Association study of CYP3A5 genetic polymorphism with serum concentrations of carbamazepine in Chinese epilepsy patients

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Abstract

Objective: To investigate the association between the CYP3A5 genetic polymorphism and the serum concentrations of carbamazepine (CBZ), to provide guidance for individualized drug dosing. Methods: Eighty-four epilepsy patients taking CBZ were included in this study. Their clinical data were recorded and CBZ serum concentrations were measured. The CYP3A5 6986 genetic polymorphism was assessed using a polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) assay. Patients were divided according to genotype into CYP3A5 expressor (CYP3A5*1/*1 genotype and CYP3A5*1/*3 genotypes) and non-expressor (CYP3A5*3/*3). The two groups were compared for the total dose of CBZ, dose of CBZ/kg body weight, serum drug concentration, dose-corrected serum concentration, and standardized serum concentration. Results: The total dose of CBZ and the dose of CBZ/kg body weight was higher in the CYP3A5 expressor group than the non-expressor (P = 0.043 and P = 0.014, respectively). The dose-corrected and standardized serum concentrations were lower in the CYP3A5 expressor group than the non-expressor (P = 0.001 and P < 0.001, respectively). There was however, no significant difference in serum drug concentration between the two groups (P = 0.487). Conclusions: There was a close relationship between CYP3A5 genetic polymorphism and the serum concentrations of carbamazepine.

INTRODUCTION

Seventy to 80% of epileptic patients have partial epilepsy.1 Carbamazepine (CBZ) is often used as monotherapy in partial epilepsy with or without secondary generalization. There are differences in therapeutic efficacy in patients taking CBZ. These differences in therapeutic response may be partly due to individual differences in serum concentrations of the drug, which may in turn be determined by the genetic polymorphisms of metabolic enzymes. CYP3A5 is one of the main enzymes that metabolize CBZ. Few studies have been previously performed to determine the relationship between CYP3A5 genetic polymorphisms and serum level of CBZ. Our objective in this study was to determine the relationship between CYP3A5 polymorphisms and CBZ serum concentrations, and the therapeutic effects of the drug in a cohort of Chinese epilepsy patients.

METHODS

Inclusion criteria

The inclusion criteria were: (1) Partial epilepsy with or without secondary generalization; (2) CBZ was taken regularly for more than a week with good adherence; (3) Other antiepileptic drugs and drugs which could affect the activities of liver drug metabolizing enzymes had not been administered; (4) There was no serious liver or renal disease.

Drug serum concentration determination

CBZ was taken (P.O.) regularly for more than a week to establish a steady state prior to drug serum concentration measurement. Venous blood was collected from patients on empty stomach in the early morning. The blood samples were pre-dose samples.

Serum concentrations of CBZ were determined using the Dimension Clinical Biochemistry System and CRBM Flex kits (particle-enhanced turbidimetric immunoassay) at the Inspection
Department, First Hospital of Jinlin University, Jilin, China. Its coefficient of variation was 31%, and the sensitivity of the analytical method was 0.5μg/ml or 2.1μmol/L. The therapeutic level of CBZ was taken as 4 to 12μg/mL. Dose-corrected serum concentration, which takes into account the effect from different doses, was calculated as the ratio of serum concentration to CBZ dose/day. Standardized serum concentration, which takes into account the effects of the dose and body weight, was calculated as the ratio of serum concentration to CBZ dose/kg body weight/day.

DNA extraction
Four milliliters of collected peripheral venous blood from epileptic patients was infused into an anticoagulation tube with 2% EDTA, and DNA was extracted using a Blood Genomic DNA Purification Kit. There was a blank control prepared to look for environmental DNA contamination and samples cross-contamination.

CYP3A5 genetic polymorphism analysis by PCR-RFLP

PCR primer: The forward primer was 5' -GGCAACATGACTTAGACAG- 3', and the reverse primer was 5' -GGTCCAAACAGGGAAGAAATA-3'.

PCR reaction system: The bulk volume of 50μL consisted of 0.7μL forward primer, 0.7μL reverse primer, 0.4μL (5 U/μL) Taq enzyme, 3μL DNA templates, 5μL 10×PCR buffer, and 39.2μL sterilized ddH₂O.

PCR reaction procedure: The reaction mixtures were kept at 95 °C for 1 min to denature the DNA fully. Denaturing was at 95 °C for 45 seconds, annealing at 57 °C for 45 seconds and extended at 72 °C for 30 seconds, repeated for 33 cycles. After these cycles were completed, the reaction mixtures were extended at 72 °C for a further 10 minutes. After amplification, the 298 bp DNA band was detected by 2% agarose gel electrophoresis for 45 min, and visualized using a gel imaging system.

Genotype determination by PCR-RFLP: The PCR product was digested by restriction enzyme Ssp I at 37 °C for 3 h. The bulk volume of the PCR-RFLP reaction system was 20 μL, consisting of 0.5μL Ssp I, 10μL gene fragment after PCR amplification, 2μL 10×NEB buffer, and 7.5μL sterilized ddH₂O. The product was detected by 3% agarose gel electrophoresis for 45 min, and visualized using a gel imaging system.

Gene Sequencing: In order to check the accuracy of PCR-RFLP, gene fragments after PCR amplification of several samples were analyzed with an automatic sequencer at Shanghai Shenggong Company.

Statistical Analysis
All data were analyzed by Statistical Package for the Social Sciences (SPSS) 13.0 software (SPSS Inc., IL, USA). Data were expressed as means ± standard deviation (SD). The Kolmogorov-Smirnov normal distribution and homogeneity tests for variance were used for a single sample of data. Student’s t-test and the chi-square (χ²)-test were used for two samples of measurement and enumeration data, respectively. Linear regression analysis was used for dependability analysis between two variables. Probability (P)-values < 0.05 were considered statistically significant.

RESULTS
Eighty-four epilepsy patients (53 males, 31 females, age range 16-77 years, body weight range 55-80 kg) treated with CBZ monotherapy were included in this analysis. Of the 84 patients, 19 suffered from generalized seizures, 65 from partial seizures and 11 had mixed seizures. One serum sample in each patient was taken for CBZ quantitation.

Clinical data of patients in the CYP3A5 expressor and non-expressor groups
According to the genotypes, patients were divided into a CYP3A5 expressor group and a CYP3A5 non-expressor group. The CYP3A5 expressor group consisted of the genotypes CYP3A5*1/*1 (AA) and CYP3A5*1/*3 (AG). The CYP3A5 non-expressor group included the CYP3A5*3/*3 genotype (GG). There were no statistically significant differences in age, gender, weight, CBZ dose, or CBZ dose/kg body weight between the two groups (Table 1, Fig. 1). The gender was compared by χ²-test, and others by Student’s t-test.
greater reduction in seizure frequency for at least one year, up to the date of the last follow-up visit. Drug inefficacy was defined as no change or less than 50% reduction in seizure frequency for at least one year, up to the date of the last follow-up visit. The efficacy comparison between the CYP3A5 expressor and non-expressor groups is shown in Table 2. There was no statistically significant difference in efficacy between the two groups ($\chi^2 = 0.005, P = 0.943$).

Products of PCR amplification and Ssp I PCR-RFLP

After PCR amplification of CYP3A5, the 298 bp DNA band was detected by 2% agarose gel electrophoresis (Figure 2). After Ssp I PCR-RFLP, two products (168bp and 130bp) of CYP3A5*3/*3 genotype (GG genotype) were obtained, three (148bp, 130bp and 20bp) of CYP3A5*1/*1 genotype (AA genotype), and four (168bp, 148bp, 130bp and 20bp) of CYP3A5*1/*3 genotype (AG genotype). The 20 bp DNA band was not detected in the electropherogram. Therefore, two bands of GG and AA genotypes, and three bands of AG genotype, could be seen in the electropherogram (Figure 3). The sequencing results are shown in Figure 4.

Genotype analysis

The percentages of AA, AG, and GG genotypes in the 84 epilepsy patients were 3.6%, 34.5%, and 61.9%, respectively. The percentages of

<table>
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<tr>
<th>Table 1: Comparison of the clinical data of patients between the CYP3A5 expressor and non-expressor groups.</th>
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<tr>
<td>CYP3A5 expressor group</td>
</tr>
<tr>
<td>Quantity</td>
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<tr>
<td>Gender</td>
</tr>
<tr>
<td>Age, years</td>
</tr>
<tr>
<td>Weight, kg</td>
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<tr>
<td>CBZ dose, mg</td>
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<td>CBZ dose, mg/kg</td>
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CBZ: carbamazepine

Figure 1. Comparison of the clinical data of patients between the CYP3A5 expressor and non-expressor groups.

Figure 3. Electropherogram of Ssp I PCR-RFLP products for CYP3A5.*3/*3 (GG genotype), CYP3A5.*1/*1 (AA genotype), and CYP3A5.*1/*3 (AG genotype).
allelomorphic gene A and G were 20.8% and 79.2%, respectively, which fit the Hardy-Weinberg genetic balance. Patients were divided into the CYP3A5 expressor group (CYP3A5*1/*1 and CYP3A5*1/*3 genotypes) and the CYP3A5 non-expressor group (CYP3A5*3/*3). There were no statistically significant differences in sex, age, or weight between these two groups.

CYP3A5 genetic polymorphism and CBZ dosage

The CBZ doses for the CYP3A5 expressor and non-expressor groups were 512.50 ± 128.89 mg and 441.35 ± 167.66 mg, respectively, and the difference was statistically significant (t = 2.054, P = 0.043). The CBZ dose/kg body weight in the CYP3A5 expressor group was 8.07 ± 2.21 mg/kg, higher than the 6.70 ± 2.53 mg/kg for the CYP3A5 non-expressor group, a statistically significant difference (t = 2.523, P = 0.014).

Association of CYP3A5 genetic polymorphism and serum drug concentration, dose-corrected serum concentration, and standardized serum concentration of CBZ

The CBZ serum drug concentrations in the CYP3A5 expressor and non-expressor groups were 7.03 ± 1.78 μg/mL and 7.37 ± 2.29 μg/mL, respectively; the difference was of no statistical significance (P = 0.487). The dose-corrected serum concentration of CBZ in the CYP3A5 expressor group was 14.08 ± 3.18 ng/(mL·mg), lower than the 18.01 ± 5.62 ng/(mL·mg) of the non-expressor group, the difference was statistically significant (P = 0.001). The standardized serum concentration of CBZ in the CYP3A5 expressor group was 0.90 ± 0.20 μg·kg/(mL·mg), lower than the 1.19 ± 0.38 μg·kg/(mL·mg) of the non-expressor group, the difference was statistically significant (P < 0.001).

DISCUSSION

CYP3A5 is located on human chromosome 9, mapped to q21.1-22.1, coding 502 amino acids. Individual and racial differences are evident in its expression and activities. While CYP3A5 is expressed in a minority of Caucasians and Asians, it is present in the majority of African Americans, and when present can be 50% of the total CYP3A enzymes in the liver. The expression of CYP3A5 is a very important factor influencing the total amount of CYP3A enzymes in the human liver, and the most important factor determining individual differences in CYP3A activities, therapeutic effects and the toxicity of drugs metabolized by CYP3A.

Table 2: Comparison of clinical efficacy to carbamazepine between the CYP3A5 expressor and non-expressor groups

<table>
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<th></th>
<th>Effective</th>
<th>Ineffective</th>
<th>Effective rate (%)</th>
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<tbody>
<tr>
<td>CYP3A5 expressor group</td>
<td>23</td>
<td>9</td>
<td>71.9</td>
</tr>
<tr>
<td>CYP3A5 non-expressor group</td>
<td>37</td>
<td>15</td>
<td>71.2</td>
</tr>
<tr>
<td>Total</td>
<td>60</td>
<td>24</td>
<td>71.4</td>
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Effective: seizure-free or a 50% or greater reduction in seizure frequency for at least one year, up to the date of the last follow-up visit; Ineffective: no change or less than 50% reduction in seizure frequency for at least one year, up to the date of the last follow-up visit, for patients treated with CBZ monotherapy.
Figure 3. Electropherogram of the PCR-RFLP products of CYP3A5 6986A/G
Notes: Marker is in the left lane; lanes 1, 5, 8, 9 of AG genotype; lanes 2-4, 6, 7, 10, 12-14, 16-18 of GG genotype; lanes 11 and 15 of AA genotype.

Figure 4. The sequencing results of CYP3A5 6986A/G
The difference in CYP3A5 activities is caused by single-nucleotide polymorphisms (SNPs). The SNP 6986 A→G (CYP3A5*3) in intron 3, is very important for CYP3A5. Several splice variant mRNAs produced by CYP3A5*3 result in a premature stop codon and leads to CYP3A5 deficiency. Therefore, CYP3A5 can be produced only when there is more than one CYP3A5*1 in allelicomorphic genes. The occurrence frequency of CYP3A5*3 in Chinese is 72.7%.4

A change in CYP3A5 activities may affect the metabolism of drugs that are substrates of this enzyme.5 The metabolism of substrates such as sirolimus, tacrolimus and amlodipine, has been shown to be altered by CYP3A5 polymorphisms. The metabolism of other substrates such as midazolam, nifedipine, and diltiazem, however, was not affected.

CBZ could be eliminated almost completely by biotransformation in the liver, mainly by the isoenzymes CYP3A4 and CYP3A5, and partially by CYP2C8 and CYP1A2.6,7,11,12 Liver enzyme induction of CBZ could enhance the metabolism itself. In this study, the doses of CBZ and of the calcineurin inhibitors require higher doses of CBZ, while those with the CYP3A5*3/*3 genotype need less, in order to reduce the risk of adverse effects of the drug and to avoid unnecessary dosing. The practical significance of this study is its usefulness for guiding clinical personalized medicine.

REFERENCES