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Effects of Pregnane X Receptor (*NR1I2*) and CYP2B6 Genetic Polymorphisms on the Induction of Bupropion Hydroxylation by Rifampin

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ABSTRACT:

We investigated genetic polymorphisms in the pregnane X receptor (*NR1I2*) in Korean individuals (n = 83) and the effects of *NR1I2* genotypes on rifampin-mediated induction of bupropion hydroxylation. The pharmacokinetics of bupropion and hydroxybupropion were evaluated after an oral dose of bupropion (150 mg) administered before and after rifampin treatment for 7 days in 35 healthy subjects. The area under the time-concentration curve (AUC) ratio of hydroxybupropion to bupropion in *CYP2B6*6* carriers was significantly lower than that in *CYP2B6*6* noncarriers in both the basal and rifampin-induced states (p = 0.012). Among the *CYP2B6*6* carriers (n = 13), the *NR1I2* TGT (−25385T + g.7635G + g.8055T) carriers exhibited a significantly lower AUC ratio, representing the CYP2B6 hydroxylation activity, compared with the TGT noncarriers, in the induced state (11.9 versus 20.3, p = 0.045). The percent difference in the AUC ratio between the basal and induced states was also significantly different (212% versus 58.8%, p = 0.006). However, no significant difference was observed among the *NR1I2* TGT genotypes for the *CYP2B6*6* noncarriers (n = 22). In conclusion, it is suggested the *NR1I2* TGT genotype decreases the bupropion hydroxylation induced by treatment with rifampin, particularly in *CYP2B6*6* carriers.

Introduction

The pregnane X receptor (PXR), also known as the steroid and xenobiotic receptor or pregnane-activated receptor, is an orphan nuclear receptor family member and is encoded by the nuclear receptor subfamily 1 (*NR1I2*) gene (Bertilsson et al., 1998; Blumberg et al., 1998; Lehmann et al., 1998). After ligand binding, PXR forms a heterodimer with the retinoid X receptor (*NR1B*), which then binds to PXR response elements located in the 5′-flanking regions of PXR target genes, resulting in transcriptional activation (Rosenfeld et al., 2003). PXR is a master transcriptional regulator of many important genes involved in xenobiotic metabolism and transport, including genes encoding cytochromes P450 (e.g., *CYP3A4*, *CYP3A5*, *CYP2B6*, *CYP2C19*, and *CYP2C8*) and transporters [e.g., *MDR1* (*ABCB1*), *MRP2* (*ABCC2*), and *OATP2* (*SLCO1B1*)] (Lamba et al., 2005).

Genetic polymorphisms in *NR1I2* may explain the interindividual variability of PXR activity, which affects the disposition and interaction of various drugs via an induction mechanism. Most previous studies have reported that variant alleles of *NR1I2* are associated with decreased rifampin-mediated CYP3A4 induction (Hustert et al., 2001; Koyano et al., 2004; Lim et al., 2005; Wang et al., 2007), but increased transcriptional activation of PXR was observed at single nucleotide polymorphisms (SNPs) of −25385C>T, −24113G>A, g.7635A>G, or g.8055C>T (Zhang et al., 2001). However, the clinical consequences of these polymorphisms have not been consistently validated, and clinically important variation has not yet been demonstrated by well controlled clinical pharmacogenetic studies.

CYP2B6 plays a primary role in the metabolism of therapeutic drugs such as cyclophosphamide, bupropion, and efavirenz (Chang et al., 1993; Kirchheiner et al., 2003; Ward et al., 2003). CYP2B6 comprises less than 1% of the total liver microsomal cytochrome P450 content and is expressed in various extrahepatic tissues, including the brain (Shimada et al., 1994). The CYP2B6 enzyme is highly polymorphic, and its genetic polymorphisms have been discovered (Lang et al., 2001). The common allele *CYP2B6*6* (c.516G>T and c.785A>G) leads to aberrant splicing of pre-mRNA, resulting in reduced expression of functional transcript and protein in human liver (Hofmann et al., 2008). Associations between *CYP2B6* genetic polymorphisms and pharmacokinetics have been reported for bupropion (Kirchheiner et al., 2003), thiotepa (Ekhart et al., 2009), and efavirenz (Rotger et al., 2007).

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ABBREVIATIONS: PXR, pregnane X receptor; SNP, single-nucleotide polymorphism; PK, pharmacokinetics; PCR, polymerase chain reaction; AUC_bup, area under the time-concentration curve of bupropion; AUC_hyd, area under the time-concentration curve of hydroxybupropion; CAR, constitutive androstane receptor; bp, base pair.

Bupropion, an atypical antidepressant and smoking cessation aid, was reportedly used as a selective marker of CYP2B6 activity in previous studies (Faucette et al., 2000). A representative PXR agonist, rifampin, has been shown to induce CYP2B6 activity as evidenced by significantly increased bupropion hydroxylation after rifampin treatment (Loboz et al., 2006). In that study, subjects with *CYP2B6*6/*6* or *CYP2B6*1/*6* exhibited decreased bupropion clearance relative to that of subjects with *CYP2B6*1/*1* after rifampin treatment, whereas clearance in the absence of rifampin treatment was similar (Loboz et al., 2006). On the basis of this preliminary result, rifampin-induced CYP2B6 activity was suggested to be associated with both the existence of the *CYP2B6*6* allele and certain other factors such as genetic polymorphism of PXR.

Both *CYP2B6* and *NR1I2* polymorphisms may be associated with the clinical pharmacokinetics and/or drug-drug interactions of bupropion; however, there have been no clinical pharmacogenetic studies of CYP2B6 substrates in the rifampin-induced state in relation to the PXR variants. Therefore, we investigated the genetic polymorphisms in PXR and examined the relationship between these genetic polymorphisms and metabolic induction of bupropion by rifampin administration in humans.

Materials and Methods

Subjects. Thirty-five unrelated Korean subjects participated in a clinical pharmacokinetics (PK) study of bupropion. All subjects were healthy, as determined by medical history, physical examination, vital signs, 12-lead ECG, drug screening, and routine clinical laboratory tests performed within 3 weeks of the start of the study. Regular heavy drinkers, smokers of more than 10 cigarettes/day, and those with a body weight differing by more than 20% from their ideal weight were excluded.

To survey *NR1I2* genetic polymorphisms in the Korean population, a total of 83 individual samples from subjects who had donated blood, including the patients in this PK study, were genotyped. *CYP2B6* genotypes were divided into *CYP2B6*6* carrier and noncarrier groups using methods described previously (Jinno et al., 2003; Hofmann et al., 2008). This study was approved by the Institutional Review Board of Seoul National University Hospital, Seoul, Korea. All procedures were performed in accordance with the recommendations of the Declaration of Helsinki on biomedical research involving human subjects and with the International Conference on the Harmonization of Technical Requirements for the Registration of Pharmaceuticals for Human Use–Good Clinical Practice guidelines. Written informed consent for participation in the study and genotyping was obtained from all subjects before enrollment.

Study Design. The subjects were admitted to the clinical research ward of the Clinical Trials Center on the night before bupropion administration. After an overnight fast, subjects were given a single dose of 150 mg of bupropion (Wellbutrin SR tablet; GlaxoSmithKline Korea, Seoul, Korea) with 240 ml of water at 9:00 AM on day 1. Subjects fasted for 4 h after drug administration, except for drinking water 2 h after dosing. Venous blood samples for PK analysis (8 ml) were collected using an intravenous catheter before dosing and 0.5, 1, 1.5, 2, 3, 4, 5, 6, 7, 9, 12, 24, 30, and 36 h after dosing. Alcohol, soft drinks, smoking, drugs, and caffeine-containing beverages were prohibited during the study. After 7 days of washout, subjects were given 600 mg of rifampin (Rifodex tablet; ChongKunDang Pharmaceutical Corp., Seoul, Korea) every morning, on an outpatient basis, on days 8 to 14. On day 15, after overnight fasting, subjects were given 150 mg of bupropion in the same manner as described for day 1.

Genotyping. *NR1I2.* Genomic DNA was extracted from peripheral whole blood using a QIAamp DNA Blood Mini Kit (QIAGEN GmbH, Hilden, Germany). Genotyping was done using TaqMan allelic discrimination assays on an AB 7500 Real-Time PCR System (Applied Biosystems, Foster City, CA). Ten microliters of PCR mixture were prepared with 5 μl of 2× TaqMan Genotyping Master Mix, 0.5 μl of 20× Drug Metabolism Genotyping Assay Mix, 3.5 μl of DNase-free water, and 1 μl of genomic DNA. Genotyping for *NR1I2* −25385C>T (rs3814055, assay no. C_27504984_30), *NR1I2* −24113G>A (rs2276706, assay no.: C_15882316_10), *NR1I2* g.7635A>G (rs6785049, assay no.: C_29280426_10), and *NR1I2* g.8055C>T (rs2276707, assay no.: C_15882324_10). Detection of SNPs was performed with validated TaqMan genotyping assays purchased from Applied Biosystems. The PCRs were as follows: initial denaturation at 95°C for 10 min, followed by 50 cycles of denaturation at 92°C for 15 s and annealing/extension at 60°C for 1 min. The allelic discrimination results were determined after amplification by performing an endpoint read. AB Sequence Detection System (SDS) 7500 software (version 1.4; Applied Biosystems) was used for the analysis.

DNA direct sequencing. To identify *NR1I2* −24020[GAGAAG]>(−) (rs3842689) sequencing was applied. The PCR method was used to amplify one *NR1I2* fragment with University of California Santa Cruz In-Silico PCR (http://genome.ucsc.edu/cgi-bin/hgPcr?command = start). The final volume of the PCR was 10 μl, consisting of 10 ng of DNA, 0.5 μM concentrations of each primer pair, 0.25 mM dNTPs, 3 mM $MgCl_2$, 1 μl of 1× reaction buffer, and 0.25 U Taq DNA polymerase (Intron Biotechnology, Seongnam-si, Gyeonggi-do, Korea). The PCR products were purified and sequenced using a BigDye Terminator Cycle Sequencing kit and an ABI 3730xl automated sequencer (Applied Biosystems). The sequencing primers were the same as those used for the PCR amplification. Mutation analyses were performed using Phred, Phrap, Consed, and PolyPhred 5.04 software (http://droog.mbt.washington.edu/PolyPhred.html).

CYP2B6. Genomic DNA was prepared from peripheral blood samples using the nucleic acid isolation device QuickGene-Mini80 (Fujifilm, Tokyo, Japan). To determine the presence of *CYP2B6*4* (c.785A>G only) and *CYP2B6*6* (c.516G>T and c.785A>G), DNA proximal to two polymorphic sites, c.785A>G (rs2279343) and c.516G>T (rs3745274), was amplified by PCR, followed by SNaPShot analysis according to the manufacturer's instructions (ABI PRISM SNaPShot Multiplex kit). The sequences of forward and reverse primers and probes were 5′-CTTTCTTGCAGCTGTTTG-3′, 5′-CCTCTGTCTTTCATTCTGTC-3′, and 5′-GGTAGGTGTCGATGAGGTCC-3′ for c.785A>G and 5′-CGTGACGTGCTGGTACA-3′, 5′-CTCCATGTCCCTGATTCTT-3′, and 5′-AGATGATGTTGGCGGTAATGGA-3′ for c.516G>T, respectively. The validity of the method was confirmed by sequencing.

Drug Concentration Analysis. The plasma concentrations of bupropion and hydroxybupropion were determined by high-performance liquid chromatography with ultraviolet detection using a modification of a previously described method (Jennison et al., 1995). Standards of bupropion and hydroxybupropion were purchased from BD Gentest (Woburn, MA), and trazodone as an internal standard was obtained from Sigma-Aldrich (St. Louis, MO). One milliliter of plasma was extracted with 10 ml of *n*-heptane-isoamyl alcohol (1000:15, v/v) including 25 μg of trazodone as an internal standard after the addition of 150 μl of 0.1 M hydrochloride. The organic phase was evaporated, reconstituted with 100 μl of the mobile phase, and injected into a high-performance liquid chromatography system (Gilson, Villiers-le-Bil, France). A mobile phase of 50 mM KH_2PO_4-acetonitile (810:190, v/v, pH 2.5) was used at a flow rate of 1.0 ml/min through a Capcell Pak C18 column (150 × 4.6-mm i.d., 5.0-mm particle size; Shiseido, Tokyo, Japan). The ultraviolet detector was set to monitor 214 nm. The lower limit of quantification for bupropion and hydroxybupropion was 2 and 10 ng/ml, respectively, and the calibration curves for bupropion and hydroxybupropion were linear from the lower limit of quantification to 1000 ng/ml (correlation coefficient >0.997). The accuracy was 91.9 to 106.1% for bupropion and 100.6 to 111.5% for hydroxybupropion, whereas the interbatch coefficients of variation were <7.7 and <9.2% for bupropion and hydroxybupropion, respectively.

Pharmacokinetics and Statistical Analysis. PK parameters were calculated using the actual sampling times. Maximum plasma drug concentrations (C_{max}) were determined from the observed values. Plasma concentrations for the terminal phase were fitted to a log-linear line using the least-squares method to obtain the terminal half-life ($t_{1/2}$). Areas under the time-concentration curve for bupropion (AUC_bup) and hydroxybupropion (AUC_hyd) were calculated using a combination of the trapezoidal rule from 0 to 36 h. AUC_hyd was divided by AUC_bup for each period, representing the activity of metabolite formation of bupropion to hydroxybupropion (formation clearance divided by metabolite clearance). Percent differences in the PK parameters between the basal and rifampin-induced states were used to assess the induction effect of rifampin treatment and were calculated as 100 × (induced − basal)/basal. WinNonlin (version 5.2; Pharsight, Mountain View, CA) was used for the PK analysis.

TABLE 1

Demographic data and CYP2B6 genotypes in the subjects of the NR1I2 TGT haplotype groups

	TGT Noncarrier (n = 22)	TGT Carrier (n = 13)	p
No. of subjects			
Male	16	5	0.0751[a]
Female	6	8	
Age (yr)	22.7 ± 1.6	23.2 ± 2.3	0.9715[b]
Weight (kg)	65.0 ± 10.1	59.5 ± 11.5	0.0941[b]
CYP2B6 genotype (n)			
**1/*1* (19) + **1/*4* (3)	13	9	0.6638[a]
**1/*6* (11) + **6/*6* (2)	9	4	

[a] Fisher's exact test.
[b] Wilcoxon rank-sum test between the two groups.

Considering the small and uneven number of subjects, comparisons of parameters between the two groups was conducted nonparametrically using the Wilcoxon rank-sum test. Comparisons of parameters between the metabolic induced state and the basal state for each subject were conducted using a paired t test. Multiple linear regression analysis was applied to evaluate the significance of effects due to genotype, gender, and body weight and their contributions to the total observed variability. $p < 0.05$ was considered statistically significant. Linkage disequilibrium and haplotype assembly analysis were performed using the Haploview 4.1 program (Broad Institute of Harvard and MIT, Cambridge, MA), on the basis of a standard expectation maximization algorithm, to reconstruct individual haplotypes from the population genotype data.

Results

Genetic Polymorphisms of *NR1I2* in the Korean Population. The frequencies of the *NR1I2* alleles −25385C, −24113G, −24020[GAGAAG], g.7635A, and g.8055C in the 83 samples were 0.681, 0.681, 0.681, 0.518, and 0.590, respectively. The distribution of the genotypes was consistent with Hardy-Weinberg equilibrium ($p > 0.05$, χ^2 test). *NR1I2* −25385C>T, −24113G>A, and −24020[GAGAAG]>(−) displayed 100% linkage ($r^2 = 1.0$), and a significant linkage ($r^2 = 0.746$) was found between g.7635A>G and g.8055C>T. SNPs in positions −25385, g.7635, and g.8055 resulted in six haplotypes: CAC, CGT, TGT, TAC, CGC, and TGC, with population frequencies of 0.428, 0.211, 0.199, 0.090, 0.042, and 0.030, respectively. The CAC haplotype was the most common and was regarded as the wild-type allele, and the CGT and TGT haplotypes were the primary variant alleles, based on their allele frequencies. Considering that the −25385C>T SNP has been reported previously to exhibit altered transcriptional activity (Zhang et al., 2001; Wang et al., 2009), the subjects were divided into TGT carrier (n = 13) and noncarrier (n = 22) groups, based on the existence of the *NR1I2* TGT haplotype, as well as the −25385C>T SNP.

Results of the Clinical Study. The demographic characteristics of the subjects and the distributions of the *CYP2B6* genotypes were not significantly different among the *NR1I2* haplotypes (Table 1). After rifampin treatment, AUC_bup markedly decreased and AUC_hyd/AUC_bup, which represents the metabolic activity of bupropion into hydroxybupropion, significantly increased ($p < 0.001$, paired t test). The percent differences were significantly greater in the *NR1I2* TGT noncarriers (228%) than in the TGT carriers (169%) ($p = 0.018$). AUC_hyd in the TGT noncarriers did not differ between the basal and induced states (0.43%, $p = 0.464$), whereas in the TGT carriers it was significantly decreased in the induced state (−24.8%, $p = 0.0002$). As a result, there were significant differences among the *NR1I2* genotypes in terms of the percent difference in AUC_hyd between the basal and induced states ($p < 0.001$). These findings are consistent with the results grouped by the −25385TT genotypes (Table 2). However, there was no significant difference in AUC_bup, AUC_hyd, or their ratios between the *NR1I2* TGT carriers and noncarriers or between the −25385CC and CT groups in either the basal or induced metabolic states (Table 2).

The effects of the *CYP2B6* and *NR1I2* genotypes were considered together and are shown by subgroup in Table 3. The *CYP2B6*6* carriers had significantly lower AUC_hyd and AUC_hyd/AUC_bup compared with the *CYP2B6*6* noncarriers in both the basal and induced states. The percent differences in AUC_hyd after rifampin treatment were significantly different among the *NR1I2* genotypes, regardless of the *CYP2B6* genotype.

There were no significant differences found in AUC_hyd/AUC_bup among the *NR1I2* TGT genotypes for the *CYP2B6*6* noncarriers. In contrast, for the *CYP2B6*6* carriers, the *NR1I2* TGT carriers had a significantly lower AUC_hyd/AUC_bup than the TGT noncarriers in the induced state (11.9 versus 20.3, $p = 0.045$), and the percent difference after rifampin induction was significantly different (212% versus 58.8%, $p = 0.006$) (Table 3). Individual plots of AUC_hyd/AUC_bup by *CYP2B6* and *NR1I2* genotypes for each period indicate much lower increases for the *NR1I2* TGT + *CYP2B6*6* carriers (Fig. 1).

The concentration-time profiles for bupropion were similar regardless of genotype for both metabolic states. However, the profiles for hydroxybupropion were different for the *CYP2B6*6* + *NR1I2* TGT carriers than for the other groups, with lower values in the basal state and much lower values in the induced state (Fig. 2). The C_{max} values of bupropion and hydroxybupropion in the basal and induced states were not significantly different between the *NR1I2* TGT carriers and noncarriers (data not shown). In the basal state, the hydroxybupropion

TABLE 2

Effects of NR1I2 genotypes on the rifampin-mediated metabolic induction of bupropion hydroxylation

	TGT Noncarrier (n = 22)	TGT Carrier (n = 13)	p^a	−25385CC (n = 17)	−25385CT (n = 17)	−25385TT (n = 1)	p^b
AUC_bup (ng · h/ml)							
Basal	1274 ± 324	1194 ± 315	0.585	1260 ± 350	1247 ± 294	929	0.5525
Induced	435 ± 196	406 ± 176	0.785	418.2 ± 212.5	432.6 ± 168.1	370	0.8505
%difference[c]	−66.7 ± 10.4*	−65.1 ± 15.8*	0.960	−67.8 ± 11.2*	−64.8 ± 14.1*	−60.2	0.607
AUC_hyd (ng · h/ml)							
Basal	9343 ± 3118	11,558 ± 4568	0.204	9290 ± 3276	10,695 ± 4112	16,053	0.231
Induced	9003 ± 2435	8541 ± 3304	0.489	8840 ± 2413	8837 ± 3202	8577	0.941
%difference	0.43 ± 23.3	−24.8 ± 11.8*	**<0.001**	−0.78 ± 20.2	−14.8 ± 23.2*	−46.6	**0.017**
AUC_hyd/AUC_bup							
Basal	7.7 ± 3.1	10.3 ± 4.6	0.088	7.8 ± 3.4	9.0 ± 3.9	17.3	0.195
Induced	26.0 ± 19.2	27.3 ± 17.0	0.759	27.9 ± 21.5	25.3 ± 15.3	23.2	0.978
%difference	228 ± 95*	169 ± 176*	**0.018**	232 ± 98.3*	182 ± 153*	34.2	**0.035**

* $p < 0.01$, paired t test between the basal and induced states.
[a] Wilcoxon rank-sum test for the *NR1I2* groups.
[b] Kruskal-Wallis test for −25385C>T groups; $p < 0.05$ indicated in bold.
[c] %difference represents the percent difference between basal and induced state, calculated as 100 × (induced − basal)/basal.

TABLE 3

Effects of the CYP2B6 genotype and NR1I2 genotypes on rifampin-mediated metabolic induction of bupropion hydroxylation

	*CYP2B6*6* Noncarrier (n = 22)		P^a	*CYP2B6*6* Carrier (n = 13)		p^a	p^b
	TGT Noncarrier (n = 13)	TGT Carrier (n = 9)		TGT Noncarrier (n = 9)	TGT Carrier (n = 4)		
AUC_bup (ng · h/ml)							
Basal	1307 ± 318	1187 ± 308	0.333	1227 ± 347	1209 ± 376	0.877	
	1257 ± 312			1222 ± 340			0.539
Induced	437 ± 188	344 ± 161	0.243	430.6 ± 216.7	545.2 ± 132.3	0.165	
	399 ± 180			466 ± 197			0.306
%difference[c]	67.2 ± 10.8	70.8 ± 12.2	0.512	65.8 ± 10.4	52.3 ± 16.8	0.199	
	68.7 ± 11.3			61.7 ± 13.7			0.649
AUC_hyd (ng · h/ml)							
Basal	10,465 ± 3112	12,796 ± 4007	0.324	7723 ± 2449	8776 ± 5073	0.940	
	11,419 ± 3609			8047 ± 3269			**0.004**
Induced	9849 ± 1923	9756 ± 3050	0.845	7782 ± 2680	5808 ± 2117	0.199	
	9811 ± 2378			7174 ± 2609			**0.013**
%difference	0.22 ± 28.0	−23.3 ± 10.5	**0.021**	0.76 ± 15.8	-28.1 ± 15.6	**0.011**	
	−9.39 ± 25.1			−8.13 ± 20.5			0.468
AUC_hyd/AUC_bup							
Basal	8.6 ± 3.5	11.3 ± 3.8	0.066	6.5 ± 2.0	8.0 ± 6.2	0.758	
	9.7 ± 3.8			6.9 ± 3.6			**0.012**
Induced	30.0 ± 23.5	34.1 ± 15.5	0.333	20.3 ± 8.9	11.9 ± 7.7	**0.045**	
	31.7 ± 20.3			17.7 ± 9.2			**0.012**
%difference	228 ± 107	218 ± 192	0.262	212 ± 78	58.8 ± 41.9	**0.006**	
	225 ± 143			164 ± 99.7			0.287

$p < 0.05$ is indicated in bold.
[a] Wilcoxon rank-sum test for the *NR1I2* groups within each *CYP2B6* genotype group.
[b] Wilcoxon rank-sum test for the *CYP2B6* groups.
[c] %difference represents the percent difference between basal and induced state, calculated as 100 × (induced − basal)/basal.

C_{max} for the TGT carriers (499.2 ± 190.9 ng/ml) was slightly higher than that for the TGT noncarriers (382.9 ± 134.3 ng/ml, $p = 0.088$).

Multiple linear regression analysis of the percent difference of AUC_hyd/AUC_bup, including the demographic and genetic factors, showed that only the *NR1I2* TGT haplotype had a significant effect ($p = 0.0049$) and explained 22.2% ($r^2 = 0.222$) of the total variability. The r^2 value increased to 0.356 when the model included body weight, the *CYP2B6* genotype, or gender variables (partial $r^2 = 0.061$, 0.048, and 0.025, respectively).

Discussion

We investigated genetic polymorphisms in *NR1I2* in the Korean population. Allele frequencies of −25385C>T and −24020 6-base pair deletion were similar to those in other ethnic groups and frequencies of g.7685A>G and g.8055C>T were slightly higher than those of Caucasian and African-American populations. Strong linkage among the −25385, −24113, and −24020 SNPs was identified, as reported previously (Zhang et al., 2001; Uno et al., 2003; Lamba et al., 2005; Wang et al., 2007).

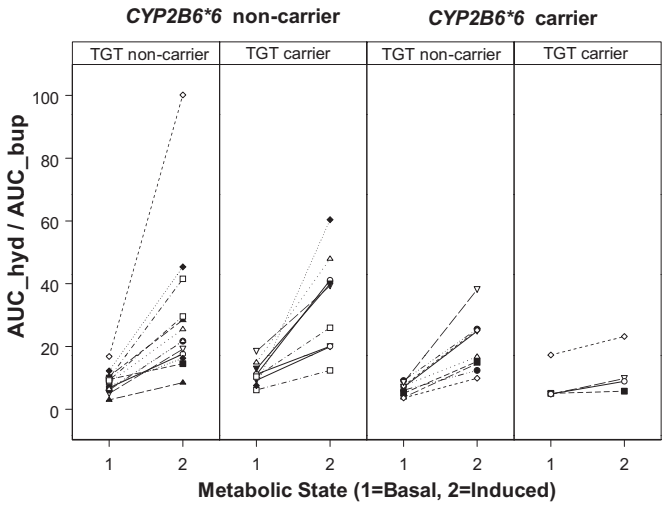

FIG. 1. Individual subject profiles showing AUC_hyd/AUC_bup ratios for the basal and rifampin-induced states in the *NR1I2* and *CYP2B6* genotype groups (n = 35).

We found that the percent differences in AUC_hyd and AUC_hyd/AUC_bup after rifampin treatment were significantly different between the *NR1I2* TGT carriers and noncarriers. These differences were highly significant in the *CYP2B6*6* carriers, with the lowest value observed in the *NR1I2* TGT carrier group (58%, the percent increase of the AUC_hyd/AUC_bup). However, significant differences were not observed among the *CYP2B6*6* noncarriers. These findings support the hypothesis that the increased metabolism of bupropion with rifampin treatment is affected by both *NR1I2* and *CYP2B6* genetic polymorphisms. Specifically, it can be postulated that reduced metabolic capacity is more pronounced in individuals with both the *CYP2B6*6* and reduced function PXR alleles.

Because Zhang et al. (2001) reported that several variant *NR1I2* genotypes exhibited increased induction activity after rifampin treatment, various studies have suggested certain effects of PXR variants, despite inconsistent results. Higher mRNA levels of intestinal CYP3A4 were associated with g.7635G and g.8055T, and greater induction of CYP3A activity was found in −25385TT subjects than in −25385CC subjects, as measured by an erythromycin breath test (Zhang et al., 2001). In addition, a recent report showed that the *NR1I2* haplotype, including TGT, was associated with weaker basal activity but greater inducible transcriptional activity of CYP3A4, based on a PK study of nifedipine (Wang et al., 2009). However, these studies were performed with CYP3A4 substrates, a very small sample size (n = 3), and limited in vivo PK information regarding the substrate drugs, and, in addition, their associated mechanism has not been experimentally demonstrated. Consistent with our findings, most studies have suggested that the same PXR genetic variants exhibited reduced PXR transcriptional activity. A 6-base pair (bp) deletion in intron 1a (−24020), which was linked

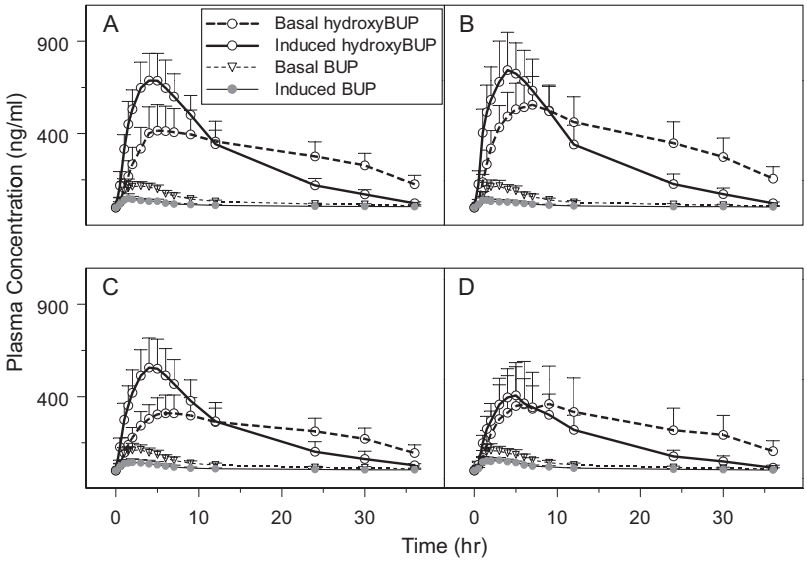

FIG. 2. Mean plasma concentration-time profiles of bupropion (BUP) and hydroxybupropion (hydroxyBUP) after oral administration of 150 mg of bupropion in the *NR1I2* and *CYP2B6* genotype groups: A, *CYP2B6*6* noncarrier + *NR1I2* TGT noncarrier; B, *CYP2B6*6* noncarrier + *NR1I2* TGT carrier; C, *CYP2B6*6* carrier + *NR1I2* TGT noncarrier; and D, *CYP2B6*6* carrier + *NR1I2* TGT carrier. The dashed and solid lines indicate basal and rifampin-induced states, respectively.

completely to −25385C>T and −24113G>A SNPs in our study, has been reported to be associated with a total loss of transcriptional activity when expressed in the HepG2 cell line (Uno et al., 2003). This observation is consistent with the results for one of our subjects with −25385TT, who had the lowest percent increase (34.2%) in AUC_hyd/AUC_bup after rifampin induction. In patients with primary sclerosing cholangitis, which is caused by abnormal bile acid detoxification, the rs6785049 (g.7635A>G) GG group exhibited a reduced survival rate associated with decreased transcription activity of PXR (Karlsen et al., 2006). Another example of clinically decreased PXR transactivation in a PXR variant was shown in patients with breast cancer who took doxorubicin. *PXR*1B* haplotypes, which consisted of g.2654T>C and IVS6-17C>T polymorphisms, were found to be associated with significantly decreased hepatic mRNA expression of PXR and its downstream target genes, *CYP3A4* and *ABCB1*. The *PXR*1B* haplotype was also significantly associated with reduced clearance of doxorubicin (Sandanaraj et al., 2008).

In the basal state, the mean value of AUC_hyd/AUC_bup was somewhat higher in the *NR1I2* TGT carriers than noncarriers (more prominently in the *CYP2B6*6* noncarriers, $p = 0.066$). The reason for this is unknown, but this phenomenon was also reported in an in vitro study of PXR.2 expression. PXR.2 is an alternatively spliced form of PXR that lacks 111 nucleotides encoding 37 amino acids in the ligand-binding domain. Basal CYP2B6 expression levels are increased in untreated PXR.2 cells: however, CYP2B6 induction activity decreased after treatment with rifampin (Lin et al., 2009). Other effects may be linked to the *NR1I2* TGT haplotype and basal metabolic activity of CYP2B6. For example, impaired transactivation of PXR may initiate other induction pathways, leading to compensation or overcompensation, depending on the circumstances.

Our results suggest that the *CYP2B6*6* allele is associated with reduced metabolic biotransformation regardless of the *NR1I2* genotype. In vitro studies of the CYP2B6 substrates, bupropion and efavirenz, have demonstrated that the **6* allele expresses up to 4-fold less protein with lower activity than the wild-type allele (Desta et al., 2007). The molecular mechanism associated with the *CYP2B6*6* allele involves aberrant splicing, leading to lower functional mRNA and protein levels as well as reduced activity (Hofmann et al., 2008). Bupropion is associated with several safety problems including seizures (Ross and Williams, 2005) and failure to achieve smoking cessation in certain groups, which may be due to differences in metabolic induction. Therefore, the knowledge of a patient's genotype may help determine the optimal regimen for bupropion, especially when drug interactions are considered. Moreover, the expression of the CYP2B6 protein and mRNA is region-specific (Gervot et al., 1999). Expression has been observed in neurons and astrocytes in the human brain (Miksys et al., 2003), the target site of bupropion, suggesting that the *NR1I2* and *CYP2B6* genotypes may be more closely associated with clinical effects than would be suggested by the apparent PK parameters obtained from plasma data. In addition, transporter activity at the blood-brain barrier must be considered in this context. Thus, the influence of *NR1I2* polymorphisms on the clinical efficacy and adverse effects of bupropion should be examined in the future in a large-scale controlled clinical study.

The primary limitation of this study was the small number of volunteers in the variant type groups. However, at a minimum it may be confidently inferred that the effect of the *NR1I2* genotype is small for *CYP2B6*6* noncarriers ($n = 22$). In addition, we were not able to examine all of the known functional polymorphisms of PXR; instead, considering the small number of subjects, we focused on five sites that are frequently found in Asian individuals. A potential role for other nuclear receptors, such as the constitutive androstane receptor (CAR), farnesoid X receptor, and glucocorticoid receptor, as well as metabolizing enzymes and drug transporters in bupropion disposition and rifampin-mediated induction, may be postulated. CAR is well known to be related to CYP2B activation (Honkakoski and Negishi, 1998). A different mechanism of induction is evident in metamizole, which does not act as a direct ligand for PXR or CAR but selectively increases human hepatic CYP2B6 and CYP3A4 expression and activity (Saussele et al., 2007). Further investigation of the in vivo effects of CAR and other factors together with PXR and CYP2B6 in the induction of bupropion hydroxylation is therefore needed.

In conclusion, our study suggests that the *NR1I2* TGT or −25385T haplotype may decrease the induced metabolism of bupropion after rifampin administration in *CYP2B6*6* carriers, whereas this effect was not significant in *CYP2B6*6* noncarriers. Controlled pharmacogenetic clinical studies and large-scale pharmacodynamic genetic polymorphism studies in patients are both needed to clarify the role of the *NR1I2* polymorphism in the efficacy, safety, and drug interactions of CYP2B6 substrate drugs.

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Authorship Contributions

Participated in research design: Chung, Cho, Yu, Shin, Jang, and H.-S. Lim.

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Performed data analysis: Chung, Kim, and K.S. Lim.

Wrote or contributed to the writing of the manuscript: Chung, Cho, Yu, Shin, and Jang.

References

Bertilsson G, Heidrich J, Svensson K, Asman M, Jendeberg L, Sydow-Bäckman M, Ohlsson R, Postlind H, Blomquist P, and Berkenstam A (1998) Identification of a human nuclear receptor defines a new signaling pathway for CYP3A induction. *Proc Natl Acad Sci USA* **95:**12208–12213.

Blumberg B, Sabbagh W Jr, Juguilon H, Bolado J Jr, van Meter CM, Ong ES, and Evans RM (1998) SXR, a novel steroid and xenobiotic-sensing nuclear receptor. *Genes Dev* **12:**3195–3205.

Chang TK, Weber GF, Crespi CL, and Waxman DJ (1993) Differential activation of cyclophosphamide and ifosphamide by cytochromes P-450 2B and 3A in human liver microsomes. *Cancer Res* **53:**5629–5637.

Desta Z, Saussele T, Ward B, Blievernicht J, Li L, Klein K, Flockhart DA, and Zanger UM (2007) Impact of CYP2B6 polymorphism on hepatic efavirenz metabolism in vitro. *Pharmacogenomics* **8:**547–558.

Ekhart C, Doodeman VD, Rodenhuis S, Smits PH, Beijnen JH, and Huitema AD (2009) Polymorphisms of drug-metabolizing enzymes (GST, CYP2B6 and CYP3A) affect the pharmacokinetics of thiotepa and tepa. *Br J Clin Pharmacol* **67:**50–60.

Faucette SR, Hawke RL, Lecluyse EL, Shord SS, Yan B, Laethem RM, and Lindley CM (2000) Validation of bupropion hydroxylation as a selective marker of human cytochrome P450 2B6 catalytic activity. *Drug Metab Dispos* **28:**1222–1230.

Gervot L, Rochat B, Gautier JC, Bohnenstengel F, Kroemer H, de Berardinis V, Martin H, Beaune P, and de Waziers I (1999) Human CYP2B6: expression, inducibility and catalytic activities. *Pharmacogenetics* **9:**295–306.

Hofmann MH, Blievernicht JK, Klein K, Saussele T, Schaeffeler E, Schwab M, and Zanger UM (2008) Aberrant splicing caused by single nucleotide polymorphism c.516G>T [Q172H], a marker of CYP2B6*6, is responsible for decreased expression and activity of CYP2B6 in liver. *J Pharmacol Exp Ther* **325:**284–292.

Honkakoski P and Negishi M (1998) Regulatory DNA elements of phenobarbital-responsive cytochrome P450 CYP2B genes. *J Biochem Mol Toxicol* **12:**3–9.

Hustert E, Zibat A, Presecan-Siedel E, Eiselt R, Mueller R, Fuss C, Brehm I, Brinkmann U, Eichelbaum M, Wojnowski L, et al. (2001) Natural protein variants of pregnane X receptor with altered transactivation activity toward CYP3A4. *Drug Metab Dispos* **29:**1454–1459.

Jennison TA, Brown P, Crossett J, and Urry FM (1995) A high-performance liquid chromatographic method for quantitating bupropion in human plasma or serum. *J Anal Toxicol* **19:**69–72.

Jinno H, Tanaka-Kagawa T, Ohno A, Makino Y, Matsushima E, Hanioka N, and Ando M (2003) Functional characterization of cytochrome P450 2B6 allelic variants. *Drug Metab Dispos* **31:**398–403.

Karlsen TH, Lie BA, Frey Frøslie K, Thorsby E, Broomé U, Schrumpf E, and Boberg KM (2006) Polymorphisms in the steroid and xenobiotic receptor gene influence survival in primary sclerosing cholangitis. *Gastroenterology* **131:**781–787.

Kirchheiner J, Klein C, Meineke I, Sasse J, Zanger UM, Mürdter TE, Roots I, and Brockmöller J (2003) Bupropion and 4-OH-bupropion pharmacokinetics in relation to genetic polymorphisms in CYP2B6. *Pharmacogenetics* **13:**619–626.

Koyano S, Kurose K, Saito Y, Ozawa S, Hasegawa R, Komamura K, Ueno K, Kamakura S, Kitakaze M, Nakajima T, et al. (2004) Functional characterization of four naturally occurring variants of human pregnane X receptor (PXR): one variant causes dramatic loss of both DNA binding activity and the transactivation of the CYP3A4 promoter/enhancer region. *Drug Metab Dispos* **32:**149–154.

Lamba J, Lamba V, and Schuetz E (2005) Genetic variants of PXR (NR1I2) and CAR (NR1I3) and their implications in drug metabolism and pharmacogenetics. *Curr Drug Metab* **6:**369–383.

Lang T, Klein K, Fischer J, Nüssler AK, Neuhaus P, Hofmann U, Eichelbaum M, Schwab M, and Zanger UM (2001) Extensive genetic polymorphism in the human CYP2B6 gene with impact on expression and function in human liver. *Pharmacogenetics* **11:**399–415.

Lehmann JM, McKee DD, Watson MA, Willson TM, Moore JT, and Kliewer SA (1998) The human orphan nuclear receptor PXR is activated by compounds that regulate CYP3A4 gene expression and cause drug interactions. *J Clin Invest* **102:**1016–1023.

Lim YP, Liu CH, Shyu LJ, and Huang JD (2005) Functional characterization of a novel polymorphism of pregnane X receptor, Q158K, in Chinese subjects. *Pharmacogenet Genomics* **15:**337–341.

Lin YS, Yasuda K, Assem M, Cline C, Barber J, Li CW, Kholodovych V, Ai N, Chen JD, Welsh WJ, et al. (2009) The major human pregnane X receptor (PXR) splice variant, PXR.2, exhibits significantly diminished ligand-activated transcriptional regulation. *Drug Metab Dispos* **37:**1295–1304.

Loboz KK, Gross AS, Williams KM, Liauw WS, Day RO, Blievernicht JK, Zanger UM, and McLachlan AJ (2006) Cytochrome P450 2B6 activity as measured by bupropion hydroxylation: effect of induction by rifampin and ethnicity. *Clin Pharmacol Ther* **80:**75–84.

Miksys S, Lerman C, Shields PG, Mash DC, and Tyndale RF (2003) Smoking, alcoholism and genetic polymorphisms alter CYP2B6 levels in human brain. *Neuropharmacology* **45:**122–132.

Rosenfeld JM, Vargas R Jr, Xie W, and Evans RM (2003) Genetic profiling defines the xenobiotic gene network controlled by the nuclear receptor pregnane X receptor. *Mol Endocrinol* **17:**1268–1282.

Ross S and Williams D (2005) Bupropion: risks and benefits. *Expert Opin Drug Saf* **4:**995–1003.

Rotger M, Tegude H, Colombo S, Cavassini M, Furrer H, Décosterd L, Blievernicht J, Saussele T, Günthard HF, Schwab M, et al. (2007) Predictive value of known and novel alleles of CYP2B6 for efavirenz plasma concentrations in HIV-infected individuals. *Clin Pharmacol Ther* **81:**557–566.

Sandanaraj E, Lal S, Selvarajan V, Ooi LL, Wong ZW, Wong NS, Ang PC, Lee EJ, and Chowbay B (2008) PXR pharmacogenetics: association of haplotypes with hepatic CYP3A4 and ABCB1 messenger RNA expression and doxorubicin clearance in Asian breast cancer patients. *Clin Cancer Res* **14:**7116–7126.

Saussele T, Burk O, Blievernicht JK, Klein K, Nussler A, Nussler N, Hengstler JG, Eichelbaum M, Schwab M, and Zanger UM (2007) Selective induction of human hepatic cytochromes P450 2B6 and 3A4 by metamizole. *Clin Pharmacol Ther* **82:**265–274.

Shimada T, Yamazaki H, Mimura M, Inui Y, and Guengerich FP (1994) Interindividual variations in human liver cytochrome P-450 enzymes involved in the oxidation of drugs, carcinogens and toxic chemicals: studies with liver microsomes of 30 Japanese and 30 Caucasians. *J Pharmacol Exp Ther* **270:**414–423.

Uno Y, Sakamoto Y, Yoshida K, Hasegawa T, Hasegawa Y, Koshino T, and Inoue I (2003) Characterization of six base pair deletion in the putative HNF1-binding site of human PXR promoter. *J Hum Genet* **48:**594–597.

Wang XD, Li JL, Su QB, Deng XY, Lu Y, Chen J, Zhang JX, Zhao LZ, Zuo Z, Chan E, et al. (2007) A pharmacogenetic study of pregnane X receptor (NR1I2) in Han Chinese. *Curr Drug Metab* **8:**778–786.

Wang XD, Li JL, Su QB, Guan S, Chen J, Du J, He YW, Zeng J, Zhang JX, Chen X, et al. (2009) Impact of the haplotypes of the human pregnane X receptor gene on the basal and St John's wort-induced activity of cytochrome P450 3A4 enzyme. *Br J Clin Pharmacol* **67:**255–261.

Ward BA, Gorski JC, Jones DR, Hall SD, Flockhart DA, and Desta Z (2003) The cytochrome P450 2B6 (CYP2B6) is the main catalyst of efavirenz primary and secondary metabolism: implication for HIV/AIDS therapy and utility of efavirenz as a substrate marker of CYP2B6 catalytic activity. *J Pharmacol Exp Ther* **306:**287–300.

Zhang J, Kuehl P, Green ED, Touchman JW, Watkins PB, Daly A, Hall SD, Maurel P, Relling M, Brimer C, et al. (2001) The human pregnane X receptor: genomic structure and identification and functional characterization of natural allelic variants. *Pharmacogenetics* **11:**555–572.

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