Note

Effects of UGT1A6, UGT2B7, and CYP2C9 Genotypes on Plasma Concentrations of Valproic Acid in Chinese Children with Epilepsy

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Summary: Valproic acid (VPA) is one of the most commonly prescribed drugs for the treatment of epilepsy. Interindividual variability in VPA dose and plasma concentration may reflect functional consequences of genetic polymorphisms in genes encoding drug-metabolizing enzymes. The aim of this study was to determine the relationship between plasma concentrations of VPA and single nucleotide polymorphisms (SNPs) involving uridine diphosphate glucuronosyltransferase (UGT) 1A6 (UGT1A6), UGT2B7, and cytochrome P450 2C9 (CYP2C9) genes in Chinese children with epilepsy. UGT1A6, UGT2B7, and CYP2C9 polymorphisms were identified by the polymerase chain reaction-restriction fragment length polymorphism approach or direct automated DNA sequencing in 98 epileptic patients treated with VPA monotherapy. Patients with double heterozygosities at nucleotide positions T19G, A541G and A552C in the UGT1A6 gene, were associated with higher VPA doses compared to those with wild type or single heterozygosity (p = 0.010). Lower adjusted plasma VPA concentrations were also observed in patients with UGT1A6 double heterozygosities than those with single heterozygosity (p = 0.027). There were no differences in VPA dose or adjusted plasma VPA concentrations among the UGT2B7*2 or CYP2C9*3 genotypic groups. These results suggest that UGT1A6 mutations affect VPA metabolism in epileptic children. It needs to be further investigated in a larger cohort of patients.

Keywords: epilepsy; valproic acid; genetic polymorphisms; UGT1A6; UGT2B7; CYP2C9

Introduction

Valproic acid (VPA) is widely used for treating children with epilepsy. Although VPA is affordable and effective in the control of seizures, there is large interindividual variability in its pharmacokinetics and pharmacodynamics. Therefore, its plasma concentration needs to be monitored as a guide of dose adjustment during the course of therapy.¹ These differences in VPA dose and plasma concentration may reflect functional consequences of genetic polymorphisms in genes encoding drug-metabolizing enzymes.

The metabolism of VPA involves three major metabolic pathways, glucuronidation conjugation, mitochondrial β-oxidation, and CYP-catalyzed terminal desaturation and hydroxylation.² The most extensive biotransformation pathway for VPA is conjugation with glucuronic acid, mainly mediated by UGT1A6, UGT1A8, and UGT2B7.³ Approximately 20–70% of a dose of VPA is excreted in the urine as glucuronide conjugates. CYP-catalyzed metabolism of VPA accounts for about 10% of the administrated dose, mainly mediated by CYP2C9.⁴ The genes coding VPA-metabolizing enzymes are highly polymorphic. It has been reported that the three most common single nucleotide polymorphisms in the coding region of the UGT1A6 gene are T19G, A541G and A552C with allele frequencies of 0.238, 0.220, and 0.247, respectively, in the Chinese population.⁵ The combinations of different variants (UGT1A6*2 to *4) result in increased glucuronidation activities.⁶,⁷ UGT2B7*2 with C802T is the most extensively studied UGT2B7 variant with an allele frequency of 32.8% in the Chinese population.⁸ The combinations of different variants (UGT2B7*2 to *4) result in increased glucuronidation activities.⁶,⁷ UGT2B7*2 with C802T is the most extensively studied UGT2B7 variant with an allele frequency of 32.8% in the Chinese population.⁸ In terms of the contribution of UGT2B7*2 to different drug metabolisms, conflicting results have been reported.⁹-¹¹ CYP2C9*3 with A1075C, the major CYP2C9 variant with an allele frequency of about 3.3% in the
Chinese population had been demonstrated to significantly impair the catalytic activities of the wild-type CYP2C9 toward various substrates both in vitro and in vivo. Previous studies reported potential contributions of the SNPs in genes encoding the major VPA-metabolizing enzymes to VPA disposition. However, the results were not entirely consistent among the studies. For example, two Chinese reports demonstrated an association between the CYP2C9*3 genotype and VPA metabolism, but the association was not observed in an Iranian study. Moreover, VPA is metabolized by multiple UGTs and CYPs. Thus, previous studies were limited in assessing the influence of these gene polymorphisms on VPA disposition since most of studies only assayed the influence of the polymorphisms of one metabolizing enzyme in adult patients with epilepsy. The present study was designed to comprehensively evaluate the effects of UGT1A6 variants including the combinations of T19G, A541G and A552C, UGT2B7*2, and CYP2C9*3 on plasma VPA concentrations in a cohort of Chinese children with epilepsy.

Methods

Patients: A total 98 epileptic patients (56 males and 42 females, mean age 7.8 ± 7.5 years, mean body weight 27.3 ± 15.5 kg) were recruited at the Shengjing Hospital, China Medical University in Shenyang, China. Patients diagnosed with partial seizures or generalized seizures were treated with VPA as monotherapy. After a minimum of one month of continuous VPA treatments, 2–3 ml blood samples were drawn from each patient to measure plasma VPA concentration. The mean VPA daily dose was 17.2 ± 15.5 mg/kg per day, and the mean VPA plasma concentration was 65.2 ± 26.8 µg/ml. Due to large interindividual variations in VPA metabolism, steady-state plasma concentrations of VPA were adjusted by the dose and body weight of each patient. Patients previously treated with any drugs known to modulate the activities of UGT1A6, UGT2B7 and CYP2C9 or with liver or renal disorders were excluded from this study. Informed consent was provided by all the patients participating in the study. Sample handling and data analysis protocols were approved by the Medical Ethical Committee of China Medical University.

VPA quantitation: Blood samples were drawn from each patient after a minimum of one month of continuous VPA treatments to ensure that plasma VPA concentrations were at steady state. Peripheral blood samples were collected from patients before VPA administration and plasma VPA concentrations were quantified using the fluorescence polarization immunoassay of the Abbott TDx system, according to the instructions of the manufacturer. The assay had a coefficient of variation (C.V.) lower than 4%, and a sensitivity of 0.7 µg/ml. Results are presented as means of duplicates for each sample. Genotyping: Genomic DNA was extracted from clotted whole blood. Prior to extraction, clotted samples were thawed, and then dispersed into small pieces through nylon mesh (pore size 77 µm). Genomic DNA was then extracted by phenol–chloroform extraction and ethanol precipitation, as previously described. The UGT1A6 T19G, A541G, A552C and CYP2C9 A1075C polymorphisms were analyzed by the polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP) method, as described previously. UGT2B7 C802T polymorphism was determined by direct automated DNA sequencing after PCR amplification using forward (5′-CTGCATAATTCTAGGACAAC-3′) and reverse (5′-CTACCATAACATCGATTGG-3′) primers.

Statistical analysis: Statistical analysis was performed using SPSS software (version 16.0; SPSS Inc., IL, USA). The patients’ demographic characteristics including age and body weight, VPA doses, plasma VPA concentrations, and adjusted plasma VPA concentrations were compared between two genotypic groups using Student’s t-test or one way ANOVA. Deviation of Hardy-Weinberg equilibrium (HWE) was examined by the chi-square test. Linkage disequilibrium between SNP pairs was carried out by using the online software SHEsis (http://analysis.bio-x.cn/myAnalysis.php). A two-tailed p-value of less than 0.05 was considered to be statistically significant.

Results

To explore the effects of genetic factors on interindividual variabilities in VPA doses and plasma concentrations in our cohort of Chinese children with epilepsy, the UGT1A6 T19G, A541G, A552C, UGT2B7 C802T, and CYP2C9 A1075C polymorphisms were analyzed by PCR-RFLP or automated DNA sequencing (Fig. 1). The frequencies of each genotype are shown in Table 1. The frequency of each genotype was consistent with the HWE. Significant linkage disequilibrium was detected among T19G, A541G, and A552C of UGT1A6 gene. The D'coefficients between T19G and A541G, T19G and A552C, and A541G and A552C were 0.938, 0.941, and 1.000, respectively. There were no significant differences in the frequencies of the UGT1A6, UGT2B7 or CYP2C9 genotypes between our study and other studies in Chinese populations. Furthermore, associations between UGT1A6, UGT2B7 and CYP2C9 SNPs and VPA doses or plasma VPA concentrations were observed in all studied epileptic children (Table 2). None of the demographic characteristics was significantly different in any genotype, indicating that these characteristics did not influence the variations of VPA dose or plasma concentrations between the genotypes (Table 2). For UGT1A6, patients with double heterozygosities at nucleotide positions T19G, A541G and A552C had significantly higher VPA doses than those with wild type or single heterozygosities
Although lower adjusted VPA concentrations were also observed in patients with UGT1A6 double heterozygosities than those with wild type or single heterozygosities (Table 2), only the differences between double and single heterozygosities were statistically significant (Fig. 1). However, these results could be biased by the 3 years mean age of the patients with UGT1A6 double heterozygosities compared to the 8 years mean age of the patients with either wild type or UGT1A6 single heterozygosity (Table 2). To test this possibility, additional statistical analyses were performed only in 0- to 4-year-old children. Interestingly, younger patients with UGT1A6 double heterozygosities still maintained higher VPA doses and lower adjusted plasma VPA concentrations than those with either wild type or single heterozygosities (Table 3).

In contrast, no significant associations were observed between VPA doses, VPA concentrations or adjusted VPA plasma concentrations and UGT2B7*2, or CYP2C9*3 genotypes in this patient cohort (Table 2, Figs. 3 and 4).

**Discussion**

Genetic polymorphism may be an important source of interindividual variability in the pharmacokinetics and pharmacodynamics of VPA. This study demonstrated that UGT1A6 double heterozygosities at nucleotide positions T19G, A541G and A552C were associated with higher VPA doses and lower adjusted plasma VPA concentrations compared to those with wild type or single heterozygosity in epileptic children. These results suggest that the increased VPA doses and the decreased adjusted plasma VPA concentra-
trations may be attributed to an increased glucuronidation rate of the drug. Previous in vitro studies also demonstrated that cells expressing recombinant UGT1A6 variants including eight possible combinations of T19G, A541G and A552C had higher VPA glucuronidation rates than those expressing the wild type. In particular, UGT1A6*2 (19G/541G/552C) showed the highest enzyme activity and intrinsic clearance values of VPA. This was further supported by two in vivo studies about A552C genotype and VPA concentration, but not supported by the other study about A541G genotype and VPA concentration in China. Interestingly, our results also showed that UGT1A6 single heterozygosity did not decrease, but rather slightly increased plasma VPA concentrations, compared with the wild type genotypic group. This tendency was also previously observed in human liver tissue samples with heterozygous expression of UGT1A6*1/*2, which exhibited lower activity than those homozygous for *1 or *2. It is explained that single heterozygous UGT1A6 variants may perturb UGT1A6 localization to the ER membrane, dimerization or stability, thus, resulting in low activity. However, due to a limited sample size, the results need to be further investigated in a larger cohort of patients.

Otherwise, this is the first study for identifying genetic factors of the interindividual variability of VPA metabolism in epileptic children. Some studies reported the effect of age on the metabolism of VPA. One study showed that there was no statistically significant difference in the rate of VPA glucuronidation in the human liver microsome bank between younger patients (2–56 years) and elderly patients (65 years onward). Another study showed that UGT1A6 and UGT2B7 had no age-dependent changes at the transcript levels or the protein levels between paediatric liver samples.
Table 3. Effects of UGT1A6 genotypes on VPA doses, VPA concentrations and adjusted VPA concentrations in epileptic children aged 0–4 years

<table>
<thead>
<tr>
<th>Genotype</th>
<th>No.</th>
<th>Age (years)</th>
<th>Weight (kg)</th>
<th>VPA dose (mg/kg per day)</th>
<th>VPA concentration (µg/ml)</th>
<th>Adjusted VPA concentration (µg/ml per mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>UGT1A6 T19G/A541G/AS52C</td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Wild type</td>
<td>16</td>
<td>2.63 ± 1.15</td>
<td>14.59 ± 4.81</td>
<td>16.76 ± 6.09</td>
<td>59.01 ± 2.11</td>
<td>4.01 ± 2.10</td>
</tr>
<tr>
<td>Single heterozygosity</td>
<td>13</td>
<td>2.00 ± 1.14</td>
<td>12.77 ± 3.88</td>
<td>17.88 ± 7.22</td>
<td>54.75 ± 24.62</td>
<td>3.43 ± 1.74</td>
</tr>
<tr>
<td>Double heterozygosities</td>
<td>5</td>
<td>2.40 ± 1.29</td>
<td>13.30 ± 2.11</td>
<td>29.35 ± 13.64</td>
<td>52.14 ± 29.90</td>
<td>2.02 ± 1.01</td>
</tr>
</tbody>
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p value
ANOVA 0.431 0.469 0.012 0.825 0.129
WT vs. SingH 0.199 0.253 0.704 0.647 0.412
WT vs. DoubH 0.733 0.551 0.004 0.591 0.045
SingH vs. DoubH 0.556 0.811 0.010 0.842 0.159

*a Wild type in UGT1A6 gene is defined as *1/*1; Single heterozygosity includes *1/*2 (n = 11), *1/*3 (n = 1), and *1/*4 (n = 1); Double heterozygosities include *2/*2 (n = 3), *2/*4 (n = 1), and *2/*10 (n = 1); *2 is defined as 19G/541G/552C; *3 is defined as 19G/541A/552A; *4 is defined as 19G/541A/552C; *10 is defined as 19G/541G/552A; WT refers to Wild type; SingH refers to single heterozygosity; DoubH refers to double heterozygosities.

Fig. 3. Differences in VPA dose, VPA concentration, and adjusted plasma concentration in various UGT2B7 genotypic groups in Chinese epileptic children
A: VPA dose, B: VPA concentration, C: adjusted VPA concentration. Horizontal lines indicate values of each group.

Fig. 4. Differences in VPA dose, VPA concentration, and adjusted plasma concentration in various CYP2C9 genotypic groups in Chinese epileptic children
A: VPA dose, B: VPA concentration, C: adjusted VPA concentration. Horizontal lines indicate mean values of each group.

-aged 7–24 months and adult liver samples (aged 25–75 years). UGT1A6 had comparatively higher activity at birth and increased to adult levels at 14 months. These results suggest that the protein levels and activities of UGT1A6 have reached the adult levels in young children after 14 months. Our results showed that UGT1A6 double heterozygosities were associated with higher VPA doses and lower VPA plasma concentrations in either the entire patient cohort or only in those aged 0–4 years. These data strongly suggest that the significant differences of VPA doses and adjusted plasma concentrations between the UGT1A6 genotypic groups are age-independent. Our findings are supported by a recent study in adult epileptic patients which also showed that homozygous carriers of the variant UGT1A6 T19G, A541G
and A552C allele tend to require higher VPA doses and lower concentration-to-dose ratios (CDRs) than noncarriers.20

Besides UGT1A6, UGT2B7 also plays an important role in the intrinsic clearance of VPA.27 To date, reports on the influence of UGT2B7 polymorphisms on substrate drug metabolism still remain inconsistent.9,10 In our study, the UGT2B7*2 genotype had no effects on plasma VPA concentrations. This is consistent with two previous studies, which also showed an insignificant difference between the UGT2B7*2 genotype and AUCs of VPA.15,20 Thus, UGT2B7*2 may have no or only minor effects on plasma VPA concentrations.

CYP2C9 is responsible for the majority (75–80%) of VPA terminal desaturation and hydroxylation.44 Two previous studies demonstrated an association between the CYP2C9*3 genotype and VPA metabolism. One indicated that the genetic polymorphisms of CYP2C19 and CYP2C9 were highly significant factors for VPA pharmacokinetics,28 the other indicated that patients with the CYP2C9*3 heterozygous genotype had higher plasma VPA concentrations than those with the wild-type genotype.18 However, no association was observed between the CYP2C9*3 genotype and plasma VPA concentrations in our study or an Iranian study.14 The discrepancy may be attributed to the sample size, age or race. Otherwise, the other CYP genes that could theoretically affect VPA plasma levels in epilepsy, including CYP2A6 and CYP2B6, may mitigate the magnitude of the CYP2C9-based VPA interactions.

In conclusion, our study demonstrates that UGT1A6 double heterozygosities at nucleotide positions T19G, A541G and A552C are associated with increased VPA metabolism, whereas UGT2B7*2 and CYP2C9*3 had no obvious impacts in Chinese children with epilepsy. It suggests that UGT1A6 mutations may be mainly responsible for the differences in plasma VPA concentrations among different genotypic groups. This might provide useful genetic information for personalized VPA therapy in epileptic children.

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References
5) Xing, Y., Yang, L., Wang, L., Shao, L., Wei, Z., Xuan, J., Li, J., Qin, S., Shu, A., He, L. and Xing, Q.: Systematic screening for polymorphisms within the UGT1A6 gene in three Chinese populations and function prediction through structural modeling. Pharmacogenomics, 10: 741–752 (2009).


