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Correlation analysis between *CYP2C9* polymorphisms and liver injury induced by flurbiprofen axetil in 100 *Han* Chinese patients

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Abstract: This study explored correlation between *CYP2C9* polymorphisms and liver injury induced by flurbiprofen axetil (FBA). A total of 100 patients undergoing primary total knee arthroplasty (TKA) were mainly administered with basic analgesic, FBA. All the patients participating in this study were required to take blood samples for detecting *CYP2C9* polymorphisms before surgery after admission. After TKA surgery, the level of glutamic-pyruvic transaminase in blood was detected to determine whether liver injury occurred in patients. The overall incidence of liver injury after TKA was 12.00% and the incidence of liver injury was 12.22% for *CYP2C9*1/*1* and 16.67% for *CYP2C9*1/*3*. The incidence of liver injury in patients with *CYP2C9*1/*3* was higher than the incidence of overall patients and patients with *CYP2C9*1/*1*, but the difference was not statistically significant. However, given the occurrence of liver injury, clinicians should still pay attention to patients with *CYP2C9*1/*3* to avoid serious liver injury that may be induced by FBA.

Keywords: Flurbiprofen axetil; total knee arthoplasty; liver injury; *CYP2C9* polymorphisms

INTRODUCTION

Flurbiprofen axetil (FBA), an ester prodrug of flurbiprofen, is one of the most commonly used nonsteroidal anti-inflammatory drugs worldwide indicated for the treatment of postoperative pain (Zhang *et al*., 2018). In recent years, FBA has been widely used in postoperative analgesia and has a great analgesic effect on a variety of postoperative pain. Wang *et al*. found that FBA might reduce postoperative delirium in patients over 70 years undergoing major noncardiac surgery (Wang *et al*., 2019); and Lin *et al*. reported that an administration strategy that maintained a relatively high plasma concentration of FBA at 6-24 hours post-operatively might reduce postoperative inflammatory pain and sufentanil-requirement in patients undergoing colorectal cancer resection (Lin, *et al*., 2014). At present, FBA with non-selective cyclooxygenase inhibition activity is widely used to relieve perioperative and postoperative pain in clinical practice (Xie, *et al*., 2021; Yao, *et al*., 2021).

Cytochrome P450 2C9 (CYP2C9) is a phase I drug-metabolizing cytochrome P450 (CYP450) enzyme isoform that plays a major role in oxidation of both xenobiotic and endogenous compounds (Van Booven, *et al*., 2010). CYP2C9 accounts for about 20% of hepatic total CYP content and metabolizes about 15% clinical drugs including numerous NSAIDs (Miners & Birkett, 1998; S. F. Zhou, Zhou & Huang, 2010). At least 71% of FBA belonging to NSAIDs is eliminated by oxidative metabolism of CYP2C9 in liver (Loisios-Konstantinidis, Cristofoletti, Jamei, Turner, & Dressman, 2020). However, CYP2C9 is a polymorphic enzyme whose gene mutation causes a decrease in the activity of the corresponding enzyme. For example, mutant-type *CYP2C9*1/*3* widely found in Asian populations has a significantly lower metabolic activity to drugs than wild-type *CYP2C9*1/*1* (Y. Zhou, Ingelman-Sundberg, & Lauschke, 2017). $AUC_{0-\infty}$ of flurbiprofen was significantly higher and all measures of flurbiprofen clearance were significantly lower in the individuals with *CYP2C9*1/*3* than in those with **1/*1* (Lee, *et al*., 2003). As a result, the difference of CYP2C9 enzyme activity directly affects blood concentration, therapeutic effect and adverse reactions of FBA. The *CYP2C9* polymorphisms is a main reason for the difference of CYP2C9 enzyme activity, so adjusting the dosage of FBA according to CYP2C9 genotype can reduce adverse reactions or improve therapeutic efficacy. In addition, Liu *et al*. pointed out that FBA-induced liver injury was a common adverse effect, the degree of injury is mainly mild and abnormal transaminase is judged as a main type of liver injury (Hao, Dai-hong, Yan-dong, Dong, & Liang, 2018). Nevertheless, a relationship between FBA and liver injury needs further study with more clinical statistical analysis, especially for association between *CYP2C9* polymorphism and FBA-induced liver injury.

In view of the major issue, namely, acute liver injury possibly induced by individual metabolic differences of FBA due to *CYP2C9* polymorphism, we selected 100 patients who received primary total knee arthoplasty (TKA) and met criteria to study the major clinical issue.

MATERIALS AND METHODS

General information

A total of 100 patients undergoing primary unilateral TKA surgery in orthopedics department from June 2020 to

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December 2021 were selected as subjects. All the patients were of Putian *Han* nationality and met the surgical indications and the patients who did not meet the inclusion criteria were excluded. Exclusion criteria in this study were as follows: (1) non-osteoarthritis lesions and various stiff knees, (2) severe lesions of bilateral knee joints, (3) severe valgus knee, (4) history of various liver diseases, including acute and chronic hepatitis, carriers of various hepatitis viruses, chronic cholecystitis, cholelithiasis, fatty liver, etc., (5) previous history of liver damage, (6) history of repeated ulcer and bleeding of digestive system, (7) history of various drug allergies, (8) recently underwent coronary artery bypass surgery, (9) history of severe heart failure and hypertension and (10) severe obesity or BMI>30kg/m^2. All the patients participating in this study were required to take blood samples for detecting *CYP2C9* polymorphisms before surgery after admission. All enrolled patients and their families were educated in clinical ethics and informed about this study so that they could understand and voluntarily participate in this study and sign the study informed consent, which is based on the requirements of Declaration of Helsinki of World Medical Association. The medical ethics of this study were approved by the Ethics Committee of Affiliated Hospital of Putian University.

Study contents

All the patients in this study underwent perioperative management in accordance with the concept of enhanced recovery after surgery (ERAS). ERAS included fasting 6 hours prior to surgery, water deprivation 2 hours prior to surgery, oral administration of 0.2g celecoxib capsule 2 hours before surgery, routine intravenous infusion of 1.0g cefazolin sodium 30 minutes before surgery and oxygen inhalation, electrocardiogram monitoring and other routine preparations before anesthesia after entering operating room. Patients were given lumbar epidural anesthesia combined with intravenous or endotracheal general anesthesia and a standardized TKA was performed by the same team of surgeons and anesthesiologists. Patients were given 10mg dexamethasone via intravenous injection and 20mg/kg tranexamic acid via intravenous drip before surgical excision and tranexamic acid was read ministered twice at the same dose at the end of surgery and 3 hours after surgery. In this study, each patient was injected with 50mg FBA intravenously immediately after surgery and was connected with a patient-controlled intravenous analgesia (PCIA) pump at the time of skin suture before the end of surgery. For each patient, FBA was administered at an interval q8h for 5 consecutive days and PCIA pump was used for 48 consecutive hours. In PCIA pumps, a total volume was adjusted to 100mL with 0.9% sodium chloride injection, a background injection dose was 2mL/h, an automatic supplemental dose was 1mL once and a locking time was 15 minutes, which could theoretically last for 48 hours based on the above settings. Theoretically, all the patients should be discharged from hospital on the 5th day after surgery and hospital stays should be adjusted appropriately when individual differences occurred. Discharge criteria were as follows: (1) The patients' incision was dry, without redness or obvious swelling; (2) The patients' diet, urine, stool and sleep were close to normal; (3) Patients can walk with the help of crutches. Discharge instructions were as follows: (1) The patient needs to continue dressing the surgical wound in the local health center, dressing every 2-3 days and stitches are removed 2 weeks after surgery; (2) Patients need to continue taking drugs for 1 week to continue the symptomatic treatment of anti-inflammatory pain relief; (3) Patients need to carry out functional exercise of the affected limb every day; (4) At the same time, patients need to combine the traditional Chinese medicine characteristics of the hospital "bamboo circle salt moxibustion" rehabilitation physiotherapy (guided by the doctor of traditional Chinese medicine rehabilitation); (5) The patient was returned to the outpatient department of our hospital one week after discharge. Details of FBA injection were provided in table 1.

Data collection and outcome measures

Drug safety assessment: The incidence of acute liver damage was mainly collected after surgery. Analysis of *CYP2C9* polymorphisms: The correlation between the adverse reactions of FBA injection and gene differences was mainly observed. Liver injury: The level of glutamic-pyruvic transaminase (GPT) in blood was detected to determine whether liver injury occurred in patients.

STATISTICAL ANALYSIS

GraphPad Prism 8 was used for data analysis in this study. Chi-square (χ^2) test was used for comparison of counting data. $P<0.05$ was statistically significant.

RESULTS

Correlation analysis between CYP2C9 polymorphisms and liver injury induced by FBA

CYP2C9 polymorphisms and preoperative/postoperative GPT levels was detected in 100 *Han* Chinese patients in this study and the detailed detected results were not showed and are available from the corresponding author upon reasonable request. Among them, 94 cases of patients were *CYP2C9*1/*1* (wild-type), 6 cases were *CYP2C9*1/*3* (mutant-type) and *CYP2C9*3/*3* (mutant-type) were not found and the genotype frequencies were 94%, 6% and 0%, respectively. Among the 94 cases of patients with *CYP2C9*1/*1*, 15 cases developed acute liver injury, 4 cases did not meet the inclusion criteria (2 fatty liver, 1 hepatitis B virus carrier, 1 cholelithiasis) and the incidence of liver injury was 12.22%. Among the 6 cases of patients with *CYP2C9*1/*3*, 1 case developed acute liver injury and the incidence of liver injury was 16.67%. Among the 100 cases of patients in control and experimental group, the overall incidence of abnormal liver function was 12.5%.

Table 1: Details of FBA injection relevant to this study.

Drug name	Dosage form	Drug specification	Indications and usage	Pharmacological action
Flurbiprofen axetil injection☆	Injection	5mL: 50mg	For the relief of postoperative pain and cancer pain	The drug is composed of lipid microspheres and flurbiprofen axetil. Flurbiprofen axetil is the precursor of flurbiprofen, which is a non-steroidal anti-inflammatory analgesic. Lipid microsphere preparation has the effect of targeting, controlled release and shortening the time of effect. The advantage of postoperative analgesia is that it has no central inhibitory effect and does not affect the recovery of patients under anesthesia. It can be used immediately after operation.

☆ Flurbiprofen axetil injection was a basic drug purchased by our hospital according to the state (Approval Number: National Drug Approval H20041508; Manufacturer: Beijing Taide Pharmaceutical Co., LTD.).

Table 2: Cases and frequency of *CYP2C9* genotypes and cases and incidence of liver injury

	Cases with different genotypes	Genotype frequency (%)	Cases with liver injury	Cases without meeting the inclusion criteria	Incidence of liver injury (%)
*CYP2C9*1/*1*	94	94	15	4	12.22
*CYP2C9*1/*3*	6	6	1	0	16.67☆
Overall	100	100	16	4	12.00

☆ The incidence of liver injury between *CYP2C9*1/*3* and *CYP2C9*1/*1* was compared by χ^2 test (P=0.3750).

There was no statistically significant difference in the incidence of liver injury between *CYP2C9*1/*3* and *CYP2C9*1/*1*. The severity of acute liver injury was mild in all cases, but the level of GPT was up to 166.3 IU/L. After discontinuation of FBA and/or application of injectable reduced glutathione for symptomatic liver protection, the level of GPT was reduced to normal range. The above results are shown in table 2.

DISCUSSIONS

More than 50 single nucleotide polymorphisms (SNPs) have been identified in the regulatory and coding regions of CYP2C9 gene (Loisios-Konstantinidis, *et al*., 2020) and the most common alleles are designated *CYP2C9*1* (wild-type), *CYP2C9*2* (R144C, rs1799853) and *CYP2C9*3* (I359L, rs1057910) (Lee, *et al*., 2003). The two above-mentioned SNPs contribute to six different genotypes that confer three functionally different phenotypes: Extensive metabolizers (*CYP2C9*1/*1*), intermediate metabolizers (*CYP2C9*1/*2*, **1/*3* and **2/*2*) and poor metabolizers (*CYP2C9*2/*3* and **3/*3*) (Loisios-Konstantinidis, *et al*., 2020). In Europeans and admixed Americans, the *2 allele dominates the genetic variability of *CYP2C9* with the allele frequencies of 11.7% and 6.6%, respectively, whereas the major allele is **3* in Asian populations with the allele frequencies of 3.4% and 11.3% in East Asians and South Asians, respectively (Y. Zhou, *et al*., 2017). The allelic frequencies of *CYP2C9* variants of the Chinese subjects (Shanghai) were 96.3%, 0.1% and 3.6% for *CYP2C9*1*, **2* and **3*, respectively (Yang, *et al*., 2003). In a total of 3122 *Han* Chinese, the frequency of wildtype *CYP2C9*1/*1* was 91.2% while the predominant genotype of a mutated *CYP2C9* was **1/*3* with a mutating frequency of 8.04% (He, *et al*., 2020). In addition, Dorji *et al*. reported that the genotype frequencies of *CYP2C9*1/*1*, **1/*3* and **3/*3* were 69.3%-99.1%, 2.3%-20.1% and 0%-2.2%, respectively, including Burmese, Chinese, Japanese, Karen ethnic minority, Korean, Malaysian, Philippino, Singaporean, Taiwanese, Thai, Indonesian and Vietnamese and the three genotype frequencies of *CYP2C9* were 90.91%, 6.45% and 0.2% in Chinese, respectively (Dorji, Tshering, & Na-Bangchang, 2019).

In this study, *CYP2C9* polymorphisms were detected in 100 *Han* Chinese patients in Putian region and we found that the genotypes of CYP2C9 were mainly wild-type *CYP2C9*1/*1* and mutant-type **1/*3* and the frequencies were 94% and 6%, respectively, which was similar to the above literature reports. Among the 100 cases of patients, the overall incidence of abnormal liver function was 12.5% and the incidence of liver injury was 12.22% and 16.67% in *CYP2C9*1/*1* and **1/*3*, respectively. In terms of the study, the incidence of liver injury in mutant-type *CYP2C9*1/*3* was higher than in wild-type **1/*1* and overall. The reason may be that the activity of the enzyme corresponding to *CYP2C9*1/*3* was decreased and flurbiprofen cannot be eliminated through normal metabolism, resulting in high plasma concentration and leading to drug-induced liver injury. The acute liver injury in all the cases were mild and the highest level of GPT was 166.3 IU/L. After discontinuation of FBA injection and/or application of reduced glutathione injection for liver-protection, the level of GPT fell to the normal range in most patients within one week, or returned to normal in some patients within two weeks. FBA belonging to NSAIDs is easy to induce drug-induced liver injury after TKA, but the degree is relatively mild and no life-threatening liver function injury has been observed at present. The level of GPT in all the patients recovered to normal after appropriate symptomatic treatment, but attention should still be paid to the use of this drug in clinical application.

For example, Yuan *et al*. reported that a case of patient was cured with FBA injection after unicompartmental knee arthroplasty and then appeared acute hepatic and renal failure (Yuan, Tao & Wan, 2022). Regular reexamination of liver function is required for patients using the drug, especially for patients carrying *CYP2C9*1/*3* and timely adjustment of therapeutic regimen is required to avoid serious adverse effects.

LIMITATION

The deficiency of the study is a single-center study with a small sample size and the clinical association between *CYP2C9* polymorphisms and acute liver injury needs to be followingly studied and further optimized, which is also our next research plan. Considering the large difference between the sample size of the study and the theoretical sample size, we must admit that there may be various biases and regarding the study of *CYP2C9* polymorphisms, we will continue to collect relevant cases for in-depth study.

CONCLUSION

In this study, there was no significant association between *CYP2C9* genotype and acute liver injury, but the incidence of acute liver injury in patients with *CYP2C9*1/*3* is relatively high, which should arouse attention from the clinicians. Patients taking FBA should have regular review in liver function, especially those carrying *CYP2C9*1/*3* and the clinicians should timely adjust the treatment regimen to avoid serious liver injury.

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