**CYP3A4*1G genetic polymorphism influences CYP3A activity and response to fentanyl in Chinese gynecologic patients**

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Received: 2 February 2009 / Accepted: 31 August 2009 / Published online: 26 September 2009 © Springer-Verlag 2009

**Abstract**

**Purpose** To investigate whether the CYP3A4*1G genetic polymorphism contributes to the variability in CYP3A activity and response to fentanyl.

**Methods** One hundred and forty-three gynecologic patients who were scheduled to undergo abdominal total hysterectomy or myomectomy with general anesthesia were enrolled in this study. Intravenous fentanyl patient-controlled analgesia was provided postoperatively for satisfactory analgesia. The degrees of pain at rest during PCA treatment were assessed with visual analog scale. The fentanyl consumption and occurrence of any adverse effects were recorded in the first 24 h postoperatively. CYP3A activity was measured by plasma 1'-hydroxymidazolam-to-midazolam ratio 1 h after intravenous administration of 0.1 mg/kg midazolam. CYP3A4*1G variant allele was genotyped using the polymerase chain reaction–restriction fragment length polymorphism method.

**Results** The frequency of the CYP3A4*1G variant allele was 0.269 in 143 Chinese gynecologic patients. The activity of CYP3A4 in patients homozygous for the *1G/*1G variant (0.34±0.15) was significantly lower than that in patients bearing the wild-type allele (*1/*1) (0.46±0.14) or in patients heterozygous for the *1/*1G variant (0.46±0.12) (P<0.05). The patients with the CYP3A4*1G/*1G genotype needed less fentanyl (227.8±55.2 μg) to achieve pain control than patients carrying the CYP3A4*1/*1 (381.6±163.6 μg) and CYP3A4*1/*1G (371.9±180.1 μg) genotypes (P<0.05) during the first 24 h postoperatively. There was no significant difference in incidence of adverse events among the different genotype groups (P>0.05).

**Conclusions** CYP3A4*1G genetic polymorphism decreases CYP3A activity and fentanyl consumption for postoperative pain control.

**Keywords** CYP3A · Phenotype · Polymorphisms · Fentanyl · Analgesia

**Introduction**

Fentanyl is a synthetic opioid that has been widely used in clinical practice for over 40 years, and it is especially effective for induction and maintenance of anesthesia or as an analgesic. However, the effective dose of fentanyl for pain control differs greatly among individuals, suggesting the presence of marked interindividual variability in the response to fentanyl treatment. Gourlay et al. [1] have reported there was a fivefold individual variation in the mean hourly fentanyl dosage requirements among patients having abdominal operations. The metabolism of fentanyl is well characterized, and the drug is metabolized in the liver predominantly by the cytochrome P450 3A4 (CYP3A4) [2]. CYP3A4 protein expression in the liver may differ up to 40-fold, leading to variations in drug metabolism and contributing to differences in individual response to the drug [3].

Interindividual variability in clinical effects of fentanyl may also result from differences in CYP3A activity due to
CYP3A4 genetic variation. Several studies have suggested that 30–85% of the interindividual variability in CYP3A4 activity is predominantly attributed to genetic factors [4, 5]. Single nucleotide polymorphisms (SNPs), as the most common form of genetic variation in the CYP3A genes, may contribute to interindividual variability in CYP3A activity and responses to fentanyl for intravenous analgesia. CYP3A4*1G is a high frequency allele in Asians, with an allele frequency of 0.249 in the Japanese [6] and 0.221 in the Chinese [7]. The CYP3A4*1G allele has been suggested to affect CYP3A activity [8], however, up to now, there has not been conclusive evidence about any effect of the CYP3A4*1G allele on the therapeutic efficacy of drugs metabolized by CYP3A [9]. There has also been no previous report on the effect of CYP3A4*1G on individual responses to fentanyl in analgesia control.

Because CYP3A4 is an important enzyme in the metabolism of fentanyl and many other drugs and CYP3A4*1G is a high frequency allele in Asians, characterization of the effects of this particular allele on fentanyl metabolism is clinically important as it will help predict factors that affect individual response to the drug. Here, we report our investigation of the impact of CYP3A4*1G polymorphism on CYP3A activity and the efficacy of fentanyl in patient-controlled analgesia (PCA).

Methods

Patients

One hundred and forty-three patients, aged 20–50 years, within ±20% of ideal body weight, and having an American Society of Anesthesiologists (ASA) physical status of I or II, undergoing selective abdominal total hysterectomy or myomectomy with general anesthesia were enrolled. The study design was approved by the Institutional Ethics Committee of Zhengzhou University, and signed informed consent was obtained from all patients. All patients were of Chinese Han nationality and live in Henan province of China. Exclusion criteria included the following: known history of psychiatric disease, significant cardiovascular disease, diabetes mellitus, alcohol or drug abuse (according to the criteria of DSM-IV), chronic analgesic use, pregnancy or nursing. Patients were also excluded from study participation for a history or laboratory evidence of liver disease (aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, bilirubin, or lactate dehydrogenase greater than two times the upper limit of normal) or renal disease (serum creatinine of >1.8 mg/dL). Patients who had consumed drugs or foods known to inhibit or induce the expression of CYP3A enzymes 2 weeks prior to surgery were also excluded. These drugs or foods included but were not limited to clarithromycin, erythromycin, grapefruit juice, HIV protease inhibitors, itraconazole, verapamil, rifampicin, phenytoin, carbamazepine, dexamethasone, and phenobarbital. The surgical procedures were performed by the same group of similarly experienced gynecologic surgeons throughout the study.

Anesthetic technique

A standardized general anesthesia protocol was used for all patients. We administered 0.1 mg/kg midazolam, 0.5 mg/kg propofol, 2 μg/kg remifentanil, and 0.6 mg/kg atracurium for induction of anesthesia. End tidal carbon dioxide was maintained between 35 and 40 mmHg by mechanical ventilation. Then 0.1–0.2 mg/kg atracurium was administered by repeated boluses and remifentanil (0.1–0.2 μg/kg/min) and propofol (6 to 8 mg/kg/h) were infused for maintenance of anesthesia.

Half an hour before completion of the surgery, a loading dose of 1 μg/kg fentanyl was given intravenously. At the end of the surgery, neuromuscular blockade was reversed with 1 mg neostigmine and 0.5 mg atropine, and the patient was extubated.

Assessment of postoperative pain

All patients received intravenous PCA with fentanyl once they were stable and awake, and reported a score >3 on a visual analog scale (VAS, 0 = no pain, 10 = unbearable pain). Postoperative PCA (1 mg fentanyl and 5 mg droperidol in 100 ml normal saline) was administered using a computer-controlled infusion pump (CADD-Legacy 6300; Deltec) programmed to give a 20-μg bolus of fentanyl solution with a lockout time of 5 min, 5 μg/h background infusion, and a maximum dose of 145 μg within a 1-h period [10, 11]. The total amount of PCA fentanyl over the first postoperative 24 h was recorded by the pump automatically.

VAS was used for assessing pain at rest during PCA every 5–10 min for the first 30 min, and 30–60 min thereafter for the first 24 h. Successful analgesia was defined as a VAS score ≤3. If the maximum permitted dose of fentanyl was reached and the patient still reported a VAS score >3, tramadol 100 mg or meperidine 1.5 mg/kg was prescribed for rescue pain control, and the patient was subsequently excluded from the study. The VAS score and incidence of any adverse effects such as nausea, vomiting, respiratory depression, and sedation in the first 24 h were recorded.

CYP3A activity

CYP3A activity was measured by determining the plasma ratio of 1′-hydroxymidazolam to midazolam 1 h after intravenous administration of 0.1 mg/kg midazolam for induction of anesthesia. Peripheral blood sample (5 ml) was collected from
each patient in heparinized tubes. After centrifugation at 3,000 rpm at 4°C for 10 min, harvested plasma was stored at −80°C until analysis. Midazolam and 1′-hydroxymidazolam concentrations were determined using liquid chromatography–mass spectrometry as Kanazawa et al. described [12] (Fig. 1).

Genotyping assays

Venous blood samples (2 ml) were collected from all patients in this study. DNA was extracted from leukocytes using a standard phenol/chloroform procedure. The CYP3A4*1G polymorphism was determined as described by Gao et al. [8]. The detection of CYP3A4*1G by polymerase chain reaction-restriction fragment length polymorphism is shown in Fig. 2.

Statistical analysis

Statistical analysis was performed with SPSS software (version 11.0; SPSS, Chicago, IL, USA). Values were reported as mean ± SD. A two-tailed P-value of <0.05 was considered to be statistically significant. Data were compiled according to the genotype, and allele frequencies were estimated from the observed numbers of each specific allele. Chi-squared test was used to verify Hardy–Weinberg equilibrium. Differences between groups were analyzed with the use of t-test and variance analysis. To test the effect of genotype on PCA fentanyl consumption, one-way analysis of variance with post-hoc Bonferroni correction for multiple comparisons was performed before and after adjustment for age, weight,
remifentanil dose during the operation, and type of surgery. The incidences of any adverse effects were analyzed using chi-squared test or Fisher exact test.

Results

In the present study, we carried out genotyping analysis of CYP3A4*1G polymorphism in 143 patients undergoing elective surgery in our medical institution. No one needed rescue management for inadequate pain control. Among these subjects, we identified three genotype groups with 75 wild-type homozygotes (*1/*1), 59 heterozygotes (*1/*1G), and 9 mutant homozygotes (*1G/*1G). The frequency of CYP3A4*1G allele was 0.269 (77/286, 95% CI 0.196, 0.342) in these patients. The allele frequency was in Hardy-Weinberg equilibrium (χ²=0.337, P>0.05). No significant differences were detected in demographic data among the groups (P>0.05) (Table 1). The fentanyl consumption was 366.6±170.7 and 372.5±167.6 µg in patients undergoing hysterectomy and myomectomy, respectively. There was no significant difference in fentanyl consumption between the two surgery type groups using t-test analysis.

We further examined the effect of CYP3A4*1G genetic polymorphism on CYP3A activity by measuring the plasma ratio of 1'-hydroxymidazolam to midazolam using the method of Kanazawa et al. [13]. The method showed a linear range of detection from 0.1 to 5.0 µg/ml for midazolam or 1'-hydroxymidazolam with the limit of detection at 1.0 ng/ml for both midazolam and 1'-hydroxymidazolam. The recovery of midazolam and 1'-hydroxymidazolam using solid-phase extraction was 100.9 and 99.4%, respectively, and the within- and between-day coefficients of variation were less than 6%. We found that the activity of CYP3A (mean ± SD) as indicated by the plasma ratio of 1'-hydroxymidazolam to midazolam was 0.45±0.13 (95% CI 0.43, 0.48; range 0.03–0.82). In addition, the Kolmogorov–Smirnov test revealed that the CYP3A activities were normally distributed (P>0.05). The CYP3A activity (mean ± SD) in the three genotype groups was 0.46±0.14 (*1/*1), 0.46±0.12 (*1/*1G), and 0.34±0.15 (*1G/*1G). Furthermore, the CYP3A activity of the *1G/*1G group was significantly lower than that of the *1/*1 or *1/*1G groups (P<0.05). No significant difference was found in CYP3A activity between the *1/*1 and *1/*1G groups (P>0.05) (Table 2).

We also analyzed pain perception in the participants by using the VAS score. We found that the VAS score (mean ± SD) was 6.2±1.3 (range 3–9) immediately after surgery and 2.1±0.7 (range 1–3) 24 h postoperatively. There was no significant difference across the genotypes (P>0.05). The fentanyl consumption (mean ± SD) was 367.9±169.5 µg (95% CI 339.9, 395.9; range 140.0–980.0 µg) in the first 24 h postoperatively. PCA fentanyl consumption (mean ± SD) was 381.6±163.6 µg in the *1/*1 group, 371.9±180.1 µg in the *1/*1G group, and 227.8±55.2 µg in the *1G/*1G group in the first 24 h. After adjustment for age, weight, remifentanil dose during the operation, and type of surgery, covariance analysis showed a significant influence of CYP3A4*1G polymorphism on fentanyl consumption. There was a significant difference in fentanyl consumption among the three groups in the first 24 h postoperatively (P<0.05). Patients in the *1G/*1G group consumed significantly less fentanyl than patients in either the *1/*1 group or the *1/*1G group (P<0.05) (Table 2).

In the first 24 h postoperatively, postoperative nausea and vomiting (PONV) occurred in 41 patients (28.7%), including 4 out of 9 patients (44.4%) in the *1G/*1G group, 15 out of 59 patients in the *1/*1G group (25.4%), and 22 out of 75 patients (29.3%) in the *1/*1 group. Mild pruritus occurred in only one patient in the *1/*1 group (0.7%). There was no significant difference in incidences of PONV between the *1G/*1G and *1/*1G groups (χ²=1.403, P=0.253), between the *1G/*1G and *1/*1 groups (χ²=0.859, P=0.449), or between the *1/*1G and *1/*1 groups (χ²=0.253, P=0.615).

Discussion

This study examined the impact of the CYP3A4*1G polymorphism on CYP3A activity and fentanyl consumption for pain control. In this study, we found that the
frequency of the *CYP3A4*1G variant allele was 0.269 in 143 Chinese gynecologic patients. Using midazolam as a probe, our study demonstrated that patients with the *CYP3A4*1G/*1G genotype exhibited significantly lower activity than patients with the *CYP3A4*1/*1 genotype and *CYP3A4*1/*1G genotype. Our findings also indicated that patients with the *CYP3A4*1G/*1G genotype need less fentanyl to achieve the same level of pain control compared with patients carrying the *CYP3A4*1/*1 and *CYP3A4*1/*1G genotypes.

CYP3A is an important liver enzyme involved in the metabolism of many clinically useful drugs including fentanyl [2]. Variations in the protein expression of CYP3A or genetic polymorphisms of *CYP3A* exert meaningful effects on patients’ responses to drugs metabolized by the enzyme [3, 13]. In healthy Chinese subjects, there is a 13-fold variation in CYP3A activity [14]. The *CYP3A4*1G variant is a high frequency allele in Asians and is one of the most frequent alleles of the *CYP3A4* gene in Chinese [15]. Our findings show that the frequency of this particular allele is 0.269 in the Chinese gynecologic patients whom we studied, which is similar to the results reported previously in a Japanese population and in a Chinese healthy population [6, 7].

Midazolam, which is metabolized to 1′-hydroxymidazol- bol by CYP3A, is a well established drug that has been used to assess the activity of CYP3A [4, 16]. Other studies [17, 18] have demonstrated that the ratio of 1′-OH-MDZ to MDZ plasma concentrations after intravenous administration can be used as a reliable index of CYP3A hepatic activity. We show here that patients homozygous for *1G/*1G variant exhibit significantly lower activity than patients homozygous for the wild-type CYP3A (*1/*1) allele or heterozygous for *1/*1G variant. Kharasch et al. [19] found that alterations in hepatic CYP3A activity were associated with noticeable changes in the metabolism of fentanyl. The reduction in CYP3A activity in our patients homozygous for the *1G/*1G variant suggests that these patients may be more sensitive to fentanyl because of reduced metabolism of the drug. Our analysis indicates that patients with the *CYP3A4*1G/*1G genotype require significantly less fentanyl for postoperative pain control than patients either in the *CYP3A4*1/*1 group or in the *CYP3A4*1/*1G group. The decreases in CYP3A activity

### Table 2

Association of *CYP3A4*1G polymorphism with postoperative pain and fentanyl consumption and CYP3A activity in gynecologic patients

<table>
<thead>
<tr>
<th></th>
<th>All subjects</th>
<th>*1/*1</th>
<th>*1/*1G</th>
<th>*1G/*1G</th>
<th>P value</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>ANOVA</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>*1/*1 vs. *1/*1G</td>
<td>*1/*1 vs. *1G/*1G</td>
<td>*1G/*1G vs. *1G/*1G</td>
<td></td>
</tr>
<tr>
<td>Initial postoperative VAS scores</td>
<td>6.2±1.3</td>
<td>6.2±1.3</td>
<td>6.1±1.3</td>
<td>6.1±1.2</td>
<td>0.846</td>
</tr>
<tr>
<td>Mean VAS scores during first 24 h</td>
<td>2.1±0.7</td>
<td>2.2±0.7</td>
<td>2.1±0.7</td>
<td>2.1±0.6</td>
<td>0.879</td>
</tr>
<tr>
<td>Fentanyl consumption during first 24 h (μg)</td>
<td>367.9±169.5</td>
<td>381.6±163.6</td>
<td>371.9±180.1</td>
<td>227.8±55.2</td>
<td>0.039</td>
</tr>
<tr>
<td>CYP3A activity (1′-OH-MDZ:MDZ)</td>
<td>0.45±0.13</td>
<td>0.46±0.14</td>
<td>0.46±0.12</td>
<td>0.34±0.15</td>
<td>0.045</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SD

VAS Visual analog scale, *1/*1 wild-type homozygote, *1/*1G mutant heterozygote, *1G/*1G mutant homozygote

*P* values are adjusted by age, weight, remifentanil dose during the operation, and type of surgery
and the reduction in the dose of fentanyl for pain control, furthermore, are not correlated with clinical demographic features of these patients such as age and body weight, and these reductions are also not correlated with the type of surgery that these patients received. We assume that decreased CYP3A activity in mutant homozygotic patients results in slower drug metabolism and subsequently higher plasma concentrations of fentanyl. Our finding is consistent with Gao et al.’s studies in the hyperlipidemic population [8]. They found the subjects with CYP3A4*1G/*1G genotype showed a higher lipid-lowering efficacy of atorvastatin compared with those with CYP3A4*1/*1G genotype or CYP3A4*1/*1 genotype. However, Hu et al. [20] found that the CYP3A4*1B (actually called CYP3A4*1G) allele may be associated with the increased level of CYP3A4 activity. Further studies with larger sample size are required to confirm our findings.

Other alleles of CYP3A gene such as CYP3A5 may also have an impact on the metabolism of fentanyl for CYP3A4 and CYP3A5 have overlapping substrate specificity. Impaired metabolism of fentanyl, for instance, was observed in subjects homozygous for CYP3A5*3 variant [21]. These findings together illustrate the need for and the importance of characterizing the effect of CYP3A polymorphisms on the metabolisms of clinically important drugs such as fentanyl as the findings from such studies will help predict patients’ response to these drugs and avoid the occurrence of toxicity in those patients with impaired drug metabolisms.

In summary, we present the first direct evidence that the CYP3A4*1G variant allele is associated with decreased CYP3A4 activity in vivo and reduction in postoperative fentanyl consumption in gynecologic patients. Our findings are of particular relevance to Chinese patients receiving postoperative analgesia as the variant allele is of high frequency in the Chinese population.

Acknowledgments This work was supported by grants from Medical Science and Technology Research Projects of Henan Province (No.200703018). The authors acknowledge the technical assistance of Shu-Sheng Zhang (Department of Chemistry, Zhengzhou University) in carrying out the LC-MS analysis.

References