Int J Clin Exp Med 2016;9(11):21549-21555
www.ijcem.com /ISSN:1940-5901/IJCEM0037558

Original Article

Individual postoperative analgesia and gene polymorphisms of CYP3A7 and MDR1: an association study

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Received April 14, 2016; Accepted September 29, 2016; Epub November 15, 2016; Published November 30, 2016

Abstract: Pain was acknowledged as a member of five vital signs. In clinical practice, anesthetic was used to alleviate pain for cancer patients undergoing surgery. Previous studies showed anesthetic sensitivity varied significantly in different races or ethnics, suggesting that anesthetic dose should be considered for individual analgesia. However, molecular mechanism of anesthetic sensitivity is still unclear. Thus, our study was focused on the association between individual postoperative analgesia and gene polymorphisms of CYP3A7 and MDR1. Consecutive gastric cancer patients were enrolled. All enrolled patients received surgery and treatment in the same hospital. Blood specimens were collected from enrolled patients. Polymorphisms of CYP3A7 and MDR1 were analyzed with PCR and PCR-RFLP. Anesthetic efficacy was evaluated for patients with different polymorphisms of CYP3A7 and MDR1, and was verified by pain scoring for fentanyl treatment. Logistic regression was performed upon age, gender, and surgery status to predict potential risk factors for high anesthetic sensitivity. Compared with gastric cancer patients with low grade of pain, patients with high grade had higher frequency of gene polymorphisms ($P < 0.01$), including genotype of CYP3A7 heterozygote, allele at T site of CYP3A7, genotype of MDR1 homozygous, genotype of MDR1 heterozygote and allele at G site of MDR1. Moreover, compared patients with AA genotype, patients with AG genotype had lower grade of pain, regardless of CYP3A7 or MDR1 ($P < 0.01$). Logistic regression demonstrated that CYP3A7 heterozygote, allele at T site of CYP3A7 and polymorphisms of MDR1 gene were risk factors upon age, gender, and surgery status for high anesthetic sensitivity. Polymorphisms of CYP3A7 and MDR1 were significantly associated with high anesthetic sensitivity in gastric cancer patients with high grade of pain. CYP3A7 heterozygote and polymorphisms of MDR1 gene could predict high anesthetic sensitivity.

Keywords: CYP3A7, MDR1, postoperative analgesia, gene polymorphism

Introduction

Pain is defined as unpleasant emotional experience and subjective feeling related to tissue injury [1]. So far, pain was acknowledged as a member of five vital signs, paralleled with body temperature, respiration, blood pressure and pulse [2]. More than 80% patients suffered from moderate or severe postoperative pain. What's more, postoperative pain prolongs the recovery and deteriorates prognosis. Thus, anesthetic was widely used for intraoperative and postoperative analgesia [3, 4]. Anesthetic sensitivity varied significantly in different races or ethnics, accordingly, suitable medicines and doses of anesthesia play pivotal roles in reducing mortality and the incidence of complications. Individual anesthesia was essential for treatment due to a large variation of anesthetic sensitivity [5-7]. However, molecular mechanisms of anesthetic sensitivity are still unclear.

Opioid is common used anesthetic in clinical practice, such as fentanyl and morphine [8, 9]. Fentanyl was first synthesized in 1960s with similar structure to morphine, but as 100 times analgesic effect as morphine [10, 11]. Current studies showed that analgesic mechanism of fentanyl are as follows: After crossing blood-brain barrier, fentanyl interacted with opioid receptors in brain and spinal cord to maintain analgesia, and P-glycoprotein, a specific translocator in blood-brain barrier, played a key role in this process [12-14].

P-glycoprotein influences sensitivity to pain, which was located on chromosome 7 and directly encoded by multi-drug resistance gene (MDRI) [15-17]. Furthermore, as a member of ATP-binding protein family, P-glycoprotein is an energy-dependent translocator [18, 19]. Besides blood-brain barrier, P-glycoprotein was widely distributed on cell membrane of intestine and liver cells, thus, P-glycoprotein regulated drug delivery of anesthesia [20-22]. Collectively, mutation or polymorphisms of MDRI could affect postoperative analgesia.

Anesthetic sensitivity also depends on drug metabolism. Cytochrome P450 proteins superfamily was main enzymes to regulate drug metabolism, including CYP3A4, CYP3A5, CYP2D6 and CYP2C7 [23, 24]. Cytochrome P450 transferred electron via interacting iron ion of heme so as to oxidized anesthetic, and finally completed hydrolysis and eliminated drug from the body [25, 26]. Accordingly, polymorphisms of CYP were possible to influence postoperative analgesia [27].

Previous study proved polymorphism of MDRI 34350T was associated with analgesia efficacy of fentanyl [28]. However, there was no studies reported the relationship between analgesia efficacy and polymorphisms of CYP. We hypothesized that more polymorphisms of MDRI and CYP were involved in anesthetic sensitivity.

In summary, our study was focused on the association between individual postoperative analgesia and gene polymorphisms. Potential polymorphisms were CYP3A7 and MDR1.

Materials and methods

Materials

Consecutive gastric cancer patients were enrolled from March 2013 to March 2016. All enrolled patients received surgery and treatment in the first affiliated hospital of Anhui medical university. Fentanyl anesthesia was performed for gastric cancer patients, and anesthetic sensitivity was graded according to standard of American Society of Anesthesiologists [29].

Our study has get approval from Ethics Committee of the first affiliated hospital of Anhui medical university, and all patients signed informed consent before participating in the study.

Inclusion criteria is as follows: 1) Normal function of vital organs, including heart, liver and kidneys; 2) No history of smoking and drinking; 3) No allergic reaction to an anesthetic; 4) No history of cardiovascular diseases and diabetes; 5) No drug history; 6) No medication of P-glycoprotein substrate before surgery.

To gastric cancer patients undergoing surgery, grade of pain is performed at 2 h before surgery as follows [22]: Grade 1 (mild pain): Feel painful but bearable. Live normally. Grade 2 (moderate pain): Feel obviously painful and unbearable. Analgesic was used to alleviate pain at times. Grade 3 (severe pain): Feel severely painful and unbearable. Analgesic was routinely used to alleviate pain.

In our study, Grade 1 and 2 were considered as the low grade of pain. Grade 3 was considered as high grade of pain.

Blood specimen collection

Blood specimens were collected from veins of patients as described previously [23]. Every patient received 5 ml blood drawing with anticoagulant heparin.

DNA extraction

Genomic DNA of patients was extracted from blood specimen with extraction kit as described previously [23].

PCR

DNA of patients was used as template. CYP3A7 and MDR1 were amplified with PCR.

Primer sequence of CYP3A7 was as follows: primer 1, 5'TTACTGTCGGAATCCTGCTC3'; primer 2, 5'CAGCTGACCTATCCATACAG3'; Primer sequence of MDR1 was as follows: primer 1, 5'GGAATCCTACCTTTCAAGCA3'; primer 2, 5'AAGGAAGGCTGGAAGAGTGC3'; Reaction system of PCR was as follows: 0.5 μL DNA template, 1.0 μL 10 × PCR buffer solution, 1.0 μL dNTP mix (1 mM), 0.5 μL primer 1 (20 μM), 0.5 μL primer 2 (20 μM), 0.05 μL Taq DNA Polymerase, 0.75 μL $MgCl_2$ (10 mM), 3 μL ddH_2O; Reaction protocol of PCR was as follows: 95°C 5 min for degeneration; 95°C 40 seconds, 56°C 30 seconds, 72°C 40 seconds (30 circles); 72°C 8 min, 4°C for storage.

Enzyme digestion

Sequence length of CYP3A7 was 170 bp after PCR. Enzyme digestion was performed as fol-

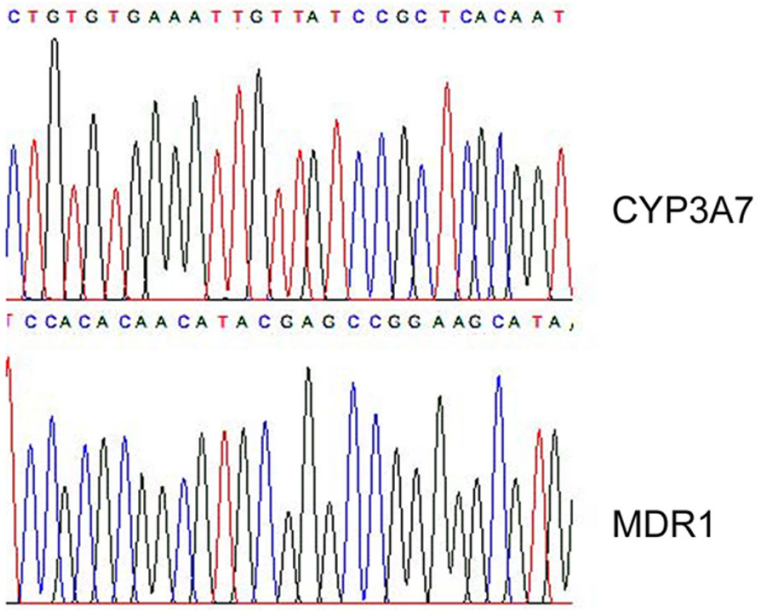

Figure 1. Sequencing data of PCR production.

Table 1. Analysis of general condition

Group	Sample	Gender (%)	Age	BMI (Kg/m^2)	Glucose (mm Hg)
High grade of pain	786	50%	56 ± 1.4	23.5 ± 3.5	5.1 ± 1.4
Low grade of pain	300	50%	58 ± 0.8	23.1 ± 2.6	5.8 ± 1.6
P value		0.76	0.96	0.36	0.29

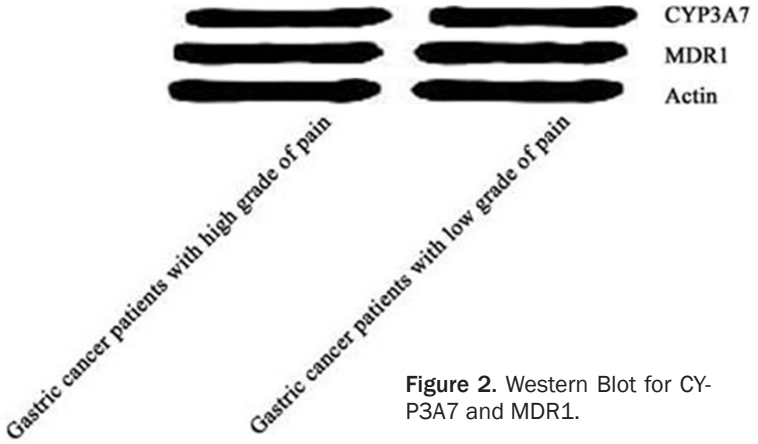

Figure 2. Western Blot for CYP3A7 and MDR1.

lows: 4 μL 10 × reaction buffer, 0.4 μL Acetylated BSA (5 μg/μL), 1 μL restriction enzyme Hsp92 II (10 U/μL), 14.6 μL ddH_2O. React at 37°C for 8 hours.

Sequence length of MDR1 was 153 bp after PCR. Enzyme digestion was performed as follows: 4 μL 10 × reaction buffer, 0.4 μL Acetylated BSA (5 μg/μL), 1 μL restriction enzyme Hsp92 II (10 U/μL), 14.6 μL ddH_2O. React at 37°C for 8 hours. React at 37°C for 8 hours.

Agarose gel electrophoresis

PCR production was examined with agarose gel electrophoresis. Experiment conditions of electrophoresis were 100 mv, 16 min. imaging system was used to capture and analyze [25].

Sequencing of PCR production

Sequencing of PCR production was accomplished by Beijing Dingguo biotechnology Co. Ltd. (China). Sequences of CYP3A7 and MDR1 were analyzed based on NCBI database (**Figure 1**).

Data statistics

SPSS17.0 software was used for data processing. Measurement data are normal distribution to X ± S. Variance analysis was performed to determine difference between groups. Logistic regression analysis was applied upon age, gender, dietary habit, and helicobacter pylori infection. *P* value < 0.05 was considered to be statistically significant.

Results

No difference was detected in general condition among patients with different grade of pain

General condition of patients was showed in **Table 1**. No difference was observed in age or gender among patients with different grade of pain.

Western blot and genotype identification for CYP3A7 and MDR1

Expressions of CYP3A7 and MDR1 were showed in **Figure 2**. No difference was detected in protein expression.

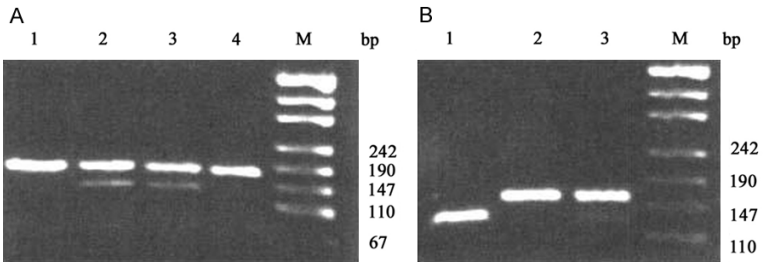

Figure 3. Genotype identification for CYP3A7 and MDR1. A. Identification for CYP3A7. M: DNA Marker. 1: CC genotype of CYP3A7. 2 & 3: TC genotype of CYP3A7. 4: TT genotype of CYP3A7. B. Identification for MDR1. M: DNA Marker. 1: MDR1 AA genotype. 2: MDR1 GG genotype. 3: MDR1 AG genotype.

Table 2. Analysis of CYP3A7 genotype for patients with different grade of pain

Groups	Samples	CC (%)	TT (%)	CT (%)
High grade of pain	786	75.2	0	24.8
Low grade of pain	300	52.8	0	47.2
	χ^2 = 12.14, P = 0.0073			

Table 3. Analysis of CYP3A7 allele frequency for patients with different grade of pain

Groups	Samples	C (%)	T (%)
High grade of pain	786	87.6	12.4
Low grade of pain	300	99.5	0.5
	χ^2 = 6.87, P = 0.0041		

Table 4. Analysis of MDR1 genotype for patients with different grade of pain

Groups	Samples	AA (%)	GG (%)	AG (%)
High grade of pain	786	71.0	11.8	18.2
Low grade of pain	300	40.1	11.0	48.9
	χ^2 = 16.3, P = 0.0038			

Table 5. Analysis of MDR1 allele frequency for patients with different grade of pain

Groups	Samples	A (%)	G (%)
High grade of pain	786	64	36
Low grade of pain	300	83	17
	χ^2 = 92.3, P = 0.0057		

Genotype identification was performed for CYP3A7 and MDR1, respectively. Examination results of two groups were showed in **Figure 3**.

Examination of CYP3A7 polymorphism for patients with different grade of pain

CYP3A7 polymorphism for patients with different grade of pain conformed to Hardy-Weinberg equilibrium. Chi-square Test was conducted to determine significant difference in allele frequency and genotype frequency between two groups (**Tables 2**, **3**, P < 0.01). It was showed that CYP3A7 genotype frequency was significantly different between gastric cancer patients with different degrees of pain after surgery (χ^2 = 12.14, P = 0.0073). Moreover, the results demonstrated that CYP3A7 allele frequency was obviously different between two groups (χ^2 = 6.87, P = 0.0041). These data together suggested that CYP3A7 allele frequency and genotype frequency play a vital role in the anesthetic sensitivity.

Examination of MDR1 polymorphism for patients with different grade of pain

MDR1 polymorphism for patients with different grade of pain conformed to Hardy-Weinberg equilibrium. Chi-square Test exhibited significant difference in allele frequency and genotype frequency between two groups (**Tables 4**, **5**, P < 0.01). The results showed that MDR1 genotype frequency was significantly different between gastric cancer patients with different degrees of pain after surgery. Moreover, MDR1 allele frequency was obviously different between the two groups. These data together suggested that MDR1 in allele frequency and genotype frequency play a vital role in the anesthetic sensitivity.

Logistic regression for association between grade of pain and gene polymorphisms

As showed in **Figure 4**, CYP3A7 heterozygote, allele at T site of CYP3A7 and genotype of MDR1 were risk factors for high grade of pain, whereas gender and age were not, suggesting significant association between pain degree and polymorphisms of CYP3A7 and MDR1.

Discussion

Pain is of great importance to clinical treatment and prediction of diagnosis [1]. Given cancer patients always need anesthetic for analgesia, dose of anesthetic has caused more attention and individual postoperative analgesia is essential for improving treatment [5].

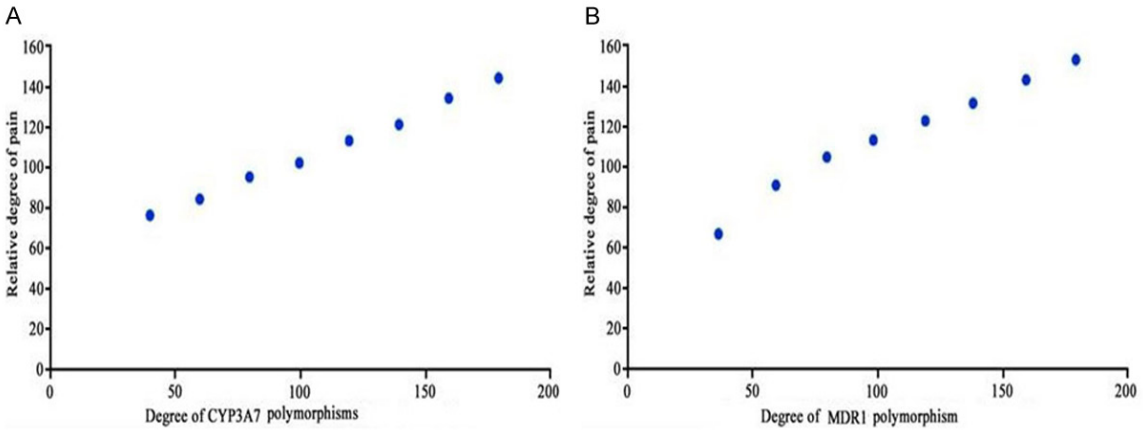

Figure 4. Logistic regression demonstrated significant association between grade of pain and gene polymorphisms according to pain score and genotype ratio. A. The effect of the CYP3A7 polymorphism on the degree of pain. B. The effect of the MDR1 polymorphism on the degree of pain.

Our study demonstrated the potential mechanism for anesthetic sensitivity and hyperalgesia, which was involved in polymorphisms of CYP3A7 and MDR1.

There are three main finding in our study. 1) Compared with patients with low grade of pain, patients with high grade had higher frequency of genotype of CYP3A7 and MDR1 heterozygote, which allele at T site of CYP3A7 and allele at G site of MDR1 respectively. All findings were consistent with previous studies [20]. 2) In gastric cancer patients, percentage of MDR1 AG genotype decreased, while MDR1 AA genotype increased. 3) CYP3A7 heterozygote, allele at T site of CYP3A7 and genotype of MDR1 were risk factors for high grade of pain, and the results were verified by logistic regression.

Previous studies have indicated that gene polymorphisms were involved in anesthetic sensitivity or hyperalgesia. Our study elucidated that in gastric cancer patients, polymorphisms of CYP3A7 and MDR1 could be predictor for anesthetic sensitivity and hyperalgesia.

There is some limitation in our study. Our study was based on a relatively small sample. Detailed mechanisms were warranted to explain how polymorphisms of CYP3A7 and MDR1 influenced anesthetic sensitivity and hyperalgesia, which need further study with transgenic or gene knock-out animal models.

In conclusion, aberrant polymorphisms of CYP3A7 and MDR1 were risk factors for high grade of pain in gastric cancer patients. Patients with those polymorphisms should be provided with more accurate strategy for individual postoperative analgesia.

Acknowledgements

This project supported by Anhui provincial outstanding young talent fund project (NO. 2011SQRL067).

Disclosure of conflict of interest

None.

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