been reported with other NSAIDs (e.g. ketoprofen and sulindac) (Foster et al., 1988; Hamman et al., 2000). It is possible that there is some substrate specificity between NSAIDs. Another possible explanation is a minor metabolism or elimination pathway by CYP2C9. A large fraction (60%) of a naproxen dose is recovered as a product of direct glucuronidation. The desmethyl metabolite, which is formed by CYPs, can account for an additional 20% of the dose recovered in urine. Even if the metabolite accounted for the entire dose recovered in bile (~20%), the contribution of CYP2C9 dependent oxidation to the overall clearance of naproxen would not exceed 40% of the dose. Other CYPs, such as CYP2C8 and CYP1A2 are also involved (Rodrigues et al., 1996; Miners et al., 1996; tracy et al., 1997). Overall, CYP2C9 plays a relatively minor role in the overall clearance of naproxen (Rodrigues, 2005).

The major limitation of this study was the small sample size of *CYP2C9*1/*3* genotype subjects. However, it is believed that an additional study will not be necessary to confirm this result due to the very similar individual pharmacokinetic parameters of naproxen in both groups.

In conclusion, naproxen, in contrast to most NSAIDs, is not affected by the *CYP2C9* genotype. While most available NSAIDs are affected by CYP2C9 and the *CYP2C9* genotype may be considered a clinically relevant risk factor, naproxen can be used as a safe NSAID without the need for a dose adjustment in those carrying the *CYP2C9*3* allele.

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