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PHARMACOKINETICS AND DISPOSITION

The impact of *CYP2D6* polymorphisms on the pharmacokinetics of codeine and its metabolites in Mongolian Chinese subjects

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Abstract
Purpose Codeine is an analgesic drug acting on μ-opioid receptors predominantly via its metabolite morphine formed almost exclusively by *CYP2D6*. Genetic polymorphisms in *CYP2D6* are associated with diminished pain relief and/or severe opioid side effects. In Chinese individuals, *CYP2D6*10* is the most common allele with reduced enzyme activity. In this study, we investigated the effect of this allele on the pharmacokinetics of codeine and its metabolites.
Method A blood sample was collected from healthy Mongolian volunteers for *CYP2D6* genotyping using a PCR-RFLP assay. A pharmacokinetic study was then carried out in three groups with *CYP2D6*1/*1* (n=10), *CYP2D6*1/*10* (n=10) and *CYP2D6*10/*10* (n=9) genotypes by collecting serial blood samples for determination of plasma levels of codeine and its metabolites, morphine, morphine 3-glucuronide (M3G) and morphine 6-glucuronide (M6G) before and after a single 30-mg oral dose of codeine phosphate. Codeine and its metabolites were measured by LC-MS/MS.
Results No significant differences were observed in the pharmacokinetic parameters of codeine in the three genotype groups. However, the C_{max} and $AUC_{0-\infty}$ of morphine, M3G and M6G were significantly different between the study groups ($P<0.05$). Compared with the **1/*1* group, the $AUC_{0-\infty}$ for morphine in the **1/*10* and **10/*10* groups decreased by ratios (95 % CI) of 0.93 (0.26–1.59) and 0.494 (0.135–0.853) respectively. Corresponding ratios for M3G were 0.791 (0.294–1.288) and 0.615 (0.412–0.818) and for M6G were 0.643 (0.39–0.957) and 0.423 (0.267–0.579).
Conclusion This study demonstrates that the *CYP2D6*10* allele plays an important role in the pharmacokinetics of the O-demethylated metabolites of codeine after oral administration.

Keywords *CYP2D6* · Polymorphism · *CYP2D6*10* · Codeine · Morphine · Pharmacokinetics

Introduction

Codeine is an effective analgesic widely used in the treatment of cough and various types of pain [1]. It is well known that O-demethylation of codeine to give morphine is mediated by the polymorphic cytochrome P450 isoenzyme 2D6 (*CYP2D6*). Although bioactivation by O-demethylation accounts for only 10 % of codeine clearance, it is considered to be essential for the analgesic, antitussive and antidiarrheal effects of codeine [2, 3].

There is substantial evidence linking *CYP2D6* genotype to variability in codeine efficacy and toxicity. Diminished pain relief even with high doses has been observed in poor metabolizers (PMs) of *CYP2D6*, whereas severe or life-threatening toxicity has been documented in ultrarapid metabolizers (UMs) even after normal doses [4, 5]. In intermediate metabolizers (IMs) of *CYP2D6*, a decrease in codeine O-demethylation is expected because of reduced enzyme activity.

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*CYP2D6*10* (100C>T; rs1065852) is the main variant allele causing reduced enzyme activity in East Asians with reported frequencies of 56.2 % in Chinese and 38.8 % in Japanese [6–9]. The *CYP2D6*10* enzyme has a P34S substitution resulting in an unstable enzyme with reduced affinity for *CYP2D6* substrates including codeine [10, 11]. Due to its instability, *CYP2D6*10* may have a more dramatic effect on the pharmacokinetics of substrates in vivo than in vitro. The aim of the present study was to investigate the effect of the *CYP2D6*10* allele on the pharmacokinetics of codeine in healthy Mongolian Chinese subjects and to delineate the impact of the number of *CYP2D6*10* alleles on the plasma concentrations of codeine and its metabolites, morphine, M6G and M3G, in these subjects.

Materials and methods

Study subjects

A single oral dose pharmacokinetic study of codeine was performed in healthy Mongolian Chinese subjects. The study was performed in accordance with the latest revisions to the Declaration of Helsinki, and ethical approval was obtained from the independent Ethics Committee of the General Hospital of Shenyang Military Region. All subjects provided their written informed consent before being enrolled in the study.

Twenty-nine healthy Mongolian Chinese subjects (15 males, 14 females, aged 20–24 years, BMI 18–24) were recruited after prescreening for *CYP2D6* genotypes. The subjects were classified into three groups as follows: Group I, *CYP2D6*1/*1*, $n=10$; group II, *CYP2D6*1/*10*, $n=10$; and group III, *CYP2D6 *10/*10*, $n=9$.

All participants were healthy according to medical history, physical examination and laboratory tests (blood chemistry, hematology and urine analysis). They were required to abstain from taking any prescription or non-prescription medications in the 2 weeks leading up to and throughout the duration of the study. They were also instructed to abstain from grapefruit, grapefruit juice, herbal dietary supplements including red yeast rice products, and caffeine-containing beverages including coffee and tea for the 3 days before the study and throughout its duration.

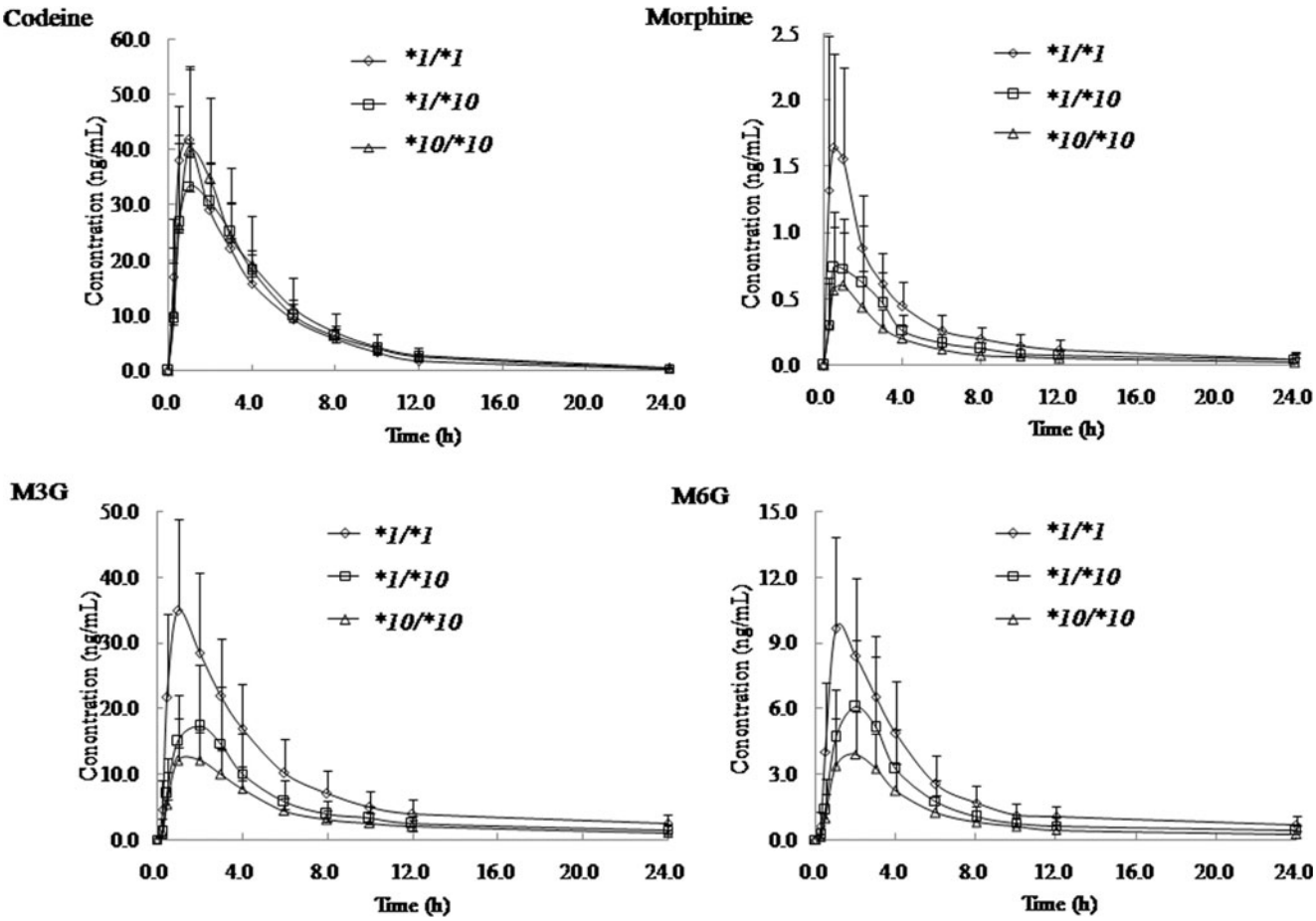

Fig. 1 Mean plasma concentration-time profiles of codeine, morphine, M3G and M6G after a single oral dose of 30 mg codeine in subjects with different *CYP2D6* genotype

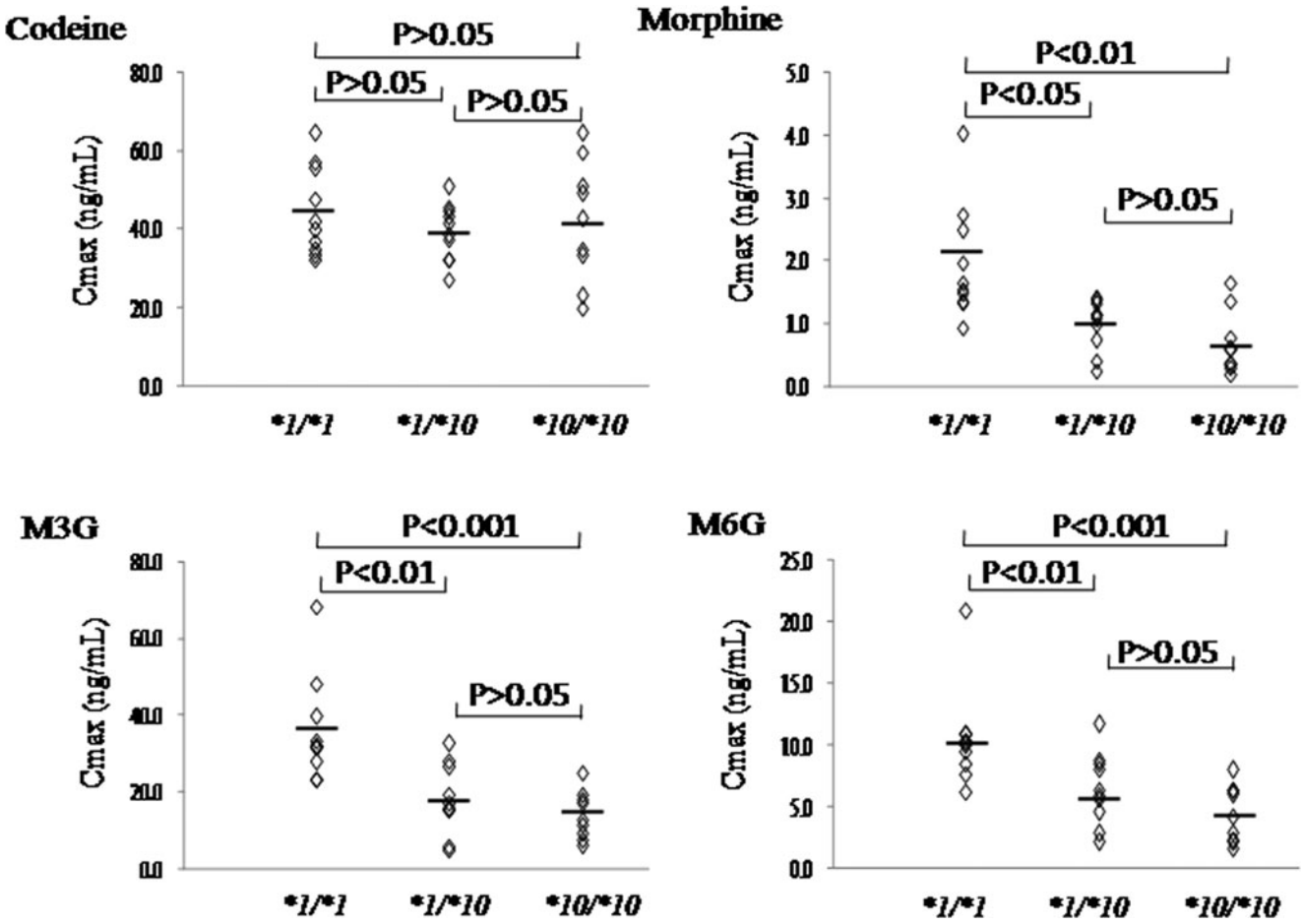

Fig. 2 Relationships between C_{max} of codeine, morphine, M3G, M6G and *CYP2D6* genotype. The horizontal bars indicate mean values

CYP2D6 genotyping

Genomic DNA was extracted from blood samples using the TIANamp Whole Blood DNA Extraction Kit (Tiangen Biotech Beijing Co., Ltd.). The *CYP2D6*10* allele was identified by the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method as described by Zuo et al. [6].

Pharmacokinetic study

After a 12 h fast, subjects were given a single codeine phosphate tablet (China National Pharmaceutical Industry Corporation Ltd.) containing the equivalent of 30 mg codeine. Blood samples (4.0 mL) were collected by venipuncture into heparinized tubes prior to dosing and at 0.25, 0.5, 1, 2, 3, 4, 6, 8, 10, 12 and 24 h after dosing. Plasma was prepared by centrifugation at 3,500 rpm for 10 min and was stored at −80 °C until analysis.

Plasma concentrations of codeine and metabolites were determined by liquid chromatography-tandem mass spectrometry (LC-MS/MS) [12] as described previously with minor revision. Briefly, 350 μl plasma was spiked with 50 μl internal standard (IS) solution (2.0 ng/ml naloxone) and subjected to solid phase extraction using an Oasis HLB extraction cartridge (1 mL, 30 mg sorbent, Waters, Eschborn, Germany). The analytes were eluted with 1 ml methanol and then evaporated to dryness in a stream of nitrogen at 50 °C. The residue was reconstituted in 100 μl methanol-0.04 % formic acid (50/50, v/v) prior to injection (30 μl) into the LC-MS system.

The LC-MS system consisted of an Agilent 1100 series HPLC (Agilent Technologies, Palo Alto, CA, USA) coupled to an Applied Biosystems Sciex API 4000 mass spectrometer (Applied Biosystems/MDS Sciex, Concord, ON, Canada) using electrospray ionization (ESI) in the positive ion mode. Chromatography was performed on a Zorbax SB-Aq C18 column (5 μm, 150×4.6 mm i.d. from Agilent Technologies) maintained at 40 °C. Gradient elution was performed using 0.04 % formic acid in purified water as solvent A and methanol as solvent B at a flow rate of 1.0 ml/min. The gradient program was as follows: 0.0–0.5 min 5 % B; 0.5–2.5 min 5 % B to 90 % B; 2.5–3.0 min 90 % B; 3.0–3.1 min 90 % B to 5 % B; 3.1–5.0 min 5 % B. An approximately 1:1 split of the column eluent was included prior to injection into the mass

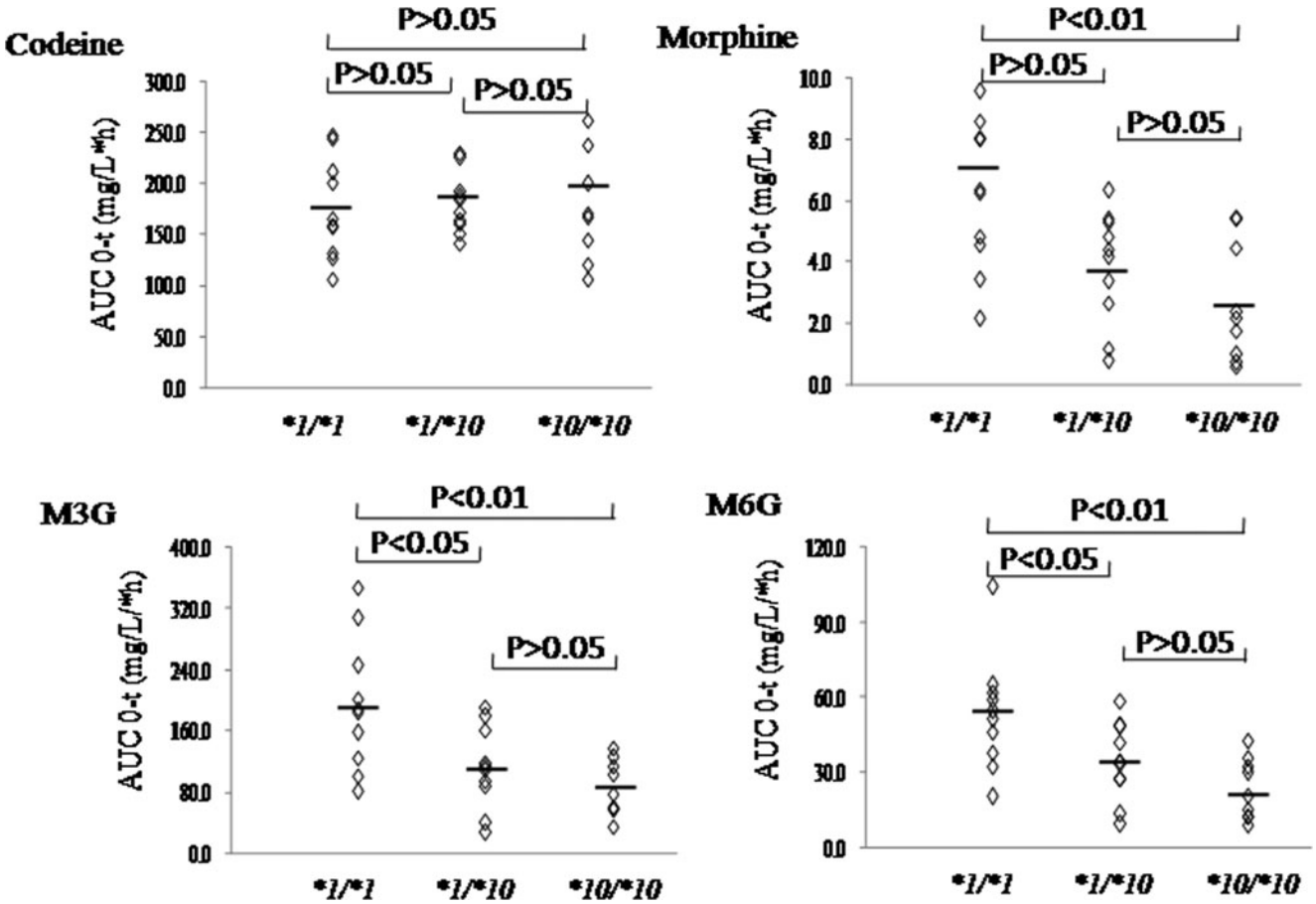

Fig. 3 Relationships between $AUC_{0\text{-}t}$ of codeine, morphine, M3G, M6G and *CYP2D6* genotype. The horizontal bars indicate mean values

spectrometer. Detection was by multiple reaction monitoring (MRM) of the following precursor-to-product ion transitions with a dwell time of 200 ms: Codeine *m/z* 300.4→215.2; morphine *m/z* 286.2→152.0; M3G and M6G *m/z* 462.2→286.2; and IS *m/z* 328.2→212.1.

The assays were linear in the range 0.05–80 ng/mL for codeine, M3G and M6G and 0.05–5.0 ng/mL for morphine with correlation coefficients (r) of>0.998 for all analytes. The lower limit of quantitation (LLOQ) for all four analytes was 0.05 ng/mL. The intra- and inter-day precision (as relative standard deviation) and accuracy (as relative error) determined using low, medium and high concentration quality control samples were≤10 % and −6.5 to 7.2 % for all analytes.

The pharmacokinetic parameters of codeine and its metabolites, including morphine, M3G and M6G, were analyzed by non-compartmental analysis using the DAS 2.1.1 software package (Mathematical Pharmacology Professional Committee of China, Shanghai, China). Peak plasma concentrations (C_{max}) of all analytes were obtained directly from the observed concentration-time data. The area under the plasma concentration-time curve ($AUC_{0\text{-}\infty}$) was calculated using the trapezoidal rule and was extrapolated to infinity. The terminal elimination rate constant (λ_Z) was determined by a linear regression of the last 4 points of the terminal elimination phase. The terminal half-life ($t_{1/2}$) was calculated as $0.693/\lambda_Z$.

Statistical analysis

All results are expressed as means ± standard deviation (SD) or means with 95 % confidence intervals (CI). Statistical analysis was performed using SPSS 17.0 for Windows (SPSS Inc., Chicago, IL, USA). Pharmacokinetic parameters of codeine and its metabolites for the three groups were compared using one-way ANOVA followed by post-hoc Bonferroni corrected *t*-tests. To evaluate trends in pharmacokinetic parameters (C_{max} and $AUC_{0\text{-}\infty}$) in relation to the number of *CYP2D6*10* alleles, the Jonckheere–Terpstra trend test was performed. A value of $P<0.05$ was considered statistically significant.

Results

All subjects completed the study and were compliant with the protocol. No clinically important adverse effects were

Table 1 Pharmacokinetic parameters of codeine, morphine, M3G and M6G according to *CYP2D6* genotype (data are means±SD)

Pharmacokinetic parameters	CYP2D6 genotype			ANOVA
	**1/*1* (*n*=10)	**1/*10* (*n*=10)	**10/*10* (*n*=9)	
Codeine				
C_{max} (ng/mL)	44.3±11.3	39.3±7.3	41.9±15.6	0.634
T_{max} (h)	0.85±0.24	1.25±0.68	1.28±0.57	
$AUC_{0\text{-}t}$ (mg/L*h)	175±49	181±30	197±81	0.687
$AUC_{0\text{-}\infty}$(mg/L*h)	178±51	183±31	199±81	0.692
$t_{1/2}$ (h)	3.45±0.83	3.68±0.55	3.89±0.71	0.392
Cl/F (L/h)	0.18±0.05	0.17±0.03	0.17±0.06	0.880
Morphine				
C_{max} (ng/mL)	2.06±0.89	0.96±0.42**	0.68±0.50***	< 0.001
T_{max} (h)	0.64±0.28	0.86±0.52	0.86±0.52	
$AUC_{0\text{-}t}$ (mg/L*h)	6.63±2.07	3.77±1.93*	2.65±1.95**	< 0.01
$AUC_{0\text{-}\infty}$(mg/L*h)	8.52±4.10	5.05±3.30	3.26±2.43*	< 0.05
$t_{1/2}$ (h)	9.40±11.7	11.5±11.1	6.84±5.46	0.598
Cl/F (L/h)	5.08±3.39	9.72±8.72	16.2±12.3*	< 0.05
M3G				
C_{max} (ng/mL)	36.0±0.89	18.2±9.0**	14.2±6.3***	< 0.001
T_{max} (h)	1.20±0.42	1.50±0.53	1.56±0.73	
$AUC_{0\text{-}t}$ (mg/L*h)	1948±86	112±54*	85.2±36.1**	< 0.01
$AUC_{0\text{-}\infty}$(mg/L*h)	209±94	134±78	109±34*	< 0.05
$t_{1/2}$ (h)	6.35±2.28	9.62±7.12	11.8±6.5*	0.126
Cl/F (L/h)	0.21±0.10	0.36±0.31	0.32±0.12	0.242
M6G				
C_{max} (ng/mL)	10.5±4.0	6.4±2.9*	4.4±1.9**	< 0.01
T_{max} (h)	1.70±0.67	2.20±1.48	1.89±0.60	
$AUC_{0\text{-}t}$ (mg/L*h)	53.2±22.7	34.2±15.7	23.3±12.1**	< 0.01
$AUC_{0\text{-}\infty}$(mg/L*h)	73.7±37.8	41.3±21.6*	27.7±15.3**	< 0.01
$t_{1/2}$ (h)	19.3±17.0	11.3±5.6	11.7±9.9	0.260
Cl/F (L/h)	0.54±0.30	1.05±0.89	1.51±0.93*	< 0.05
$AUC_{0\text{-}\infty(Morphine+M3G+M6G)}$ (moL/L*h)	0.64±0.27	0.40±0.27*	0.31±0.10**	< 0.05

*P<0.05; **P<0.01; ***P<0.001

observed. The mean plasma concentration-time profiles of codeine, morphine, M3G and M6G in the three groups are shown in Fig. 1 with corresponding pharmacokinetic parameters presented in Table 1. There were no significant differences in the C_{max} and $AUC_{0\text{-}\infty}$ of codeine but corresponding C_{max} and $AUC_{0\text{-}\infty}$ values of morphine, M3G and M6G were significantly different (P< 0.05). Compared to the *CYP2D6*1/*1* group, the $AUC_{0\text{-}\infty}$ of morphine in the **1/*10* and **10/*10* groups [(mean (95 % CI)] were 0.93 (0.26–1.59) and 0.494 (0.135–0.853), respectively. Corresponding values for M3G were respectively 0.791 (0.294–1.288) and 0.615 (0.412–0.818) and for M6G were 0.643 (0.39–0.957) and 0.423 (0.267–0.579). There was a significant trend towards higher C_{max} and $AUC_{0\text{-}\infty}$ values of morphine, M3G and M6G in individuals with more *CYP2D6*10* alleles suggesting a gene-dose effect as shown in Figs. 2 and 3. However, the differences in the levels of metabolites of codeine did not correlate with the $t_{1/2}$ and CL/F values of codeine, which is consistent with the fact that>80 % of the codeine dose is metabolized by other routes, including glucuronidation at the 6-OH position and N-demethylation to norcodeine.

Discussion

In comparison with the *CYP2D6*1* allele, *CYP2D6*10* possesses Pro34Ser and Ser486Thr amino acid substitutions that reduce its metabolic capacity for *CYP2D6*

substrates. *CYP2D6*10* appears to be the most clinically important allele in Asian (or East Asian) populations, given the high prevalence of IMs with one or two copies of the allele, but PMs are found in very low frequency in these populations. However, no formal statistical evaluation of the relationship between *CYP2D6*10* allele and codeine metabolism has appeared to date.

Kirchheiner et al. (2007) studied the pharmacokinetics of codeine in one *CYP2D6*1/*10* carrier and one *CYP2D6*2/*10* carrier but this small sample size was insufficient to accurately assess the relationship between the *CYP2D6*10* allele and codeine metabolism in vivo [13]. Tseng et al. (1996) described codeine pharmacokinetics in relation to the presence of *CYP2D6*10* but the pharmacokinetics of M3G and M6G were not included [14]. The present study focused on the effect of the *CYP2D6*10* allele on the levels of codeine and its O-demethylated metabolites.

The results show that the *CYP2D6*10* allele significantly decreases the plasma levels of morphine, M3G and M6G levels but not those of codeine. In addition, a strong correlation between the number of **10* alleles and the plasma concentrations of all O-demethylated metabolites was observed. The C_{max} and AUC values of morphine showed linear relationships with the number of **10* alleles (0, 1 and 2) (Table 1) demonstrated by the Jonckheere–Terpstra trend test ($P < 0.05$). In addition, the total concentration of morphine and its glucuronides (a measure of the total O-demethylation of codeine) was lower in the **1/*10* and **10/*10* groups by 38 % and 52 % respectively relative to the **1/*1* group. In addition, there was a significant difference in the C_{max} and $AUC_{0\text{-}\infty}$ values of the O-demethylated metabolites between wild-type **1/*1* carriers and **10* carriers, especially in those homozygous for *CYP2D6*10* ($P < 0.01$). This is expected given the decreased enzyme activity of *CYP2D6*10* toward codeine in vivo.

As the analgesic effect of codeine is mediated largely by morphine and M6G, a decrease in the O-demethylation of codeine has the potential to cause analgesic failure. This is the case for patients carrying the *CYP2D6*10* allele and particularly for those with the *CYP2D6*10/*10* genotype. Although the results of our limited pharmacokinetic study cannot predict whether a dosage adjustment is likely to be needed, it is clear that patients carrying the *CYP2D6*10* allele should be closely monitored for less than optimal response to codeine, and should be offered an alternative analgesic, such as morphine, if required. Like codeine, a number of other opioids such as tramadol, hydrocodone and oxycodone are O-demethylated by *CYP2D6* to active metabolites with much stronger μ-receptor affinity than the parent drugs [15, 16]. It is possible the findings in the present study will be valuable in terms of the clinical use of these drugs in patients with different *CYP2D6* genotypes.

Conclusion

In conclusion, this study demonstrates that the *CYP2D6*10* allele is associated with a decrease in the extent of O-demethylation of codeine in healthy Mongolian Chinese subjects. Larger studies are warranted to assess the analgesic effect of codeine in extensive and intermediate metabolizers before the need for dosage adjustment can be assessed.

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