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RESEARCH ARTICLE

Effects of *CYP2D6* and *CYP2C19* genetic polymorphisms and cigarette smoking on the pharmacokinetics of tolperisone

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Abstract

Tolperisone, a muscle relaxant used for post-stroke spasticity, is metabolized to its main metabolite by CYP2D6 and to a lesser extent by CYP2C19 and CYP1A2. We investigated the effects of *CYP2D6* and *CYP2C19* genetic polymorphisms and cigarette smoking on tolperisone pharmacokinetics. A 150 mg oral dose of tolperisone was given to 184 healthy Korean subjects and plasma concentrations of tolperisone were measured by liquid chromatography-tandem mass spectrometry (LC-MS/MS). A 3.14-fold significant increase in $AUC_{0-\infty}$ was observed in the *CYP2D6*10/*10* group compared with the *CYP2D6*wt/*wt* group, whereas a 3.59-fold increase in $AUC_{0-\infty}$ was observed in CYP2C19PMs compared to CYP2C19EMs. Smokers had a 38.5% decrease in $AUC_{0-\infty}$ when compared to non-smokers. When these effects were combined, *CYP2D6*10/*10*-CYP2C19PM-Non-smokers had a 25.9-fold increase in $AUC_{0-\infty}$ compared to *CYP2D6*wt/*wt*-CYP2C19EM-Smokers. Genetic polymorphisms of *CYP2D6* and *CYP2C19* and cigarette smoking independently and significantly affected tolperisone pharmacokinetics and these effects combined resulted in a much greater impact on tolperisone pharmacokinetics.

Keywords Tolperisone · CYP2D6 · CYP2C19 · CYP1A2 · Smoking · Genetic polymorphism · Pharmacokinetics

Introduction

Tolperisone is a centrally acting muscle relaxant that inhibits spinal reflexes by voltage-gated sodium and calcium channel blockade (Kocsis et al. 2005). Tolperisone has been used as oral and intravenous formulations to treat muscular hypertonicity and muscle spasms associated with various neurological and locomotor disorders since the 1960s. However, in 2012, European Medicines Agency restricted the clinical use of tolperisone to symptomatic treatment of post-stroke spasticity in adults due to increased reports of hypersensitivity reactions such as urticaria, pruritus, dyspnea, angioedema, erythema, rash, and anaphylactic reaction/shock (http://www.ema.europa.eu/docs/en_GB/document_library/Press_release/2012/06/WC500129069.pdf). Adverse reactions of tolperisone unrelated to hypersensitivity are usually mild and include excessive sweating, abdominal pain, nausea, vomiting, diarrhea, flatulence, and xerostomia (Vora 2010). However, an acute overdose of tolperisone can result in serious clinical sequelae such as coma, seizures, respiratory depression, and cardiac arrest (Martos et al. 2015).

Upon oral administration, tolperisone is rapidly absorbed and has a low bioavailability of 16.7% due to its extensive metabolism (Miskolczi et al. 1987). Tolperisone is metabolized to hydroxymethyl-tolperisone by multiple cytochrome P450 (CYP) enzymes (Dalmadi et al. 2003). The majority of tolperisone metabolism is mediated by CYP2D6, and to a lesser extent by CYP2C19 and CYP1A2 (Dalmadi et al. 2003). CYP2D6 and CYP2C19 are highly polymorphic enzymes, and more than 172 *CYP2D6* and 39 *CYP2C19* allelic variants have been identified to date in the coding region of the gene (http://www.pharmvar.org). The genetic polymorphisms of CYP enzymes significantly affect the pharmacokinetics and pharmacodynamics of substrate drugs (Bae et al. 2009, 2011, 2012, 2020; Choi et al. 2014; Kim et al. 2022; Cho et al. 2023a, b; Kang et al. 2023). Cigarette smoking has been known to induce CYP1A2 activity. In

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heavy smokers smoking > 20 cigarettes per day, a 1.72-fold increase in CYP1A2 activity compared to nonsmokers has been observed (Tantcheva-Poór et al. 1999). Upon cessation of cigarette smoking, the CYP1A2 activity decreases and a new steady state of the enzyme activity is reached within approximately 7 days (Faber and Fuhr 2004).

Our previous study examined tolperisone pharmacokinetics in 15 healthy subjects and observed wide interindividual variability in tolperisone pharmacokinetics, in which we suggested that such variability could be due to the genetic polymorphism of the involved drug-metabolizing enzymes (Bae et al. 2007). Subsequently, we proposed the significant effects of genetic polymorphism of *CYP2D6* or *CYP2C9* on the pharmacokinetics of tolperisone in the American Society for Clinical Pharmacology and Therapeutics (ASCPT) 2013 Annual Meeting (Byeon et al. 2013a, b). As wide variations in the pharmacokinetics of tolperisone among individuals were observed, we aimed to more accurately and descriptively clarify the effects of *CYP2D6* and *CYP2C19* genetic polymorphisms and other factors on tolperisone pharmacokinetics by recruiting even more subjects, examining the combined effects of both CYP enzymes' genetic polymorphisms and exploring the effects of other factors, such as smoking, on the pharmacokinetics of tolperisone.

Materials and methods

Subjects

One hundred eighty-four healthy Korean individuals carrying the *CYP2D6*wt/*wt* (**wt* = **1* or **2*) ($n = 51$), *CYP2D6*wt/*10* ($n = 79$), or *CYP2D6*10/*10* ($n = 54$) were included in this study. The participants were further divided by their *CYP2C19* genotypes into extensive metabolizers (EMs) (*CYP2C19*1/*1*), intermediate metabolizers (IMs) (*CYP2C19*1/*2* or **1/*3*) and poor metabolizers (PMs) (*CYP2C19*2/*2*, **2/*3*, or **3/*3*) and by smoking status. To minimize the variability, participants carrying the *CYP2C19*17* allele have been excluded from this study. Participants' genomic DNA was isolated from peripheral blood leukocytes using the Wizard Genomic DNA Kit (Promega, Madison, WI, USA). Polymerase chain reaction-restriction fragment length polymorphisms (PCR–RFLP) and long PCR methods, as previously described, were used for the analyses of *CYP2D6*2*, **3*, **5*, **10* and **X×N* and *CYP2C19*2*, **3*, **17* alleles (de Morais et al. 1994a, b; Johansson et al. 1996; Lundqvist et al. 1999; Mendoza et al. 2001; Sim et al. 2006).

All participants were healthy according to medical history, physical examination, and routine laboratory tests (blood chemistry, hematology and urine analysis). They were asked to refrain from any medication, caffeine, grapefruit products, and alcoholic beverages for at least 1 week before and throughout the study period. All participants provided verbal and written informed consent. The study was performed in accordance with the guidelines of the Declaration of Helsinki and was approved by the Institution Ethics Committee of Sungkyunkwan University, Suwon, Korea.

Study design

Each subject received a 150 mg oral dose of tolperisone (Midocalm, Han Lim Pharm. Co., Seoul, Korea) with 240 mL of water after an overnight fast. Subjects maintained the fasting state for 4 h after drug administration. Venous blood samples (7 mL) were obtained before and 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, 6, 8, 10 and 12 h after tolperisone administration. The blood samples were centrifuged immediately, and plasma samples were stored at − 70 °C until analysis.

LC/MS–MS assay

Tolperisone plasma concentrations were determined by liquid chromatography-tandem mass spectrometry (LC-MS/MS) according to our previously reported method (Choi et al. 2012b).

Pharmacokinetic analysis

Pharmacokinetics parameters of tolperisone were estimated by noncompartmental methods using the BA Calc 2007 analysis program (KFDA, Seoul, Korea). Actual blood sampling times were used for the analysis. The C_{max} was used as the observed value. The AUC was calculated using the linear trapezoidal rule. The elimination rate constant (k_e) was estimated from the least squares regression slope of the terminal plasma concentration. The $AUC_{0-\infty}$ was calculated as $AUC_{0-\infty} = AUC + C_t/k_e$, where C_t is the last plasma concentration measured. The elimination half-life ($t_{1/2}$) was calculated as $t_{1/2} = \ln 2/ k_e$ and the apparent oral clearance (CL/F) of tolperisone was calculated as $CL/F = dose/AUC_{0-\infty}$.

Statistical analysis

All pharmacokinetic data are expressed as mean ± SD. Differences in pharmacokinetic parameters between the genotype groups were assessed using a one-way analysis of variance with the Bonferroni t-test or Kruskal–Wallis one-way analysis of variance with the Mann–Whitney rank-sum test after normality and equal variance tests. Data were analyzed using the statistical program SigmaPlot 12.0 for Windows (version 12.0, Systat Software, Chicago, Illinois, USA). *P* values < 0.05 were considered statistically significant.

Results

A total of 184 healthy Korean subjects (170 males and 14 females) participated in the study. The mean age of the participants was 23.0 ± 2.1 years and the mean height and weight were 174. ± 6.6 cm and 67.8 ± 8.1 kg, respectively. All subjects completed the study according to the protocol without observed adverse events or hypersensitivity reactions.

After administration of a tolperisone 150 mg oral tablet, marked differences were observed in the participants' pharmacokinetic parameters based on their *CYP2D6* genotypes. The *CYP2D6*10/*10* group had significantly higher C_{max}, AUC_{0-12} and $AUC_{0-\infty}$ and lower CL/F than *CYP2D6*wt/*wt* and *CYP2D6*wt/*10* groups (Table 1, Fig. 1A). In particular, the $AUC_{0-\infty}$ of the *CYP2D6*10/*10* group was 3.14-fold and 1.96-fold higher than *CYP2D6*wt/*wt* and *CYP2D6*wt/*10* groups, respectively. Of note, there were considerable variations in the individual $AUC_{0-\infty}$ values within each genotype group, as indicated by a large range of the standard deviations and dispersion of individual $AUC_{0-\infty}$ plots in Fig. 1A.

When the pharmacokinetic parameters were analyzed according to the *CYP2C19* genotype, C_{max}, $AUC_{0-12,}$ and $AUC_{0-\infty}$ were significantly increased and CL/F was significantly decreased in the CYP2C19PM group, as compared to the CYP2C19EM and CYP2C19IM groups (Table 1, Fig. 1B). The $AUC_{0-\infty}$ in the CYP2C19PM group were 3.59-fold and 2.50-fold higher in comparison to the CYP2C19EM and CYP2C19IM groups, respectively. Likewise, considerable variability was observed in the $AUC_{0-\infty}$ values in each *CYP2C19* genotype group, as indicated by the large range of standard deviations and dispersion of individual $AUC_{0-\infty}$ plots in Fig. 1B.

To examine the effects of combined *CYP2D6* and *CYP2C19* genotypes on the pharmacokinetics of tolperisone, participants were divided into nine subgroups based on the *CYP2D6-CYP2C19* genotype status (Table 2). Compared to the *CYP2D6*wt/*wt*-CYP2C19EM group, all other *CYP2D6-CYP2C19* genotype groups showed a statistically significant increase in C_{max}, $AUC_{0-12,}$ and $AUC_{0-\infty}$ and a decrease in CL/F. In comparison to the *CYP2D6*wt/*wt*-CYP2C19EM group, 3.58-fold, 5.11-fold, and 10.49-fold higher $AUC_{0-\infty}$ values were observed in the *CYP2D6*10/*10*-CYP2C19EM group, *CYP2D6*wt/*wt*-CYP2C19PM group, and *CYP2D6*10/*10*-CYP2C219PM group, respectively. Plotting of the individual $AUC_{0-\infty}$ values continued to show wide variability in distribution within the same genotype groups, especially in the groups containing the CYP2C19PM genotype (Fig. 2).

To further examine the additional factors that may affect tolperisone pharmacokinetics, the effects of cigarette smoking were assessed. The pharmacokinetic parameters were stratified according to the participant's smoking status (Table 3). Compared to non-smokers, the smokers had significantly decreased C_{max}, $t_{1/2}$, AUC_{0-12}, and $AUC_{0-\infty}$ values, and there was a 1.54-fold increase in CL/F. The $AUC_{0-\infty}$ of smokers was decreased by 38.5% compared to non-smokers.

To account for the effects of all altered activities of the CYP enzymes studied, we categorized participants based on the *CYP2D6* genotype-*CYP2C19* genotype-smoking status. Regardless of the CYP enzyme functions, all of the nine *CYP2D6-CYP2C19* genotype groups exhibited significant pharmacokinetic differences between smokers and non-smokers, including the group with the greatest CYP activity (*CYP2D6*wt/*wt*-CYP2C19EM) (Fig. 3A) and

Table 1 Pharmacokinetic parameters of tolperisone in different *CYP2D6* or *CYP2C19* genotypes after single administration of 150 mg tolperisone

Variable	*CYP2D6*			*P* value	*CYP2C19*			*P* value
	**wt/*wt* (n = 51)	**wt/*10* (n = 79)	**10/*10* (n = 54)		*EM* (n = 73)	*IM* (n = 78)	*PM* (n = 33)	
C_{max} (ng/mL)	54.1 ± 67.5	83.7 ± 81.6	151.2 ± 123.6***,###	< 0.001	56.6 ± 50.0	88.7 ± 74.1	196.6 ± 155.1***,###	< 0.001
t_{max} (hr)	0.9 ± 0.3	0.9 ± 0.4	1.0 ± 0.3	N.S	0.9 ± 0.4	0.9 ± 0.3	1.0 ± 0.2	N.S
$t_{1/2}$ (hr)	3.0 ± 1.7	3.1 ± 1.5	2.9 ± 1.1	N.S	2.8 ± 1.3	3.1 ± 1.5	3.3 ± 1.5	N.S
CL/F (L/hr)	3493.9 ± 3174.7	2104.3 ± 2423.8**	927.9 ± 801.4***,#	0.001	2672.8 ± 2390.3	2235.1 ± 2922.6	760.3 ± 703.4**,#	< 0.001
AUC_{0-12} (ng·hr/mL)	91.7 ± 108.3	147.4 ± 165.4	289.5 ± 235.9***,###	< 0.001	104.8 ± 104.3	152.1 ± 130.0	377.1 ± 304.0***,###	< 0.001
$AUC_{0-\infty}$ (ng·hr/mL)	95.3 ± 110.6	152.5 ± 170.5	299.3 ± 245.9***,###	< 0.001	109.0 ± 109.5	156.4 ± 131.9	391.5 ± 315.6***,###	< 0.001

**wt* = **1* or **2*. The three genotype groups were compared by one-way ANOVA with post hoc Bonferroni *t*-test

N.S not significant

Each data were expressed as mean ± SD

**P* < 0.05; ***P* < 0.01; ****P* < 0.001, compared with *CYP2D6*wt/*wt* or *CYP2C19EM*. #*P* < 0.05; ##*P* < 0.01; ###*P* < 0.001, compared with *CYP2D6*wt/*10* or *CYP2C19EM*

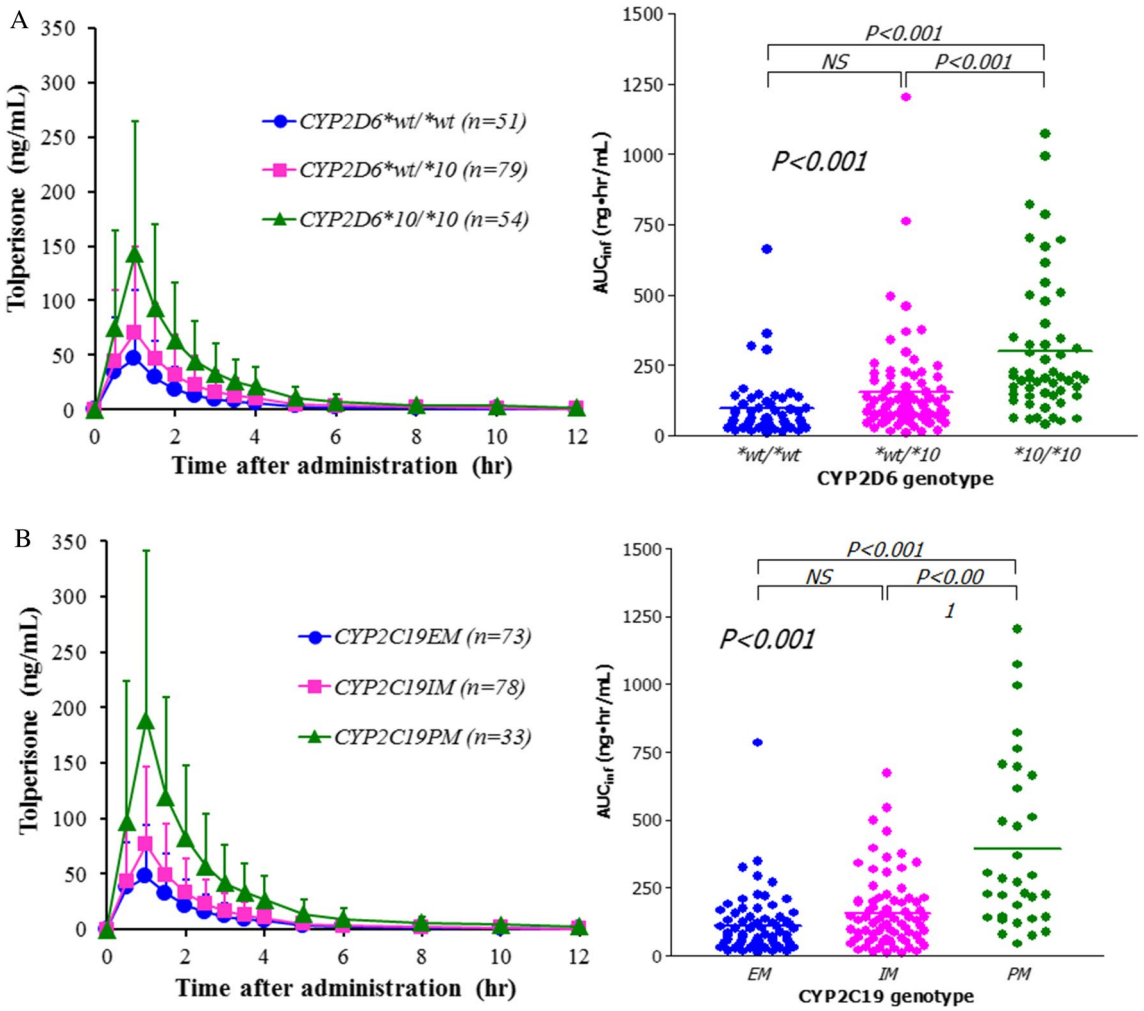


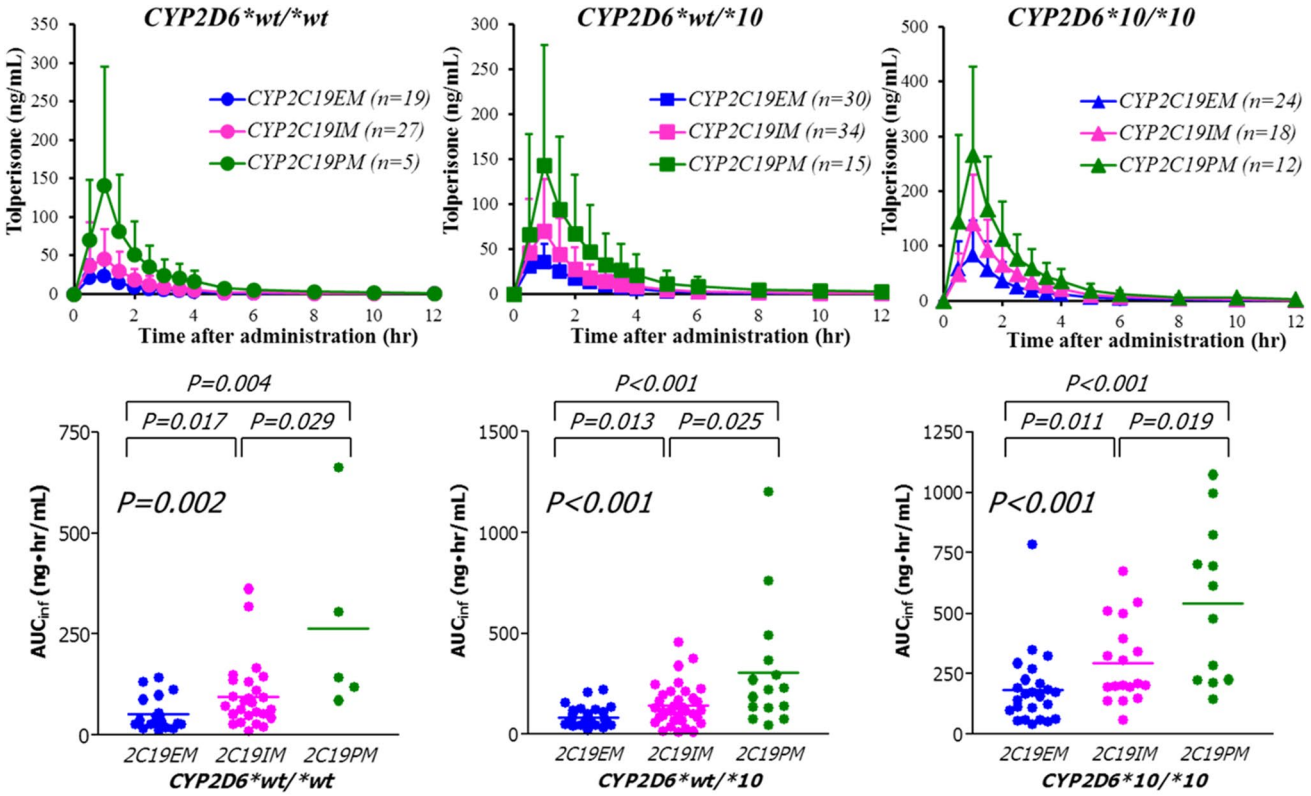
Fig. 1 Plasma concentration profiles and $AUC_{0-\infty}$ of tolperisone in different *CYP2D6* (**A**) or *CYP2C19* (**B**) genotypes after single administration of 150 mg tolperisone. *NS* not significant. The three genotype groups were compared by one-way ANOVA with post hoc Bonferroni t-test

Table 2 Pharmacokinetic parameters of tolperisone in different *CYP2D6-CYP2C19* genotypes after single administration of 150 mg tolperisone

	C_{max} (ng/mL)	CL/F (L/hr)	AUC_{0-12} (ng·hr/mL)	$AUC_{0-\infty}$ (ng·hr/mL)
*CYP2D6*wt/*wt-CYP2C19EM* (n = 19)	27.8 ± 24.6	4920.1 ± 3266.0	48.9 ± 40.2	51.4 ± 41.4
*CYP2D6*wt/*wt-CYP2C19IM* (n = 27)	56.0 ± 55.2**	2960.4 ± 2986.6*	91.7 ± 81.5*	95.1 ± 82.3*
*CYP2D6*wt/*wt-CYP2C19PM* (n = 5)	144.0 ± 152.0**	955.0 ± 607.4**	254.2 ± 233.1**	262.9 ± 238.6**
*CYP2D6*wt/*10-CYP2C19EM* (n = 30)	47.5 ± 29.8**	2320.1 ± 1389.3**	82.2 ± 45.6**	85.6 ± 47.9**
*CYP2D6*wt/*10-CYP2C19IM* (n = 34)	83.2 ± 66.3***	2402.5 ± 3342.2***	139.0 ± 103.4***	142.8 ± 104.0***
*CYP2D6*wt/*10-CYP2C19PM* (n = 15)	148.5 ± 132.7***	996.9 ± 878.6***	297.0 ± 299.5***	308.5 ± 309.5***
*CYP2D6*10/*10-CYP2C19EM* (n = 24)	90.7 ± 65.1***	1334.5 ± 949.0***	177.3 ± 146.4***	183.9 ± 155.0***
*CYP2D6*10/*10-CYP2C19IM* (n = 17)	144.0 ± 88.7***	748.1 ± 524.8***	274.2 ± 161.7***	280.8 ± 165.0***
*CYP2D6*10/*10-CYP2C19PM* (n = 13)	272.2 ± 160.2***	412.4 ± 281.0***	516.8 ± 297.1***	536.8 ± 310.6***

Each data were expressed as mean ± SD. Each group was compared by Mann–Whitney Rank Sum Test

*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$, compared with *CYP2D6*wt/*wt-CYP2C19EM*



NS, Not significant. The three genotype groups were compared by Kruskal-Wallis one-way ANOVA test with post hoc Mann-Whitney test.

Fig. 2 Plasma concentration profiles and $AUC_{0-\infty}$ of tolperisone in different CYP2D6-CYP219 genotypes after single administration of 150 mg tolperisone

Table 3 Pharmacokinetic parameters of tolperisone in smokers and non-smokers after single administration of 150 mg tolperisone after single administration of 150 mg tolperisone

Variable	Smoker (n = 55)	Non-smoker (n = 129)	*P* value
C_{max} (ng/mL)	66.6 ± 62.6	107.6 ± 110.0	0.010
t_{max} (hr)	1.0 ± 0.3	0.9 ± 0.3	N.S
$t_{1/2}$ (hr)	2.7 ± 1.6	3.1 ± 1.4	0.044
CL/F (L/hr)	2839.2 ± 2893.2	1847.9 ± 2303.6	0.014
AUC_{0-12} (ng·hr/mL)	121.3 ± 134.3	196.0 ± 209.2	0.016
$AUC_{0-\infty}$ (ng·hr/mL)	125.0 ± 136.9	203.1 ± 217.1	0.015

Each data were expressed as mean ± SD. The two groups were compared by Student's t-test

N.S not significant

the group with the least CYP activity (*CYP2D6*10/*10*-CYP2C19PM) (Fig. 3B). *CYP2D6*wt/*wt*-CYP2C19EM-Smokers, the group with the greatest expected CYP enzyme activities, had C_{max} and $AUC_{0-\infty}$ of 13.6 ± 5.2 and 25.3 ± 5.8 while the *CYP2D6*10/*10*-CYP2C19PM-Non-smokers, the group with the least expected CYP enzyme activities, had C_{max} and $AUC_{0-\infty}$ of 336.0 ± 165.8 and 636.9 ± 301.7, which resulted in a 27.4-fold significant increase in C_{max} and 25.2-fold significant increase in $AUC_{0-\infty}$ in the *CYP2D6*10/*10*-CYP2C19PM-Non-smokers group compared to the *CYP2D6*wt/*wt*-CYP2C19EM-Smokers group (Table 4, Fig. 4). In addition, significant increases in $T_{1/2}$, AUC_{0-12} and significant decrease in CL/F were observed in the *CYP2D6*10/*10*-CYP2C19PM-Non-smokers compared to the *CYP2D6*wt/*wt*-CYP2C19EM-Smokers (data not shown). Even when the *CYP2D6-CYP2C19*-Smoking status were combined, the $AUC_{0-\infty}$ values within the group continued to show wide interindividual variations, which was most prominent in those who had decreased CYP enzyme activities (Fig. 3A, B).

Discussion

Tolperisone is metabolized to its main metabolite, hydroxymethyl-tolperisone, mainly by CYP2D6, and to a lesser extent, by CYP2C19 and CYP1A2 (Dalmadi et al.

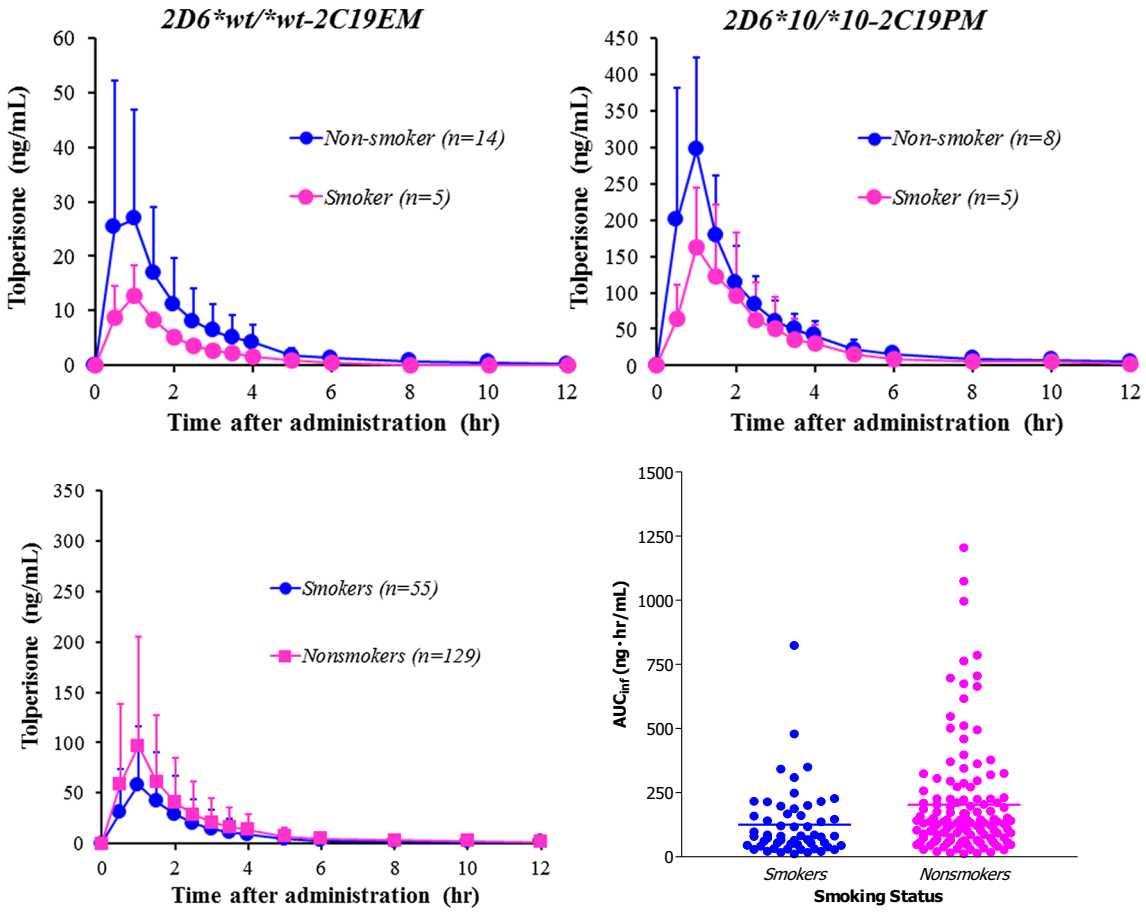


Fig. 3 Plasma concentration profiles∞ $AUC_{0-\infty}$ of tolperisone in the greatest expected CYP activity and the least expected CYP activity genotype groups by smoking status after single administration of 150 mg tolperisone

Table 4 C_{max} and AUC_{inf} of tolperisone in the fastest metabolizer group and the slowest metabolizer group in this study

	C_{max} (ng/mL)	$AUC_{0-\infty}$ (ng·hr/mL)
*CYP2D6*wt/*wt-CYP-2C19EM* -Smoker	13.6 ± 5.2	25.3 ± 5.8
*CYP2D6*10/*10-CYP-2C19PM* -Nonsmoker	336.0 ± 165.8*	636.9 ± 301.7*

2003). Among these, CYP2D6 and CYP2C19 have a high frequency of genetic polymorphisms in East Asians. *CYP2D6*10*, which has reduced affinity for the substrates, is the most prevalent allele with a frequency of 42–51% in East Asians, followed by *CYP2D6*1* (34%) and **5* (6%) (Ingelman-Sundberg 2005, CPIC codeine supplement 2014). In a study evaluating genetic polymorphism of CYP enzymes in 672 unrelated Chinese, null function alleles *CYP2C19*2* and **3* were found in frequencies of 32.5–49.4% and 2.1–5.2%, respectively (Zuo et al. 2012). Based on our previous study in Koreans, *CYP2D6*wt/*wt, *wt/*10*, and **10/*10*

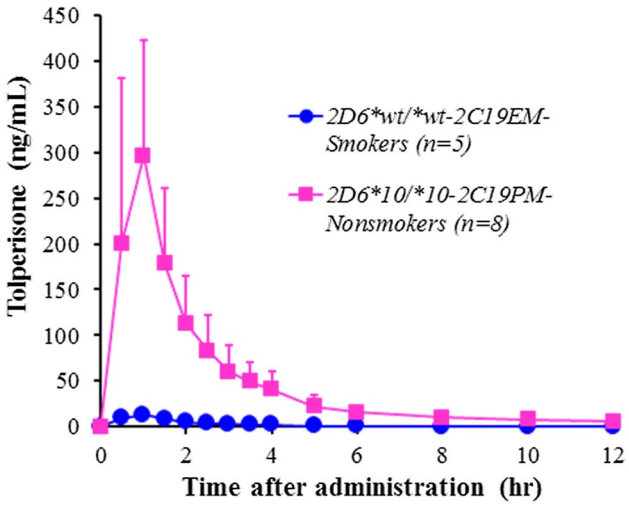


Fig. 4 Plasma concentration profiles of the fastest metabolizer group (*CYP2D6*wt/*wt*-CYP2C19EM-Smokers) and the slowest metabolizer group (*CYP2D6*10/*10*-CYP2C19PM-Non-smokers) in this study after single administration of 150 mg tolperisone

genotype groups were observed in frequencies of 22.1%, 43.7%, and 22.3%, respectively (Byeon et al. 2018), while CYP2C19 EMs, IMs and PMs were observed in frequencies of 38.8%, 46.0% and 14.0%, respectively (unpublished data).

In 2013, we presented the significant effects of *CYP2D6* or *CYP2C19* genetic polymorphisms on the pharmacokinetics of tolperisone at the ASCPT Annual Meeting (Byeon et al. 2013a, b). Recently, a similar study by Pawloswska et al. (2015) was conducted with a very limited number of subjects. The study was conducted in 28 healthy Polish subjects who received oral tolperisone and observed significant differences in AUC and C_{max} of tolperisone between the *CYP2D6*1/*1* and **1/*4*, **1/*5* genotype groups but only a change in oral clearance (CL/F) between the *CYP2C19*1/*1* and **1/*2* genotype groups. As wide interindividual variability exists in tolperisone pharmacokinetics and discrepancies exist in the effects of CYP genetic polymorphisms, investigation of such effects in a large number of subjects and examination of the combined effects of both *CYP2D6* and *CYP2C19* genetic polymorphisms along with additional factors that could influence tolperisone pharmacokinetics were necessary. Therefore, this study reports the results of the study conducted in 187 subjects which examined the effects of the pharmacokinetics of tolperisone based on the participants' *CYP2D6* and *CYP2C19* genotype and smoking status.

While there was a significant 3.14-fold increase in $AUC_{0-\infty}$ in the *CYP2D6*10/*10* group compared to the *CYP2D6*wt/*wt* group and a 3.59-fold increase in $AUC_{0-\infty}$ in the CYP2C19PM group compared to the CYP2C19EM group, the genetic polymorphisms in either *CYP2D6* or *CYP2C19* alone could not fully explain the interindividual variability in tolperisone pharmacokinetics as there was considerable variability even within the same genotype group. When the genetic polymorphism effects were examined in the combined *CYP2D6-CYP2C19* genotype groups, there was a 10.49-fold difference in $AUC_{0-\infty}$ when comparing the individuals with wild type of both *CYP2D6* and *CYP2C19* (*CYP2D6*wt/*wt*-CYP2C19EM) to the individuals with the least functional *CYP2D6* and *CYP2C19* activity in the study (*CYP2D6*10/*10*-CYP2C219PM). However, considerable variability in individual $AUC_{0-\infty}$ values was present even when the *CYP2D6-CYP2C19* genotypes were combined.

To further evaluate whether other factors contribute to tolperisone pharmacokinetics, smoking status was assessed in the present study. The US FDA suggests smoking as a CYP1A2 inducer in vivo (http://www.fda.gov/Drugs/DevelopmentApprovalProcess/DevelopmentResources/DrugInteractionsLabeling/ucm093664.htm) and smoking is known to induce CYP1A2 activity by polycyclic aromatic hydrocarbons found in cigarette smoke (Kalow et al. 1991). Actual phenotyping of CYP1A2 was not performed specific on cigarette smoking in this study. However, as there were 55 smokers in the present study who were restricted from smoking activity only during the actual study procedures that compromised drug administration and blood draws and comparison of the pharmacokinetic data of the smokers versus non-smokers showed a significant decrease in tolperisone $AUC_{0-\infty}$ by 38.5% in smokers, we presumed that this difference was due to the increased metabolism of CYP1A2 from cigarette smoking. When the effects of smoking were assessed in nine different CYP2D6-CYP2C19 genotype groups, all groups, including the group with the greatest CYP activity and the least CYP activity had significantly decreased tolperisone exposure in smokers compared to non-smokers, which suggests that smoking affects the pharmacokinetics of tolperisone regardless of the subject's *CYP2D6* or *CYP2C19* genotype status. When the effects of *CYP2D6* and *CYP2C19* genotypes and smoking status were all combined, there was an immense 25.2-fold increase in the $AUC_{0-\infty}$ in *CYP2D6*10/*10*-CYP2C19PM-Non-smokers compared to the *CYP2D6*wt/*wt*-CYP2C19EM-Smokers. However, there were still considerable variations in the individual $AUC_{0-\infty}$ values within the same subgroup, which suggest that other factors besides these three factors contribute to the pharmacokinetics of tolperisone.

Generally, individuals carrying two of the reduced function alleles (i.e. *CYP2D6*10*, **17*, **41*) are considered IMs, while individuals carrying two of the null function alleles (i.e. *CYP2D6*4, *5, *6*) are considered PMs. In Asians, CYP2D6PMs are very rare (< 1%), while in Europeans, 8% of the population are PMs (Sistonen et al. 2007; Choi et al. 2012a). As the frequency of CYP2D6PMs in Koreans is very low, no PMs were included in this study, but in Europeans where CYP2D6PMs exist more frequently, CYP2D6PM-CYP2C19PM-Non-smokers would result in even greater increase in tolperisone pharmacokinetics than the 25.2-fold increase in $AUC_{0-\infty}$ observed difference in *CYP2D6*10/*10*-CYP2C19PM-Non-smokers compared to the *CYP2D6*wt/*wt*-CYP2C19EM-Smokers in this study. Additionally, in CYP2D6PMs who take CYP2C19 strong inhibitors such as fluconazole or fluvoxamine, or in CYP2C19PMs who take CYP2D6 strong inhibitors such as bupropion, fluoxetine, paroxetine or quinidine, or in those who take drugs that are inhibitors of both CYP2D6 and CYP2C19, such as ticlopidine, regardless of their genotypes, it is expected that administration of tolperisone would result in largely enhanced exposure. As this study was conducted as a single-dose study, no adverse reactions were observed, but patients in the clinical setting who take tolperisone three times daily chronically with decreased CYP functions could be at a much greater risk of adverse reactions from tolperisone.

Although the mechanism of tolperisone hypersensitivity is unknown, it has been hypothesized that hapten formations from tolperisone metabolites activating the immune system by

covalent modification of proteins, structural similarity of tolperisone to the topical lidocaine, or tolperisone's intrinsic vasodilatory activity could contribute to the cause of the hypersensitivity reaction (http://www.ema.europa.eu/docs/en_GB/document_library/Referrals_document/Tolperisone_31/WC500141050.pdf; Ribi et al. 2003). As the exact cause of the hypersensitivity reaction related to tolperisone is yet to be determined, it could be valuable to explore whether there is an association between high exposure to tolperisone in subjects with certain genotypes and the occurrence of hypersensitivity reactions.

Personalized medicine (or personalized pharmacotherapy) aims to determine the most adequate treatment and dose regimen to obtain the maximum efficacy and minimum side effects for patients by taking into account their characteristics such as comedications, concomitant diseases, phenotype, and genotype (Marsousi et al. 2017). The development of innovative methods to optimize drug safety and efficacy in specific populations or individuals is of great clinical importance. In recent years, physiologically-based pharmacokinetic (PBPK) modeling has been suggested as a promising approach that can predict drug exposure in specific populations or individuals and realize personalized pharmacotherapy (Cho et al. 2021a, b, 2022; Jung et al. 2021; Kim et al. 2021; Whang et al. 2022; Lee et al. 2022). Therefore, PBPK modeling including pharmacogenetic information for tolperisone can be a promising method to overcome the very large individual variation in tolperisone's pharmacokinetics.

In conclusion, the genetic polymorphism of *CYP2D6* and *CYP2C19* and cigarette smoking independently affected the pharmacokinetics of tolperisone to a significant degree. When the effects of altered function of two, or all three of the CYP enzymes were combined, there was a much more pronounced change in the pharmacokinetics of tolperisone, and such individuals with altered function CYP enzymes, especially when multiple altered functions are combined, could be at an increased risk of adverse reactions with tolperisone.

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Data availability Not applicable.

Declarations

Conflict of interest The authors declared no competing interest for this work.

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