Impact of CYP2C19 and CYP2C9 gene polymorphisms on sodium valproate plasma concentration in patients with epilepsy

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ABSTRACT

Background Valproic acid (VPA) is a broad spectrum anticonvulsant drug, which could be partially metabolised by cytochrome P450 (CYP) 2C9 and 2C19 enzymes. This study was designed to investigate the relationship between CYP2C19 and CYP2C9 gene polymorphisms and the plasma concentrations of VPA in subjects with epilepsy.

Methods Eighty-three subjects with epilepsy aged 18–92 years were enrolled in this study. All were treated with sustained-release VPA monotherapy. Based on the genotypes of CYP2C19 and the ability to metabolise substrates, the subjects were divided into poor metabolisers, intermediate metabolisers and extensive metabolisers. Sanger sequencing was used to detect the genotypic and allelic frequencies of CYP2C19 (*1, *2 and *3) and CYP2C9 (*13) of the patients. Automatic immunity analysis was used to find steady-state trough plasma concentrations of VPA. By adjusting the plasma concentrations of VPA with body weight and total daily dose of VPA, the concentration-to-dose ratio of VPA (CDRV) was obtained. Data were analysed using SPSS software.

Results The genetic frequencies of CYP2C19*2, CYP2C19*3 and CYP2C9*13 were 33.1%, 3.0% and 5.4%, respectively, among patients with epilepsy from Yunnan province, China who used VPA therapy. The CDRV was significantly lower in the CYP2C19 extensive metabolisers (3.33±1.78) than it was in the CYP2C19 intermediate metabolisers (4.45±1.42) and the CYP2C19 poor metabolizers (6.64±1.06). The CYP2C19*2 and CYP2C19*3 alleles were correlated with the plasma VPA concentration, while the CYP2C9*13 allele had no effect on the plasma VPA concentration (p=0.809).

Conclusions The genetic polymorphisms of CYP2C19 significantly affect the VPA plasma concentration, and the dosage of VPA for intermediate and poor metabolisers could be lower than for extensive metabolisers. CYP2C9*13 carrier was not closely related to plasma concentrations of VPA in patients with epilepsy.

INTRODUCTION

As a branched short-chain fatty acid, valproic acid (VPA) is widely used as an antiepileptic drug and a mood stabiliser. It performs well in preventing partial seizures, absence seizures and generalised seizures in patients with epilepsy. It can be administered intravenously or orally. The therapeutic range for VPA (total) is generally considered to be 50–100 µg/mL. A dose above 150 µg/mL is associated with serious toxicity including hepatotoxicity, pancreatitis and other metabolic disorders.[1] The dose of VPA and the plasma concentration may be significantly different among individuals (10-fold difference from mean dose of adults) due to genetic polymorphisms of cytochrome P450 (CYP) 2C19 and 2C9, or interactions with other drugs.[2–4] Optimising the VPA dose with genetic variation could play a key role in individualised care to avoid adverse drug reactions or treatment failure and maximise drug efficacy.[5]

The metabolism of VPA is complex. The major metabolic pathways of VPA comprise glucuronidation and mitochondrial β-oxidation, while CYP-mediated oxidation is only a minor pathway.[1 6] CYP2C9 plays a key role in the biotransformation of VPA in humans, transforming VAP into 4-ene-VPA and 5-OH-VPA, while CYP2C19 transforms a small amount of VPA into 4-OH-VAP. There are significant racial differences in VAP transformation due to gene polymorphism.[7]

Genetic polymorphism of CYP2C19 has been extensively studied and at least 69 allelic variants have been defined currently by the Human Cytochrome P450 (CYP) Allele Nomenclature Database (https://www.pharmvar.org/htdocs/archive/cyp2c19.htm). Based on the genotypes of CYP2C19 and the ability to metabolise substrates, individuals can be divided into poor metabolisers (PMs), intermediate metabolisers (IMs) and extensive metabolisers (EMs). The genotype of PMs consists of two loss-of-function alleles (eg, *2/*2, *2/*3, *3/*3), resulting in significantly reduced or absent CYP2C19 activity. IMs have one wild-type allele and one variant allele that encodes reduced or absent enzyme function (eg, *1/*2, *1/*3), resulting in decreased CYP2C19 activity. EMs are homozygous for the CYP2C19*1 allele, which is associated with functional CYP2C19-mediated metabolism.[8]

CYP2C19*2 (c.681G>A; rs4244285) is the most common CYP2C19 loss-of-function variant. The CYP2C19*2 allele frequencies are approximately 12%–15% in Caucasians and African-Americans, and 29%–35% in Asians. CYP2C19*3 (c.636 G>A; rs4986893) is another commonly tested loss-of-function allele. The frequencies of CYP2C19*3 are 2%–9% in Asians but <1% in other parts of the world.[9] Furthermore, the CYP2C19 genetic polymorphisms significantly influence the pharmacokinetic variability of VPA based on a previous study.[2] Nevertheless, there is no full available data about the genetic variation of CYP2C19 in the population of Yunnan province in China. The association between CYP2C19

genotypes and pharmacokinetic variability of VPA therefore remains to be further explored.

Although CYP2C9 is in the secondary metabolic pathway of VAP in adults, genetic variations related to change in CYP2C9 activity may cause substantial inter-individual differences on pharmacokinetics and adverse reactions of valproate. Several mutant alleles have been identified including high frequency alleles CYP2C9*2 and CYP2C9*3, the functional statuses of which are associated with decreased activity of CYP2C9.[10][11] However, the association between CYP2C9*13 (c.269T>C; rs72558187) and VPA pharmacokinetics is unknown.

The aims of this study were to determine (1) the genetic frequencies of CYP2C19*2 and CYP2C19*3 in patients with epilepsy using VAP therapy and (2) the association between CYP2C19 and CYP2C9 genotypes and the plasma concentrations of VPA in patients with epilepsy.

MATERIALS AND METHODS
Study design
The inclusion criteria included: (1) patients with a diagnosis of seizures; (2) age 18 or older; and (3) patients regularly taking sustained-release valproate twice daily for at least 1 month. The exclusion criteria included: (1) patients who were taking other antiepileptic drugs; (2) those with serious liver, renal or cardiac diseases; (3) patients with poor adherence; and (4) patients with serious adverse drug reactions to VPA. Ethical approval for the study was obtained from the ethics committee of the Kunming First People's Hospital Research Centre. Informed consent was obtained from all participants.

A total of 83 eligible patients were enrolled from the First People's Hospital of Kunming in Yunnan province, China from March 2016 to May 2017. The demographic data including age, gender, body weight and dosage regimen of patients are shown in table 1. Steady-state trough blood samples were collected 1 hour before the administration of the morning dose. After centrifugation at 12 000 rpm for at least 4 min at room temperature, the plasma was used to determine the VPA concentration by fluorescence polarisation immunoassay and the remaining blood samples were stored at −80°C for later genome DNA isolation and genotype detection.

Genotyping
The genotype of CYP2C19 and CYP2C9 were detected by Sanger sequencing. Genome DNA of individuals was isolated using a blood DNA isolation kit (DP349, Tiangen, China) . CYP2C19*2 (c.681G>A; rs4244285), CYP2C19*3 (c.636 G>A; rs4986893) and CYP2C9*13 (c.269T>C; rs72558187)

flank sequences were amplified by polymerase chain reaction (PCR) with the following primers: rs4244285 forward: AAAGCAGGTATAAGTCTAGGAAATG, rs4244285 reverse: ATAAAGTCCCGAGGGGTTGTT, rs4986893 forward: CTTC ACCCTGTGATCCCACTT, rs4986893 reverse: GTACTTCA GGGCTTGGTCAAT, rs72558187 forward: GGTCTATGG-CCCTGTGTTCA, rs72558187 reverse: ACATGCACACCT ACCAAATCC.

PCR products were purified using the TIANgel Purification Kit (DP 219, Tiangen, China) and sent to Generalbiol Company for sequencing. Mutation alleles were assayed by Lasergene software based on sequence results.

Plasma trough concentration of VPA
The total plasma trough concentrations of VPA were analysed by semi-quantitative chemiluminescent microparticle immunoassay (ARCHITECT i1000SR immunoassay analyser, Abbott Laboratories, Abbott Park, Illinois, USA) using ARCHITECT iValproic Acid (produced by Denka Seiken, Tokyo, Japan and distributed by Abbott Laboratories). Routine calibration curve and quality control samples were applied to ensure the accuracy and precision of the method. To minimise the inter-individual variations, the plasma concentrations of VPA were adjusted by total daily dose and body weight to obtain the concentration-to-dose ratio of VPA (CDRV).

Statistical methods
Data analysis was performed with IBM SPSS Statistics 25.0 (SPSS, IBM Corporation, Chicago, Illinois, USA). Genotype distributions and allele frequencies of CYP2C19 G681A, CYP2C19 G636A and CYP2C9 T269C of participants were determined, and their 95% CIs were calculated. Hardy–Weinberg equilibrium was determined for all the genotypes using χ^2 testing. Continuous variables including age, body weight, plasma trough concentration of VPA, mean VPA concentration and CDRV were calculated and presented as mean±SD, and the statistical analysis of CDRV among EMs, IMs and PMs was performed by one-way analysis of variance (ANOVA) followed by the Games–Howell test. The statistical analysis of CDRV between CYP2C9*13 carriers and non-carriers was performed using the independent sample t-test. The significance threshold was set at 0.05.

RESULTS
Patient characteristics
The demographic characteristics of the patients are shown in table 1. A total of 83 steady-state concentrations were collected. The plasma trough concentrations of VPA of the participants was 56.3±26.1 µg/mL and the mean value was within the 50–100 µg/mL therapeutic concentration of VPA. The CDRV value was 4.3±1.9 $\mu g \cdot kg/mL \cdot mg$

Genotype distributions of CYP2C19*2 (c.681G>A; rs4244285), CYP2C19*3 (c.636 G>A; rs4986893) and CYP2C9*13 (c.269T>C; rs72558187)
The genotype frequencies are shown in table 2. CYP2C19 G681A and CYP2C9 T269C polymorphisms were consistent with Hardy–Weinberg equilibrium (p>0.05). Genotype distributions of CYP2C19 G681A of the 83 patients showed that 40 (48.2%) subjects were GG, 31 (37.3%) were GA and 12 (14.5%) subjects were AA. For CYP2C19 G636A, 79 (95.2%) subjects were GG, 3 (3.6%) were AG and 1 (1.2%) was GG. Genotype distributions of the CYP2C9 T269C of the patients showed that 75 (90.4%) were TT, 7 (8.4%) were TC and 1 (1.2%) was CC.

Table 1 Demographic characteristics of the patients

Variable	Enrolled patients (n=83)
Mean±SD age, years	50.0±18.7
Male sex, n (%)	58 (69.9%)
Female sex, n (%)	25 (30.1%)
Mean±SD body weight, kg	62.0±9.2
Total daily dose of VPA, mg	872.3±355.8
VPA concentration, µg/mL	56.3±26.1
Patients with VPA concentration <50 µg/mL*	36
Patients with VPA concentration >100 µg/mL*	0
CDRV (mg/L per g/kg/day)	4.3±1.9

*The valproic acid reference range is 50~100 µg/mL.[21]
CDRV, concentration-to-dose ratio of VPA; VPA, valproic acid.

Table 2 Genotype distribution and allele frequency of CYP2C19 G681A, CYP2C19 G636A and CYP2C9 T269C polymorphisms

Genotypes	N (%)	Allele frequency (%)	P value
CYP2C19 G681A			
GG (*1)	40 (48.2%)	33.1	0.152
GA (*2)	31 (37.3%)		
AA (*2)	12 (14.5%)		
CYP2C19 G636A			
GG (*1)	79 (95.2%)	3.0	0.001*
GA (*3)	3 (3.6%)		
AA (*3)	1 (1.2%)		
CYP2C9 T269C			
TT	75 (90.4%)	5.4	0.106
TC	7 (8.4%)		
CC	1 (1.2%)		
*Statistically significant (Chi-square test).			

The allele frequencies of CYP2C19 681A, CYP2C19 636A and CYP2C9 269C were 33.1%, 3.0% and 5.4%, respectively.

Concentration-to-dose ratio of VPA (CDRV) in different genotype groups

Among the 83 patients, the frequency of EMs (CYP2C19 *1 homozygote), IMs (CYP2C19 *1/*2, *1/*3) and PMs (CYP2C19 2/*2, *2/*3, *3/*3) were 44.6%, 39.8% and 15.6%, respectively (table 3). The CDRV was significantly lower in EM individuals (3.33±1.78 µg·kg/mL·mg) than in IMs (4.45±1.42 µg·kg/mL·mg, p=0.013) and PMs (6.64±1.06 µg·kg/mL·mg, p=0.000), and the CDRV was significantly lower in IM individuals than in PMs (p=0.000). The CDRV was not significantly different between CYP2C9 wild-type homozygotes (4.08±1.97 µg·kg/mL·mg) patients and CYP2C9*13 carriers (3.91±0.99 µg·kg/mL·mg), p=0.809.

DISCUSSION

CYP2C9 and CYP2C19 are the predominant enzymes in the CYP-mediated metabolism of VPA. Several reports have shown that CYP2C19*2 and CYP2C19*3 are the most common alleles among the Chinese population and their prevalence varies according to geographical region and ethnic group.[5 12 13] Yunnan province is famous for ethnic diversity in China. There are 26 ethnic groups in Yunnan, which accounts for nearly half of the country's 56 recognised ethnic groups. Our study has shed light on the genotype distributions of the CYP2C19 G681A, CYP2C19 G636A polymorphisms in a small sample of people from Yunnan and provides knowledge of tailoring drug therapy based on genotype to individual patients, with the potential of improving the safety and efficacy of VPA.

The allele frequency of CYP2C19*3 is consistent with previous observations (3% vs 2%–7.1%).[5 9] However, the allele frequency of CYP2C19*2 in this investigation was higher than that reported in other studies (33.1% vs ≈29%).[9 14] The CDRV was significant lower in EMs (3.33±1.78 µg·kg/mL·mg) than in IMs (4.45±1.42 µg·kg/mL·mg, p=0.013) and PMs (6.64±1.06 µg·kg/mL·mg, p=0.000). In contrast, the mean VPA dose in EMs was larger than in IMs and PMs. It indicates the influence of CYP2C19*2 and CYP2C19*3 carriers on CYP2C19 metabolism associated with VPA plasma concentration. Although a previous study has noted that CYP2C9*3 and CYP2C9*13 alleles appear to be associated with the decreased metabolism of irbesartan,[15] the effects of CYP2C9*13 on the VPA concentration in patients with epilepsy has rarely been explored. Our data showed CYP2C9*13 (c.269T>C; rs72558187) genotype frequencies of 90.4% (TT), 8.4% (TC) and 1.2% (CC), which are higher than previous studies.[16 17] However, the CDRVs showed no significant difference (p=0.809) between CYP2C9 wild-type homozygote (4.08±1.97 µg·kg/mL·mg) patients and CYP2C9*13 carriers (3.91±0.99 µg·kg/mL·mg). Jiang et al showed that the CYP2C9*3 allele influenced the pharmacokinetic variability of VPA in Chinese epileptic patients, but in this study we did not include this allele. The influence of the CYP2C9*3 allele on CDRVs is not excluded in our work. The limited number of patients included in this work also limits the accuracy of the impact of the CYP2C9*3 allele on the VPA concentration. Further studies with more samples are needed to illustrate the effect of the CYP2C9*13 allele on the VPA concentration in patients with epilepsy.

This research has certain limitations. First, glucuronidation and mitochondrial beta-oxidation (approximately 70%–90%) are the major VPA metabolic pathway in adults.[18] We did not

Table 3 Effects of the CYP2C19 and CYP2C9 genotypes on standardised VPA concentrations in the patients with epilepsy

Genotypes	N (%)	Mean age (years)	Mean VPA dose (mg/kg per day)	Mean VPA concentration (µg/mL)	CDRV (µg·kg/mL·mg)	P value
CYP2C19 G681A/ G636A						
EMs*	37 (44.6%)	50.70±17.91	16.28±7.50	48.73±24.08	3.33±1.78	EMs vs IMs p=0.013§
IMs†	33 (39.8%)	48.73±19.05	12.80±5.02	57.15±25.07	4.45±1.42	EMs vs PMs p=0.000§
PMs‡	13 (15.6%)	51.15±24.62	11.54±3.12	75.55±25.58	6.64±1.06	IMs vs PMs p=0.000§
CYP2C9 T269C						
Non-carriers¶	75	49.72±22.98	14.14±6.50	56.21±26.40	4.08±1.97	p=0.809††
Carriers**	8	52.50±17.17	14.25±4.52	56.92±24.64	3.91±0.99	

*EM: extensive metaboliser genotype: CYP2C19 *1/*1.
†IM intermediate metaboliser genotype: CYP2C19 *1/*2 (n=31) and CYP2C19 *1/*3 (n=2).
‡Poor metabolizer genotype: CYP2C19 *2/*2 (n=11), CYP2C19 *3/*3 (n=1) and CYP2C19 *2/*3 (n=1).
§Games–Howell test, mean difference is significant at the 0.05 level.
¶Non-carriers: CYP2C9*1/*1.
**Carriers: *1/*13 (n=7) and *13/*13 (n=1).
††Independent sample t-test, mean difference is significant at the 0.05 level.
EMs, extensive metabolisers; IMs, intermediate metabolisers; PMs, poor metabolisers; VPA, valproic acid.

investigate the contribution of uridine diphosphate glucurono-syltransferase polymorphisms which are the most confounding factors to interindividual variations in valproate pharmaco-kinetics.[19 20] Second, the sample is too small to derive greater statistical power because of the limited number of patients eligible for the trial, but the results do provide some valuable information on the CYP2C19*2, CYP2C19*3 and CYP2C9*13 allele frequency in Yunnan province, and our research related CYP2C19 genotype to VPA concentrations, which may provide guidance on VPA dosing in epilepsy.

CONCLUSION

The genetic polymorphisms of CYP2C19 could play a key role in individualised dosing regimens of VPA. Allele CYP2C19*2 and CYP2C19*3 carriers have a higher trough plasma VPA concentration than CYP2C19 wild-type patients, which indicates that the dose of VPA in CYP2C19*2 and CYP2C19*3 carriers could be lower than for EM patients. The CYP2C9*13 genotype had no influence on the VPA plasma concentration in this study.

Key messages

What is already known on this subject

⇒ The dose of VPA and plasma concentration could be significantly different among individuals due to genetic polymorphisms.

⇒ Exploration of the relationship between CYP2C19 and CYP2C9 gene polymorphisms and the serum concentrations of VPA in patients with epilepsy could provide clues for precise drug administration.

What this study adds

⇒ The genetic polymorphisms of CYP2C19 could play a key role in VPA individualised dosing regimens.

⇒ Allele CYP2C19*2 and CYP2C19*3 carriers have a higher trough plasma VPA concentration than CYP2C19 wild-type patients, which indicates that the dose of VPA in CYP2C19*2 and CYP2C19*3 carriers could be lower than for EM patients.

⇒ The CYP2C9*13 genotype had no influence on the VPA plasma concentration in this study.

Data availability statement All data relevant to the study are included in the article or uploaded as supplementary information. All data relevant to the study are included in the article. Raw data are available upon reasonable request.

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