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Effect of the cytochrome P450 2D6*10 genotype on the pharmacokinetics of tramadol in post-operative patients

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The cytochrome P450 2D6 (CYP2D6) is the most highly polymorphic isoenzyme of the cytochrome P-450-system, which affects the metabolism of one-fourth of all prescription drugs. Tramadol, a narcotic-like pain reliever used to treat moderate to severe pain, is primarily metabolized by CYP2D6. The CYP2D6*10 allele is the most common allele in the Chinese population. Therefore, we investigated the effects of CYP2D6*10 on tramadol pharmacokinetics in 45 post-operative patients who had undergone gastrointestinal tract surgery. Tramadol was administered to the patients after the operation, and the plasma concentrations of tramadol and *O*-desmethyltramadol were subsequently evaluated at 12 time points. Pharmacokinetic analyses were performed using non-compartmental methods. The area under the curve (AUC), plasma clearance (CL), elimination half-life ($T_{1/2}$), mean residence time (MRT), peak concentration, and peak time of tramadol and *O*-desmethyltramadol were calculated. CYP2D6*10 was genotyped by polymerase chain reaction-restriction fragment length polymorphism. The frequency of CYP2D6*10 alleles was 51% in the 45 patients. The patients were divided into three groups according to their CYP2D6*10 genotype: wild-type, heterozygous, and homozygous mutant. Pharmacokinetic parameters were compared among the three groups. The analyses showed that $T_{1/2}$, MRT, and AUC of tramadol were larger, and CL was lower in homozygous mutant patients compared to the wild-type group ($P < 0.05$). These results show that the CYP2D6*10 genetic polymorphism has a significant impact on the pharmacokinetics of tramadol in Chinese post-operative patients.

1. Introduction

Tramadol is a centrally acting synthetic opioid analgesic that is extensively metabolized by either CYP2D6 to *O*-demethyltramadol (M1) or CYP3A to *N*-demethyl tramadol (M2) in the human liver (Pedersen et al. 2006). The M1 metabolite is pharmacologically active. In animal models, it is up to six times more potent than tramadol in producing analgesia, and 200 times more potent in binding to the μ-opioid receptor (García-Quetglas et al. 2007). The activity of the CYP2D6 isoenzyme plays a vital role in the analgesic effect of tramadol (García-Quetglas et al. 2007; Zhou, 2009).

The current line of thinking is that the marked individual and interethnic variabilities in the activity of CYP2D6 are mainly caused by genetic polymorphisms (Frank et al. 2007; Sheng and Xiao 2008). As a result of the polymorphic expression of CYP2D6, the frequency of the poor metabolizer (PM) phenotype is 5–10% in Caucasians, mainly due to the CYP2D6*3, *4 and *5 alleles (Neafsey et al. 2009). Whereas in Asians, the PM phenotype does not exceed 2%, and virtually no CYP2D6*3 or * 4 alleles are present, although this population has the CYP2D6*14 PM allele, which has not been seen in Caucasians (Ji 2008). In the Chinese population, CYP2D6*10 is the most common allele with a frequency of 50–70%. This allele has a C188T mutation, resulting in a 9–56-fold reduction in enzyme activity (Neafsey et al. 2009; Gan et al. 2002; Wang et al. 2006). Therefore, the effects of allele polymorphisms on the blood concentration and pharmacokinetics of tramadol may ultimately influence its therapeutic effects.

Previous studies have reported that the genetic polymorphisms of CYP2D6 affect tramadol pharmacokinetics in healthy subjects (Pedersen et al. 2006; García-Quetglas et al. 2007; Gan et al. 2002; Kirchheiner et al. 2008; Allegaert et al. 2005; Yu et al. 2008; Li et al. 2009; Slanar et al. 2007). Gan et al. (2002) studied the correlation between tramadol pharmacokinetics and the CYP2D6*10 genotype in healthy orthopedic patients. Stamer et al. (2007) examined plasma concentrations of tramadol and *O*-desmethyltramadol enantiomers in post-operative patients with different CYP2D6 genotypes.

2. Investigations, results and discussion

In this study, the effect of the CYP2D6*10 genetic polymorphism on the pharmacokinetics of tramadol in post-operative patients undergoing gastrointestinal tract operation was evaluated.

All patients were compared in age, body weight, and body height using the Kruskal-Wallis test. Pharmacokinetic analysis was performed using non-compartmental methods. The area under the curve (AUC), plasma clearance (CL), elimination half-life ($T_{1/2}$), mean residence time (MRT), peak concentration (C_{max}),

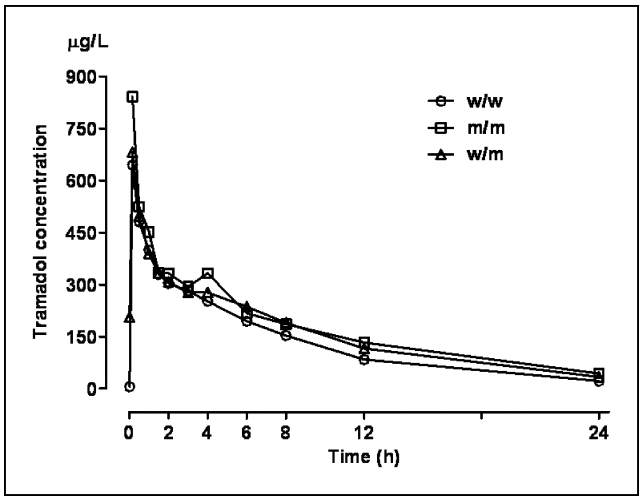

Fig. 1: Mean plasma concentration-time curve of tramadol.

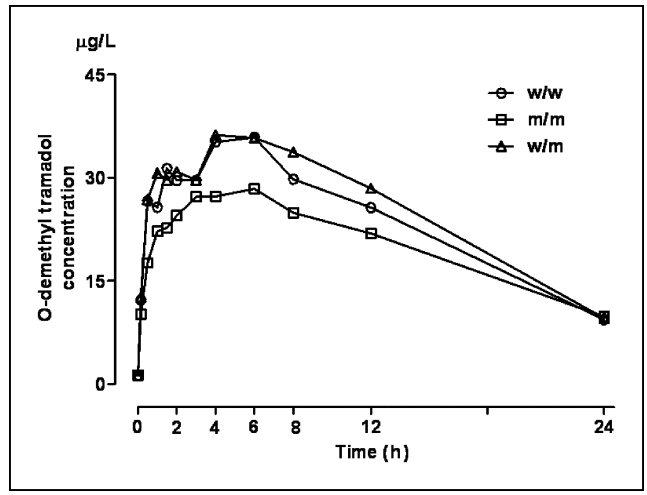

Fig. 2: Mean plasma concentration-time curve of O-demethyltramadol.

and peak time (T_{max}) of tramadol and *O*-desmethyltramadol were calculated using non-compartmental methods with DAS 2.1.1 software (Tongji Medical College, Huazhong University of Science and Technology). Results are expressed as mean values ± SD. The pharmacokinetic parameters were compared with the Mann–Whitney *U*-test when values were not distributed normally using SPSS 1.7 (IBM SPSS Statistics). P values less than 0.05 were considered statistically significant. The statistical deviation of CYP2D6*10 from Hardy–Weinberg equilibrium was determined using the chi-square goodness of fit test.

There were no statistically significant differences in age, body weight, and body height among the patients (Table). The patients were divided into three groups according to their CYP2D6*10 genotype: wild-type (w/w; n = 14), heterozygous (w/m; 16), or homozygous mutant (m/m; 15). The distribution of the CYP2D6*10 allele was consistent with Hardy–Weinberg equilibrium ($P > 0.05$).

At 0, 0.15, 0.5, 1, 1.5, 2, 3, 6, 8, 12, and 24 h, the plasma concentrations of 45 patients were added and averaged, and the mean plasma concentration-time curves of tramadol and O-desmethyltramadol were plotted according to time, as shown in Figs. 1 and 2. The $AUC_{0-\infty}$ of tramadol in the m/m group was the largest with a 43.89% increase, compared to a 15.29% AUC increase in the m/w group, the $T_{1/2}$ and MRT were longest in the m/m group, CL was the lowest in m/m group (Table). $T_{1/2}$, $Auc_{0-\infty}$ of *O*-desmethyltramadol were the largest and CL was the lowest in m/w group, MRT was the longest in m/m group.

The pharmacokinetic parameters of tramadol and *O*-demethyltramadol were compared with the Mann–Whitney *U*-test between the w/w-m/m and w/w-m/w groups at a time. The $T_{1/2}$ and MRT, and $AUC_{0-\infty}$ of tramadol were significantly larger ($P < 0.05$), and the CL was lower in m/m patients compared to w/w patients ($P < 0.05$), there were no statistically significant differences in the C_{max} and T_{max} ($P > 0.05$). The $T_{1/2}$ and $Auc_{0-\infty}$ of *O*-demethyltramadol were larger and CL was lower in m/w group compared with the w/w group ($P > 0.05$).

The CYP2D6*10 allele is the most common allele in the Chinese population, with a frequency of 50% to 70% (Gan et al. 2002; Wang et al. 2006). The present investigation revealed that the CYP2D6*10 allele frequency is 51% in mainland Chinese, which is in accordance with previously published reports (Ji 2008; Wang et al. 2006). However, the frequency of this allele in Chinese people living in Hong Kong, Taiwan, and Singapore is 64.71%, 70.0%, and 62.0%, respectively (Garcia-Barceló et al. 2000). These differences demonstrate the marked individual and interethnic variability of the CYP2D6*10 allele.

In our study, the pharmacokinetic parameters of tramadol were markedly different between the m/m and w/w groups, suggesting that the CYP2D6*10 genetic polymorphism might cause significant changes in tramadol pharmacokinetics. In addition, tramadol is mainly metabolized to M1 by CYP2D6 in human liver. Mutations in this gene causes its enzyme activity to decrease, and increases in the numbers of genetic mutations might result in significantly decreased activity of the enzyme. This may cause tramadol to not be as quickly metabolized to *O*-demethyl tramadol, thereby affecting the analgesic effect of tramadol. The CYP2D6*10 genetic polymorphism might be one of the genetic factors producing individuality.

Figure 2 and the Table show that the C_{max} and AUC of M1 of the w/m group were the highest and largest, followed by the m/m and w/w groups, The CYP2D6*10 genetic polymorphism might be one of the genetic factors producing individuality, but CYP2D6*10 alleles did not affect the pharmacokinetics of *O*-demethyltramadol. This might be due to the fact that *O*-demethyltramadol is mainly eliminated in the liver and kidney, which might contribute to individual differences of elimination. In a previous study, Stamer et al. (2007) reported the blood concentrations of tramadol and M1 in patients with different CYP2D6 genotypes who had recovered from major abdominal surgery, and found that the CYP2D6 genotype influenced the efficacy of tramadol treatment. In most previous investigations that included healthy volunteers, CYP2D6 genetic polymorphisms influenced tramadol pharmacokinetics. These findings appear to explain the differences in the disposition of tramadol. Therefore, combining CYP2D6 inhibitors with tramadol may lead to an improvement in the clinical outcome of pharmacotherapy. Genotyping information might provide guidance for determining the appropriate dose of CYP2D6 to administer to different patients.

Numerous studies have focused on the effect of CYP2D6 genetic polymorphisms on tramadol pharmacokinetics in healthy volunteers (Pedersen et al. 2006; García-Quetglas et al. 2007; Gan et al. 2002; Kirchheiner et al. 2008; Allegaert et al. 2005; Yu et al. 2008; Li et al. 2009; Slanar et al. 2009), however, few studies have investigated the impact of CYP2D6 polymorphisms on tramadol in patient populations. This trial investigated the patient suffering from various medical conditions and taking other drugs according to the clinical conditions of diseases.

The frequency of CYP2D6*3, *4, *5, and *6 genotypes is very low in Chinese populations (Neafsey et al. 2009; Ji 2008; Ji et al. 2002), so in this trial, we focused on the most frequent genotype, CYP2D6*10. Our results demonstrate that this genetic polymor-

Table: Demographic data and pharmacokinetic parameters

Group	w/w(n = 14)	m/w(n = 16)	m/m(n = 15)	*p-value*
Age(year)	53.57 ± 12.71	51.56 ± 10.22	53.87 ± 10.03	0.856
Sex (m/f)	8/6	8/8	6/9	0.607
Body height (cm)	163.86 ± 8.07	162.19 ± 5.91	161.47 ± 7.19	0.533
Body weight (kg)	58.18 ± 7.67	56.03 ± 5.75	60.57 ± 9.53	0.428
BMI	21.67 ± 2.46	21.33 ± 2.19	23.21 ± 3.08	0.102
Tramadol				
$T_{1/2}$(h)	5.50 ± 2.01	7.04 ± 2.35	8.54 ± 4.038	0.009[a] 0.061[b]
$AUC_{0-\infty}$ (μg/l·h)	3903.07 ± 1167.27	4500.73 ± 1221.38	5616.03 ± 2058.75	0.032[a] 0.135[b]
CL (L/h·kg)	28.17 ± 10.50	24.25 ± 8.43	20.34 ± 7.73	0.032[a] 0.135[b]
MRT (h)	7.83 ± 1.99	9.64 ± 3.02	11.86 ± 5.50	0.005[a] 0.088[b]
Cmax (μg/l)	643.82 ± 169.97	681.69 ± 192.91	719.36 ± 231.89	0.485[a] 0.430[b]
Tmax (h)	0.29 ± 0.25	0.25 ± 0.17	0.22 ± 0.14	0.539[a] 0.768[b]
M1				
$T_{1/2}$(h)	8.73 ± 2.78	11.92 ± 7.38	11.34 ± 5.91	0.295[a] 0.299[b]
$AUC_{0-\infty}$(μg/l·h)	624.27 ± 305.53	870.06 ± 422.92	753.01 ± 257.99	0.383[a] 0.244[b]
CL (l/h·kg)	232.32 ± 178.20	133.76 ± 43.82	146.48 ± 44.09	0.383[a] 0.244[b]
MRT (h)	13.52 ± 3.54	18.17 ± 10.62	18.28 ± 9.34	0.055[a] 0.157[b]
Cmax (μg/l)	40.16 ± 14.13	42.72 ± 15.03	36.89 ± 16.90	0.432[a] 0.835[b]
Tmax (h)	2.83 ± 1.81	3.26 ± 2.78	4.87 ± 3.26	0.087[a] 0.768[b]

[a] p-value of Mann–Whitney U-test between w/w-m/m
[b] p-value of Mann–Whitney U-test between w/w-m/w

phism influences tramadol pharmacokinetics in post-operative patients.

3. Experimental

Forty-five patients (22 males and 23 females) scheduled for gastrointestinal tract surgery were selected. Enrollment criteria included an American Society of Anesthesiologists grade I to II, age range of 35 to 75 years old, body mass index less than or equal to 30, and the ability to speak and read Mandarin Chinese. Subjects were excluded if they were addicted to drinking, smoking, drug addiction, and tolerance before, had epilepsy, a history of psychotic disorders, had abnormalities in liver or renal function, or were using drugs that inhibit or induce CYP2D6 and CYP3A4 enzyme activities. The genotyping has been described previously (Wang et al. 1993).

After a single bolus injection of 100 mg tramadol in the post-operative patients, 2 ml peripheral blood was collected at 0, 0.15, 0.5, 1, 1.5, 2, 3, 6, 8, 12 and 24 h. After centrifugation for 10 min at 4000 x g, the plasma was stored at –80 °C for subsequent analyses.

Tramadol and *O*-demethyltramadol plasma concentrations were determined using high performance liquid chromatography (HPLC), mass spectrometry, TSQ Quantum Access MS/MS mass spectrometry, Lequan HPLC Mass Spectometer Workstation (Themo Scientific Co, USA), and Zorbax SB-C_{18} chromatographic columns (2.1 mm × 50 mm ID, 1.8 μm) (Agilent Technologies). The mobile phase consisted of 0.1% formic acid and 5 mM ammonium acetate solutions (0.1% formic acid acetonitrile solutions and gradient elution). The flow rate of the mobile phase was 0.3 ml/min, the column temperature was at 30 °C, and atmospheric pressure chemical ionization (APCI) mass spectrometry was carried out. Reaction ions monitoring (SRM), positive ion mode, discharge current 4.0 V, vaporization temperature 400 °C, sheath gas (N_2) pressure: 40 Arb, auxiliary gas (N_2) pressure: 8 Arb, capillary temperature 280 °C, Collision gas pressure 1.0 mTorr, the ionic reaction of quantitative analysis were respectively m/z tradamol: 264.2→58.6, *O*-demethyltramadol: 250.0→58.6, fluconazole: 307→220; collision energy: tradamol 32 eV, *O*-demethyltramadol 16 eV, fluconazole 17 eV, scan times 0.1 s, scan width (m/z): 0.01.

Briefly, 50 μl plasma were added to 150 μl of the acetonitrile solutions containing 10 ng/ml of the internal standard fluconazole, and then mixed for 20 s and centrifuged at 20000 x g for 10 min at 4 °C. The supernatant (100 μl) was added to empty vials, and 10 μl was used for injections.

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