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Influence of genetic polymorphisms on the pharmacokinetics of celecoxib and its two main metabolites in healthy Chinese subjects

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ABSTRACT

Celecoxib is a selective cyclooxygenase-2 inhibitor used extensively for the treatment of rheumatism and osteoarthritis. The aim of this study was to evaluate the influence of the genetic polymorphisms of *CYP2C9*, *CYP2D6* and *CYP3A4* on the pharmacokinetics (PK) of celecoxib and its two main metabolites, hydroxyl-celecoxib and carboxy-celecoxib, in healthy Chinese subjects, based on a bioequivalence study of celecoxib. This study was an open-label, two-period, crossover study. 52 healthy Chinese male subjects were recruited and were genotyped for *CYP2C9*3, *CYP2C9*13, *CYP2D6*10 and *CYP3A4*18 by using polymerase chain reactions (PCR). They were randomly divided into two groups and each group received either 200 mg test formulation followed by reference formulation or vice versa with a one-week washout period. Safety and tolerability were monitored throughout the study and no severe adverse events were observed. Genotyping using PCR revealed that none of the subjects carried the *CYP3A4*18 and *CYP2C9*13. Therefore, the influence of the *CYP2C9*3 and *CYP2D6*10 on the PK of celecoxib and its metabolites in Chinese was studied. Compared with *CYP2C9*1/*1 group, pharmacokinetic parameters of celecoxib such as AUC_{0-48} and C_{max} was increased by 90.6% and 45.8%, the $t_{1/2}$ was extended by 21.8% and the *CL/F* was decreased by 51.1% in *CYP2C9*1/*3 group. In terms of hydroxy-celecoxib, compared with *CYP2C9*1/*1 group, the C_{max} was decreased by 17.2%, the $t_{1/2}$ prolonged 42.1% in *CYP2C9*1/*3 group. In terms of carboxy-celecoxib, the AUC_{0-48} was increased by 25.2%, the $t_{1/2}$ prolonged 16.1% and the *CL/F* was decreased by 21.2% in *CYP2C9*1/*3 group. Except for the $t_{1/2}$ of hydroxy-celecoxib, no statistically significant difference was observed in other pharmacokinetic parameters of hydroxy-celecoxib and carboxy-celecoxib between the two *CYP2C9* genotypic groups. This study revealed that there was no significant influence of *CYP2D6*10 on the metabolism of celecoxib, and the expression of *CYP2C9*3 led to increased drug exposure and slowed drug disposition in healthy Chinese male subjects.

1. Introduction

Celecoxib, 4-[5-(4-methylphenyl)-3-(trifluoromethyl)-pyrazol-1-yl] benzene sulfonamide, is the first selective cyclooxygenase-2 (COX-2) inhibitor approved for treatment of rheumatism and osteoarthritis. Traditional non-steroidal anti-inflammatory drugs (NSAIDs) inhibit COX-1 and COX-2 to produce anti-inflammatory, analgesic and antifebrile effects together with serious adverse effects (Fanelli et al., 2013; Lindhardsen et al., 2013). As a selective COX-2 inhibitor, celecoxib is more effective and safe with less adverse effects than traditional NSAIDs (Essex et al., 2013), and is used extensively for treating postoperative analgesia (Matsota et al., 2013), rheumatic arthritis (McCormack, 2011), epithelial tumor (Jendrossek, 2013), atherosclerosis (Chenevard et al., 2003), etc.

Celecoxib is metabolized mainly by oxidation pathway to two metabolites, including hydroxylation of celecoxib to form hydroxy-celecoxib followed by further oxidation to form carboxy-celecoxib (Paulson et al., 2000). High inter-individual variability in the pharmacokinetic parameters was largely attributed to the genetic polymorphism of Cytochrome P450 proteins (CYPs) (Prieto-Perez et al., 2013; Sandberg et al., 2002; Tang et al., 2001). Pharmacokinetics of celecoxib after oral administration in adults (Park et al., 2012) and in children (Stempak et al., 2002) were reported. But no results of pharmacokinetics of celecoxib in Chinese were reported. Previous studies investigated polymorphism of different subtypes of CYPs and their relationship with the pharmacokinetics of celecoxib. Oxidative metabolism of celecoxib is primarily catalyzed by CYP2C9. *CYP2C9*3* lowers *CYP2C9* activity, significantly decreasing the clearance with a median value of 30 l/h in carriers of the wild-type genotype, 21 l/h in heterozygous carriers of one *CYP2C9*3* allele and 9 l/h in homozygous carriers of *CYP2C9*3*, but *CYP2C9*2* has no significant influence on celecoxib pharmacokinetics in Caucasians (Kirchheiner et al., 2003; Prieto-Perez et al., 2013; Sandberg et al., 2002; Stempak et al., 2005; Tang et al., 2000, 2001).

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Other alleles that decrease CYP2C9 activity in vivo are *CYP2C9*8, *CYP2C9*11 and *CYP2C9*13 (Human CYP2C9 Allele Nomenclature, 2014), but *CYP2C9*8 has not been found in Chinese, while *CYP2C9*11 has only been discovered in few cases of Chinese Tibetan (Wang et al., 2012), and *CYP2C9*13 has a very low frequency in Chinese (Si et al., 2004). So in relation to *CYP2C9*, we only investigated the influence of *CYP2C9*3 and *CYP2C9*13 polymorphism on the pharmacokinetics of celecoxib in Chinese. Although the influence of *CYP2C9* polymorphism on the pharmacokinetics of celecoxib has been investigated before in Caucasians, it has not been studied in Chinese. Therefore, we hypothesized that with different CYP activities among different races, there might be some differences in the pharmacokinetics of celecoxib between Chinese and Caucasians. In vitro studies showed that CYP3A4 also plays a part in the oxidative metabolism of celecoxib (Sandberg et al., 2002; Tang et al., 2000). In Chinese, the prevalent allele of *CYP3A4* is *CYP3A4*18* which decreases CYP3A4 activity in vivo, and other alleles of *CYP3A4* are *CYP3A4*5*, *CYP3A4*6*, and *CYP3A4*21* with unclear CYP3A4 activity and low frequencies of not more than 1% (Human CYP3A4 Allele Nomenclature, 2014; Zhou et al., 2011). Celecoxib can inhibit the metabolism of CYP2D6 substrates (Werner et al., 2003). The *CYP2D6*10* exists widely in Chinese (Qin et al., 2008), and decreases CYP2D6 activity (Sakuyama et al., 2008). Other alleles of *CYP2D6* in Chinese have very low frequencies and don't result in a loss of enzymatic activity (Qin et al., 2008; Sakuyama et al., 2008). However, the frequency of *CYP2D6*10* is rare in Caucasians (Sachse et al., 1997), the correlation between *CYP2D6*10* and the pharmacokinetics hasn't been discussed before.

The present study aims to evaluate the safety and investigate the influence of the genetic polymorphisms of *CYP2C9*, *CYP2D6*, and *CYP3A4* on the pharmacokinetics of celecoxib and its main metabolites in healthy male Chinese subjects, based on a bioequivalence study of celecoxib. This study would provide the important scientific basis for the clinical application and help clinicians with dose adjustment of celecoxib in Chinese.

2. Materials and methods

2.1. Subjects

52 healthy Chinese male subjects were recruited in this study. All the subjects satisfied the inclusion criteria: 18–40 years old, a body weight not less than 50 kg, a body mass index (BMI) between 19 and 24, non-smoker and not addicted to drinking alcohol. None of them had a history of cardiac, hepatic, renal, pulmonary, gastrointestinal, nervous system, metabolic disorder or drug allergy. All the subjects were in good health according to the complete medical history, and physical and laboratory examination. None of the subjects took any medicine two weeks before the study. All subjects gave written informed consent. The study protocol was approved by an Independent Ethics Committee, Institute of Dermatology, Chinese Academy of Medical Sciences, Nanjing, China and performed according to the Declaration of Helsinki (World Medical, 2013) and the International Conference on Harmonisation Good Clinical Practice Guidelines (International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use, 2001).

2.2. Study design

This was a single-dose (200 mg), open-label, stratified randomized, two-period, crossover and self-controlled bioequivalence study. 52 eligible subjects were divided into 2 groups randomly. Each subject was administered either test formulation (Jiangsu Chiatai Qingjiang Pharmaceutical Co., Ltd., T) or reference formulation (Celebrex®, Pfizer, R) in period 1 and after a one-week washout period the subject that took T now took R and vice versa in period 2. In each period, they were all required to fast overnight for at least 10 h and each subject was administered 200 mg celecoxib capsule (T or R) along with 250 ml of water the following day. Blood samples (4 ml) were collected from cubital veins immediately before and 0.5, 1, 1.5, 2, 2.33, 2.67, 3, 3.5, 4, 5, 6, 8, 10, 13, 24, 36 and 48 h after drug administration. Subjects could drink water freely 2 h after drug administration and 4 h after drug administration they had lunch together. The subjects were not allowed to do strenuous exercises or to be bed ridden. Following centrifugation at 4000 rpm for 5 min, the plasma samples were separated and stored at −70 °C until pharmacokinetic analysis. All laboratory parameters were monitored during screening and after the study. Vital signs and adverse events were recorded before and throughout the study.

For pharmacokinetic study, plasma samples were assayed for celecoxib after administration of T and R to 51 subjects. After genotyping, the plasma of 20 selected subjects (4 subjects without *CYP2C9*3 and *CYP2D6*10, 5 subjects with *CYP2C9*1/*1 and *CYP2D6*1/*10 genotypes, 5 subjects with *CYP2C9*1/*1 and *CYP2D6*10/*10 genotypes, 3 subjects with *CYP2C9*1/*3 and *CYP2D6*1/*10 genotypes, and 3 subjects with *CYP2C9*1/*3 and *CYP2D6*10/*10 genotypes) from the 51 subjects were assayed for the metabolites of celecoxib, hydroxyl-celecoxib and carboxy-celecoxib, to further study the influence of genetic polymorphism on the pharmacokinetics of celecoxib in healthy Chinese subjects.

2.3. Safety and tolerability

Drug administration and plasma sample collection were done in phase I clinical wards of clinical drug trial department at the Institute of Dermatology, Chinese Academy of Medical Sciences, Nanjing, China. The wards are equipped with emergency rescue equipments and drugs. The adverse events were observed under the supervision of the clinicians. Once adverse events occurred, first aid measures and therapy were conducted. At the end of each period blood biochemistry and electrocardiogram (ECG) were reexamined.

2.4. Celecoxib and its metabolites assay

The UPLC–MS/MS method used for the assay of celecoxib was different from the one used for hydroxy-celecoxib and carboxy-celecoxib. In each method, samples were first thawed at ambient temperature and vortex-mixed, the analytes were extracted by protein precipitation from plasma with methanol, and determined using the Waters ACQUITY UPLC® system (Milford, MA) coupled with a Waters® Xevo TQ MS (Milford, MA); chromatographic separation was performed on a Waters ACQUITY UPLC® BEH C_{18} Column (1.7 μm, 2.1 mm × 150 mm) with a gradient elution system of methanol and a specific concentration of acetic acid (0.05% for celecoxib assay and 0.01% for hydroxy-celecoxib and carboxy-celecoxib assay); the tandem mass spectrometer was operated in electrospray ion (ESI) negative mode at a unit resolution using multiple reaction monitoring (MRM) data acquisition; and data collection was conducted using MassLynx™ V4.1. The monitoring transition for celecoxib, hydroxy-celecoxib, carboxy-celecoxib and internal standard (IS) glipizide was 380.10 → 316.13, 396.02 → 302.07, 410.01 → 366.07 and 444.30 → 319.16 respectively.

The plasma assay methods were fully validated. The analytes and the internal standard (IS) were baseline separated with symmetrical narrow peaks. The calibration curves were linear over the concentration range of 2–1500 ng/ml for celecoxib, 0.25–150 ng/ml for hydroxy-celecoxib and 2–1000 ng/ml for carboxy-celecoxib respectively with correlation coefficients (r^2) >0.996. Low (5 ng/ml, 0.5 ng/ml, 6 ng/ml), medium (150 ng/ml, 10 ng/ml, 50 ng/ml) and high (1200 ng/ml, 100 ng/ml, 800 ng/ml) quality control samples for celecoxib, hydroxy-celecoxib and carboxy celecoxib, respectively were prepared. The within-run and between-run precisions were <4.2% and <6.6% for celecoxib, <11.9% and <7.3% for hydroxy-celecoxib, and <9.2% and <5.8% for carboxy-celecoxib (relative standard deviation %, RSD %), respectively. Matrix effect was not observed. The average recovery of