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Original Article

The cytochrome P450 2D6*10 genetic polymorphism alters postoperative analgesia

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Abstract: The present study was aimed to investigate the effects of the cytochrome P450 (*CYP*) 2D6*10 genetic polymorphism on postoperative patient-controlled morphine usage. A total of 114 patients were selected, and 102 patients completed the study. Polymerase chain reaction-based restriction fragment length polymorphism (PCR-RFLP) was used to determine the CYP2D6*10 genotype, and patients were categorized into three groups according to CYP2D6 genotype: heterozygous (m/w), wild-type homozygous (w/w), and mutant homozygous (m/m). Total morphine usage and visual analogue score (VAS) were determined 72 hours after the operation and compared across the three genotype groups. Statistical methods used to analyze results were the χ^2 test, analysis of variance, and multiple linear regression analysis; $P<0.05$ was considered to be statistically significant. The cumulative use of morphine in the m/w group was significantly higher than that in the m/m group between $T_{0.5}$ and T_{4h} ($P<0.05$). There were no significant differences in the loading dose of morphine or VAS among the different genotypes within 72 hours of operation. Patients carrying the CYP2D6*10 m/w genotype required higher doses of morphine at $T_{0.5}$~T_{4h} compared to the m/m group, and therefore received a higher cumulative dose of morphine post-operation. This phenomenon may be due to a decreased ability to synthesize endogenous opioid peptide.

Keywords: Cytochrome P450, CYP2D6*10, genetic polymorphisms, morphine, postoperative analgesia

Introduction

Cytochrome P450 (CYP450) is a heme-thiolate monooxygenase produced in the liver and intestinal tract and involved in the metabolism of various substances, including environmental compounds and drugs. Within the superfamily of hemoproteins, there are multiple CYP subfamilies, which include the enzymes CYP3A4, CYP3A5, CYP2D6, CYP2C9, and CYP2C19 [1].

CYP450 has been shown to play an important role in the production of endogenous opioid peptides (EOPs), produced by polymorphonuclear leukocytes (PMNs); CYP2D6 and CYP450 are responsible for opioid metabolism [2]. Unlike local anesthetics, non-steroidal anti-inflammatory drugs, and other opioids, EOPs have no side effects (like respiratory depression, vomiting, or addiction) [3]. Since EOPs and morphine utilize the same receptors [4], an individual's EOP levels can be estimated by measuring the amount of morphine [5, 6]. This study sought to explore the difference in EOP levels produced by individuals harboring the CYP2D6*10 polymorphism versus wild-type CYP2D6 after undergoing general anesthesia, thereby providing a basis for individualized pain management options.

Study participants and research methods

Study participants

In this prospective study, 114 patients of Han nationality receiving general anesthesia in the Affiliated Haikou Hospital of Xiangya Medical College in Central South University between January 2014 and September 2014 were selected randomly to participate. 49 (42.98%) were male and 65 (57.02%) were female; mean patient age was 57±12.73 years. Patients

Table 1. Study participant information [($\bar{X}$±s), (n, %)]

Variable	Genotype			*F* or χ^2	*P*
	m/w (N=38)	w/w (N=34)	m/m (N=30)		
Age (years)	57.31±12.05	53.94±12.89	52.17±10.13	2.816	0.094
Gender				4.759	0.093
Male	15 (39.47)	22 (64.71)	14 (46.67)		
Female	23 (60.53)	12 (35.29)	16 (53.33)		
ASA				0.365	0.833
I	7 (18.42)	5 (14.71)	4 (13.33)		
II	31 (81.58)	29 (85.29)	26 (86.67)		
Operative site					0.401*
Abdominal	6 (15.79)	2 (5.88)	4 (13.33)		
Spinal	32 (84.21)	32 (94.12)	26 (86.67)		
Smoking				5.571	0.062
Yes	27 (71.05)	18 (52.94)	13 (43.33)		
No	11 (28.95)	16 (47.06)	17 (56.67)		
Alcohol				2.956	0.228
Yes	25 (65.79)	16 (47.06)	15 (50.00)		
No	13 (34.21)	18 (52.94)	15 (50.00)		
BMI (kg/m^2)	23.81±3.29	23.60±3.05	23.27±3.31	2.930	0.085
Surgery duration (min)	165±79	139±69	124±68	2.785	0.103
Anesthesia duration (min)	173±63	181±64	148±69	2.721	0.114

Note: *Fisher's exact test.

Table 2. Cumulative doses of morphine PCA at each time point among genotypes (mg)

Genotype	$T_{0.5h}$	T_{4h}	T_{12h}	T_{24h}	T_{48h}	T_{72h}
m/w (N=38)	7.66±1.30	10.51±2.84*	13.21±4.97	14.83±5.99	18.58±7.96	19.82±8.31
w/w (N=34)	7.28±1.21	9.86±2.86	12.96±4.60	14.85±6.11	17.54±6.70	18.98±7.64
m/m (N=30)	7.40±1.15	8.70±1.49	12.04±4.48	14.36±6.81	16.93±7.37	18.40±7.95

Note: *Compared with group m/m, P<0.05.

included in the study agreed to use morphine for 72 hours post-surgery, which was done under general anesthesia. All participants were between 30 and 70 years old, weighed 45-85 kg, and fit under the American Society of Anesthesiologists (ASA) class I or II. No patients had history of alcohol abuse, drug addiction, epilepsy, mental illnesses, or severe cirrhosis, and had liver function classified as Child-Pugh class A. Patients that did not receive postoperative analgesia with morphine, who had a history of chronic pain or long-term oral administration of drugs, had severe cardiopulmonary dysfunction, experienced reoperation within 3 months of surgery, and having depression or other mental illness were excluded from the study. The surgical procedures included open abdominal surgery and surgery involving the extremities. The patients receiving surgeries that caused severe pain were excluded. Ultimately, 102 patients completed the trial, of whom 36 underwent intestinal surgery, 27 underwent gastric surgery, 5 underwent spleen surgery, 4 underwent hip joint surgery, and 8 underwent lumbar surgery. The differences among surgeries were not statistically significant. This study was approved by the Affiliated Haikou Hospital of Xiangya Medical College in Central South University Ethics Committee and informed consent was obtained from all participants.

Methods

Genotyping

Before surgery, 2 mL of venous blood were collected from each patient and added to a tube

Table 3. VAS among genotypes

Genotype	$T_{0.5h}$	T_{4h}	T_{12h}	T_{24h}	T_{48h}	T_{72h}
m/w (N=38)	2.87±1.90	2.21±0.86	2.20±0.78	2.19±0.83	1.69±0.85	1.46±0.69
w/w (N=34)	3.29±2.21	1.90±0.76	1.97±0.84	1.90±0.71	1.51±0.70	1.36±0.80
m/m (N=30)	3.09±2.08	2.29±1.16	1.98±1.08	1.79±0.96	1.47±0.98	1.49±0.98

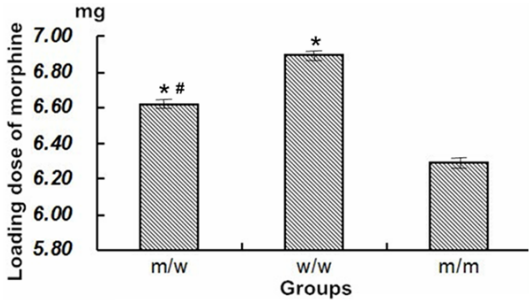

Figure 1. PCA morphine doses among CYP2D6 genotypes. Note: *Compared with group m/m, P>0.05, #Compared with group w/w, P>0.05.

with an anticoagulant. The blood and the anticoagulant were fully mixed and stored at -20°C until ready for use. DNA was extracted from patients' whole blood according to the instructions in the Whole Blood Genomic DNA Purification Mini Kit (TaKaRa Code D332A, TaKaRa Bio. Dalian, China). CYP2D6*10 was genotyped using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). The sequences of the forward and reverse primers were as follows: (F) 5'-ATCATCAGCTCCCTTTATAAG-3' and (R) 5'-CTGTGGTTTCACCCACC-3', respectively. The PCR conditions used here included an initial incubation for 5 min at 95°C, and followed by 32 cycles of 30 sec at 95°C, 10 sec at 59°C and 20 sec at 72°C. After a final incubation for 10 min at 72°C, the amplicons were analyzed by agarose electrophoresis (1.0%). The PCR product was digested by Hph I enzyme to determine the genotypes. Possible genotypes were heterozygous (m/w), homozygous wild-type (w/w), and homozygous mutant (m/m). This was a double-blind study in which all participating surgeons, anesthesiologists, and other medical workers did not know patients' genotypes when administering anesthesia.

Anesthesia and analgesia

For surgery, 1-2 mg of Midazolam (Jiangsu Nhwa Pharmaceutical Co., Ltd, Xuzhou, China) was injected intravenously before anesthesia. Anesthesia was administered with 0.08-0.10 mg/kg vecuronium bromide (Wuhan Dahua Pharmaceutical Co., Ltd, Wuhan, China), 1-2 mg/kg propofol (Wuhan Sanhuan Pharmaceutical And chemical Co., Ltd, Wuhan, China), and 5 μg/kg fentanyl (Xian Janssen Pharmaceutical Ltd., Xian, China), and mechanical ventilation was performed after endotracheal intubation. Less than 2.5% sevoflurane (Jinan Wedo Industrial Co., Ltd, Jinan, China) was inhaled, 0.1-0.2 μg/kg/min remifentanil (shanghai haosu chem-tech co. Ltd, Shanghai, China) was injected intravenously, and vecuronium bromide was continuously injected intravenously during surgery. Approximately 30 min before the surgery ended, 120 mg of flurbiprofen axetil (Beijing Tide Pharmaceutical Co., LTD, Beijing, China) and 3 mg of granisetron (Sichuan Taiji Pharmaceutical Co Ltd., Chendou, China) were injected intravenously. Approximately 10 min before the surgery ended, 0.1 mg/kg morphine (Hubei Keyi Pharmaceutic Co., LTD, Wuhan, China) was injected intravenously. After surgery, a patient-controlled analgesia (PCA) pump (Nantong Apon Medical Devices Co., Ltd., Nantong, China) was connected carrying 2 mg/mL morphine. The PCA dose was 5 mL at a time limited to 8 min, or 25 mL/min, and a total volume of 150 mL. After patients awoke, their endotracheal tubes were pulled out and they were sent to a post-anesthesia care unit for 30 minutes of observation.

Post-operative observations

After patients entered the post-anesthesia care unit, physiological indexes including pulse rate, blood pressure, oxygen saturation, and consciousness were evaluated. A visual analogue score (VAS) was used to measure pain intensity, in which a 0 score indicated no pain and a 10 score indicated the most severe pain. Whether fentanyl was used for analgesia depending on the patients' pain intensity scoring. If the VAS was more than 4 points, 25-50 μg of fentanyl were injected intravenously; if

Table 4. Multiple linear regression analysis of physiological factors and the cumulative amount of morphine administered between $T_{0.5}$ and T_{4h}

Variable	*B*	$S_{\bar{X}}$	*t*	P	Standardized *β*
Age (years)	0.091	0.047	1.692	0.121	0.014
Gender	0.230	0.125	1.860	0.069	0.007
BMI (kg/m^2)	0.492	0.286	1.702	0.094	0.183
Surgery duration (min)	-0.415	0.301	-1.475	0.119	-0.290
Anesthesia duration (min)	-0.091	0.298	-1.364	0.130	-0.043
Smoking (Yes)	0.194	0.231	1.392	0.124	0.197
Alcohol (Yes)	0.178	0.291	1.698	0.098	0.201
m/w	0.215	0.137	2.170	0.032	0.569

patients presented with nausea and vomiting, they were given 3 mg of granisetron intravenously; if the VAS was 4 points or less, and there was no nausea, vomiting, or loss of consciousness, the patient was sent back to the ward. At 0.5 h, 4 h, 12 h, 24 h, 48 h, and 72 h, records were made for the patients' VAS as well as an assessment of adverse drug effects including nausea, vomiting, itchy skin, drowsiness, and respiratory depression.

Statistical analysis

Double data entry was performed using EpiData version 3.1 (EpiData Software, Odense, Denmark) to create a data bank, and logic checks were performed. SAS 9.2 (SAS Institute Inc., Cary, NC) software was used to analyze data by chi-square test and analysis of variance. A p value less than 0.05 was considered to indicate a statistically significant difference.

Results

Participants' genotypes

CYP2D6*10 genotyping revealed that 38 patients were heterozygous m/w, 34 patients were homozygous w/w, and 30 patients were homozygous m/m. No significant differences were found related to age, sex, ASA classification, surgical sites, smoking habits, drinking habits, body mass index (BMI), the duration of surgery, or the duration of anesthesia (**Table 1**).

The PCA morphine dose was significantly altered among different genotypes

The cumulative amounts of PCA morphine administered between $T_{0.5}$ and T_{4h} in patients carrying the m/w genotype were significantly higher in comparison to those carrying the m/m genotype (P<0.05). At other time points, the differences among patients were not statistically significant. The individual loading doses of PCA morphine were not significantly different, nor were the VASs at each time point (**Tables 2**, **3**; **Figure 1**).

Multiple linear regression analysis of physiological variables and the cumulative amounts of morphine administered between $T_{0.5}$ and T_{4h}

Multiple linear regression analysis was used to investigate the effects of various physiological factors [age, sex, ASA classification, surgical sites, smoking habits, drinking habits, BMI, duration of surgery, duration of anesthesia, and CYP2D6*10 genotype (w/m)] upon the cumulative amounts of morphine administered between $T_{0.5}$ and T_{4h}. The results showed that the w/m genotype was a significant factor influencing the amounts of morphine required (P<0.05). Furthermore, the standard partial regression coefficient indicated that the w/m genotype could increase the cumulative amounts of morphine administered between $T_{0.5}$ and T_{4h} (**Table 4**).

Discussion

Each variant of the polymorphic *CYP2D6* gene shows marked individual differences in the metabolism of drugs. Over 100 variants of *CYP2D6* have been identified, of which 15 contribute to abnormal enzyme activity [7, 8]. CYP2D6 variants are classified into four types according to activity in the metabolism of drugs: poor metabolizer (PM), intermediate metabolizer (IM), extensive metabolizer (EM), and ultrarapid metabolizer (UM) [9]. Gene mutations can lead to differences in the activity and amount of the enzyme, thereby giving rise to individual responses to drugs, so *CYP2D6* may provide key insights into the design of individualized medicine.

Here, we showed a significant difference in the effects of morphine among patients carrying different *CYP2D6* genotypes. Compared to

patients carrying an m/m genotype, the cumulative amounts of morphine administered between $T_{0.5}$ and T_{4h} were significantly higher in patients with an m/w genotype. Multiple linear regression analysis of the cumulative amounts of morphine administered between $T_{0.5}$ and T_{4h} showed that the w/m genotype was correlated with increased morphine usage. These results suggest that the CYP2D6*10 polymorphism contributes to pain sensitivity and tolerance during postoperative acute pain, which may be due to changes in the production of endogenous opioid peptides.

Interestingly, CYP2D6 UM has been shown to produce more endogenous morphine than other CYP2D6 metabolizers, thereby lessening the need for exogenous opioid [10]. Endogenous opioid peptides (EOPs) are opioid active substances naturally produced in mammals, and act as neurotransmitters playing crucial roles in regulating sensory, motor, neurological, and immune systems. These are just as effective at reducing pain as exogenous morphine [11]. Previous studies have shown that patients with CYP2D6 PM have a lower tolerance to pain than those with CYP2D6 EM due to a genetic defect causing the enzyme to produce less EOP [12].

CYP2D6 is known to play a key role in the synthesis of EOPs in the liver and brain and on the surface of polymorphonuclear leukocytes (PMNs) [13-15]. In our study, there was no difference in VAS 4 h after surgery, but the patients with the m/w genotype required higher doses of exogenous morphine than patients with the m/m genotype, suggesting that patients with m/w produced a lower level of EOPs than patients with m/m. As a gene fragment forming an IM, CYP2D6*10 can decrease the activity of CYP2D6. Patients with CYP2D6*10 have been shown to experience reduced efficacy when taking dextromethorphan, and patients with IM experience an increased incidence of complications, leading to increased risks and inefficiencies in clinical care [16].

In summary, 4 h after general anesthesia, patients carrying the m/w genotype required a higher level of exogenous morphine than those carrying the m/m genotype. Furthermore, multiple linear regression analysis of the cumulative amounts of morphine administered between $T_{0.5}$ and T_{4h} showed that the w/m genotype was closely correlated with the amount of morphine used. Therefore, it is possible that the m/w genotype produces a lower level of EOPs than the m/m genotype. Further studies will investigate this theory and explore the effects of CYP2D6 on the metabolism of EOPs and illuminate new possibilities for individualized pain management.

Disclosure of conflict of interest

None.

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