ELSEVIER

Brain & Development xxx (2017) xxx–xxx

BRAIN & DEVELOPMENT
Official Journal of the Japanese Society of Child Neurology

www.elsevier.com/locate/braindev

Original article

Correlations between UGT2B7*2 gene polymorphisms and plasma concentrations of carbamazepine and valproic acid in epilepsy patients

Hui Zhang [a], Wenfang Zhang [b,*], Yuechun Li [a], Jie Yan [a], Jinfeng Zhang [a], Baojun Wang [a]

[a] Department of Neurology, Baotou Central Hospital, Baotou 014040, China
[b] Department of Neurology, The Fourth Affiliated Hospital of Baotou Medical College, Baotou 014032, China

Received 9 October 2016; received in revised form 19 August 2017; accepted 7 September 2017

Abstract

Purpose: The study aims to detect the polymorphisms in uridine diphosphate glucuronyl transferase (UGT) 2B7*2 and investigate the corresponding effects on the blood concentrations of valproic acid (VPA) and carbamazepine (CBZ).

Methods: A chemiluminescence immunoassay analyzer was used to detect the plasma concentrations of VPA or CBZ in patients. Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method was used to analyze UGT2B7*2 gene polymorphisms.

Results: A total of 117 patients were enrolled under the VPA group, out of which 84 patients were aged 6 years or older. Comparison of the blood concentrations of VPA showed significant differences among patients with the three standard genotypes (mutant, heterozygous, and wild-type) based on one-way ANOVA ($F = 4.386$, $p = 0.016$). In addition, comparison of the blood concentrations among the three genotypes in the CBZ group (78 patients) showed no significant differences based on analysis using ANOVA ($F = 0.897$, $p = 0.412$).

Conclusion: The UGT2B7*2 gene polymorphisms significantly affect the standard blood concentrations of VPA, but not CBZ.

Keywords: UGT2B7*2; Valproic acid; Carbamazepine; Gene polymorphism; Epilepsy

1. Introduction

Uridine diphosphate glucuronyl transferase (UGT) is an enzyme that catalyzes the addition of glucuronic acid to a substrate in a process known as a glucuronidation reaction, which plays a major role in phase II drug metabolism [1]. In this study, we selected a gene from the UGT gene family—the uridine diphosphate glucuronosyltransferase 2 family, polypeptide B7 (UGT2B7). UGT2B7 is known to glucosidase hyodeoxycholic acid in the liver, as well as glucuronidase steroid hormones and fatty acids [2]. Current studies have already investigated the polymorphism UGT2B7*2 802 C>T [3]. Previous studies have demonstrated that the amino acid change at position 268 from histidine (His) to tyrosine (Tyr) can modify the enzymatic activity of UGT287*2. The UGT2B7*2 gene has several known polymorphisms that result in abnormalities in the metabolism of nicotine [4] and anti-AIDS drugs (such as Efavirenz [5]).

* Corresponding author.
E-mail address: wenfangzhangdoc@126.com (W. Zhang).

https://doi.org/10.1016/j.braindev.2017.09.004
0387-7604/

Furthermore, certain studies have demonstrated that the UGT287*2 genotype can influence the pharmacokinetics of morphine [6], epirubicin, and tamoxifen [7].

Valproic acid (VPA) and carbamazepine (CBZ) are common and highly effective anti-epileptic drugs. Once absorbed into the blood, VPA is primarily metabolized in the liver. VPA has a half-life of about 15 h, with effective blood concentrations ranging from 50 to 100 μg/ml. VPA combines with glucuronic acid and is eventually excreted by the kidneys during phase II metabolism. Uridine diphosphate glucuronyl transferase (UGT) is the most common phase II metabolic enzyme and functions to increase the solubility of metabolic substrates via glucuronidation, thereby facilitating the discharge of the metabolic substrates. UGT activity is known to be influenced by gene polymorphisms and thus exerts different metabolic activities towards the substrates. Recent studies have suggested that the UGT287*2 polymorphisms affect the plasma concentrations of VPA [8,9]; however, one study demonstrated that the polymorphisms had no effect on VPA metabolism [10–12]. These conflicting results could be due to the complex nature of VPA metabolism, which is in turn influenced by valproate pharmacokinetics, co-administration with other drugs, and various exogenous and endogenous factors that can act as enzyme inducers or inhibitors. Therefore, further studies are necessary to fully investigate VPA metabolism.

Carbamazepine (CBZ) is one of the most widely prescribed anticonvulsants despite the high incidence of idiosyncratic side effects. CBZ metabolism is complex, and studies have identified more than 30 CBZ metabolites, with CBZ N-glucuronide being the most abundant metabolite. CBZ can be metabolized by liver, kidneys, and intestinal microsomes. UGT2B7 is the only isoform that can catalyze the formation of CBZ glucuronide [13]. Recent studies have shown that polymorphisms in EPHX1, UGT2B7, and SCN1A can exert additional effects on the steady-state plasma concentrations of CBZ [14]. However, Yogita Ghodke Puranik reported no impact of UGT2B7 genotypes on CBZ metabolism [15]. Therefore, we carried out this study to investigate the correlation between UGT2B7 genotypes and CBZ metabolism.

In view of this, we investigated UGT2B7*2 polymorphisms in epilepsy patients and analyzed the correlations between UGT2B7*2 gene polymorphisms and standard blood concentrations of VPA and CBZ.

2. Materials and methods

2.1. Subjects

A total of 195 epilepsy patients medicated with VPA or CBZ alone in the outpatient and ward of Baotou Central Hospital from August 2013 to November 2014 were enrolled in the present study. This study was conducted in accordance with the declaration of Helsinki. This study was conducted with approval from the Ethics Committee of Baotou Medical College. Written informed consent was obtained from all participants.

2.2. Specimen collection

All patients received mediation for over one month. Fasting peripheral venous blood was sampled from each patient in the morning to measure steady-state blood concentrations. Blood concentrations were then normalized according to patient's body weight and daily dose. Blood specimens (2 ml) were placed in ethylenediaminetetraacetic acid (EDTA) anticoagulant tubes and stored at −80 °C for subsequent batch DNA extraction.

2.3. Serum concentration detection of VPA and CBZ

Serum detection of VPA and CBZ was performed using an automatic chemiluminescence immunoassay analyzer (Siemens, Germany). The detection range and therapeutic range of VPA were 1–150 μg/ml and 50–100 μg/ml, respectively. The detection range and therapeutic range of CBZ were 0.25–18 μg/ml and 4–10 μg/ml, respectively.

2.4. DNA extraction

DNA was extracted using blood genomic DNA extraction kit [Sangon Biotech (Shanghai) Co., Ltd.]. Briefly, the erythrocyte lysate, white blood cell lysate, and protein lysate were added to remove red blood cells, to lyse leukocytes, and to remove proteins, respectively. Genomic DNA could then be obtained after the washing steps.

2.5. PCR amplification

PCR amplification primers were synthesized by Sangon Biotech (Shanghai) Co., Ltd. The primer sequences were as follows: 5′-TGC CTA CAC TAT TCT AAC C-3′ and 5′-TCT CTG AAA ATT CTG CAC T-3.'

Each PCR reaction was carried out in 50-μl reaction volumes containing 5 μl of DNA template, 1.5 μl each of upstream and downstream primer, 25 μl of PCR Smart Mix solution, and 17 μl of ddH_2O.

The following PCR amplification profile was used: 94 °C for 2 min; 35 cycles of 94 °C for 30 s, 54 °C for 30 s, and 72 °C for 1 min; and an extension step at 72 °C for 5 min. The PCR products (580 bp) were then obtained and stored at −20 °C.

2.6. Polymorphism detection

The resulting PCR products were enzymolyzed using the BseGI endonuclease (Thermo, USA) and subse-

Please cite this article in press as: Zhang H et al. Correlations between UGT2B7*2 gene polymorphisms and plasma concentrations of carbamazepine and valproic acid in epilepsy patients. Brain Dev (2017), https://doi.org/10.1016/j.braindev.2017.09.004