Pharmacological Reports 69 (2017) 504–511

Contents lists available at ScienceDirect

Pharmacological Reports

journal homepage: www.elsevier.com/locate/pharep

Original article

Influence of genetic variants of *CYP2D6*, *CYP2C9*, *CYP2C19* and *CYP3A4* on antiepileptic drug metabolism in pediatric patients with refractory epilepsy

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ARTICLE INFO

Article history:
Received 26 July 2016
Received in revised form 10 January 2017
Accepted 16 January 2017
Available online 19 January 2017

Keywords:
Refractory epilepsy
CYPs
SNPs
Anticonvulsant drugs

ABSTRACT

Background: Identified the polymorphisms of *CYP2D6*, *CYP2C9*, *CYP2C19* and *CYP3A4,* within a rigorously selected population of pediatric patients with drug-resistant epilepsy.
Method: The genomic DNA of 23 drug-resistant epilepsy patients and 7 patients with good responses were analyzed. Ten exons in these four genes were genotyped, and the drug concentrations in saliva and plasma were determined.
Results: The relevant SNPs with pharmacogenomics relations were *CYP2D6*2* (rs16947) decreased your activity and *CYP2D6*4* (rs1065852), *CYP2C19*2* (rs4244285) and *CYP3A4*1B* (rs2740574) by association with poor metabolizer. The strongest risk factors were found in the AA genotype and allele of SNP rs3892097 from the *CYP2D6* gene, followed by the alleles A and T of SNPs rs2740574 and rs2687116, respectively from CYP3A4.
The most important concomitance was between homozygous genotype AA of rs3892097 and genotype AA of rs2740574 with 78.3% in drug-resistant epilepsy patients as compared to 14.3% in control patients.
Conclusion: The results demonstrated the important role of the *CYP 3A4*1B* allelic variant as risk factor for developing drug resistance and *CYP2D6, CYP2C19* SNPs and haplotypes may affect the response to antiepileptic drugs.

Introduction

About 35% of patients with epilepsy are refractory to treatment despite several polytherapy regimens [1–3]. Clinically, drug resistance is associated with the time of onset (before the first year), type (usually febrile seizures), the high frequency of seizures prior to drug administration and the presence of structural lesions. Pharmacokinetic theory proposes that the overexpression of transporter proteins in the blood brain barrier and the expression of certain allelic variants of metabolizing enzymes (CYP450) modify the concentrations of AEDs that enter the brain [4].

The CYP450 enzymes (CYPs) are accountable for the metabolism of approximately 90% of all clinically prescribed drugs; the first three CYP families are part of oxidative enzymes (traditionally called metabolic enzymes phase I). CYP34A4 is the most important hepatic CYP, and represents more than a third of hepatic CYPs. Others, CYP1A2, CYP2B6, CYP2C9, CYP2C19 and CYP2D6, are

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http://dx.doi.org/10.1016/j.pharep.2017.01.007
1734-1140/

conclusively important for the metabolism of drugs; these first families of CYPs include genes that are highly polymorphic, meaning there are frequent genetic variations that affect their function [4–7]. *CYP2D6* is highly polymorphic, with over 100 known allelic variants [8]. Some polymorphisms lead to a complete loss *of CYP2D6* function, while others reduce its activity. These polymorphisms seem to cause large inter-individual and ethnic differences in *CYP2D6* activity in vivo. *CYP2C9* and *CYP2C19* are responsible for the catalysis of the oxidation and metabolic clearance of up to 20% of clinically important anticonvulsant drugs, as phenytoin [9,10]. *CYP2C9*2* and *CYP2C9*3* are recognized as the main *CYP2C9* variants [11] and have reduced catalytic activity compared with the wild type (*CYP2C9*1*) [12]. *CYP2C19* acts on 5–10% of drugs in current clinical use, including antidepressants and barbiturates [13]. At least 28 variant alleles for *CYP2C19* have been identified, the most extensively described of which are *CYP2C19*2* and *CYP2C19.* Both *CYP2C19*2*, which causes a 40-nucleotide deletion and a frame shift, and *CYP2C19*3,* which leads to a premature stop codon, result in the production of a truncated protein without enzymatic activity [14]. More than 30 SNPs have been reported in CYP3A4; the most well characterized are *CYP3A4*1B* and CYP3A4*22 due to their effects on the functional activities of the encoded enzymes. The variability of CYP3A5 protein expression is attributed to three alleles (*CYP3A5*3, CYP3A5*6 and CYP3A5**7), all of which are associated with the reduced/abolished expression of CYP3A5. The distribution of CYP3A alleles varies across ethnic groups. For instance, 8.8% and 8.0% of Mexican subjects from Mestizo and Tepehuano, respectively, carried *the CYP3A4 * 1B* allele [15].

Variations in the *CYP2D6, CYP2C9, CYP2C19* and *CYP3A4* genes could influence inter-individual variations in AED metabolism that may be responsible for the drug-responsive or drug-resistant phenotype. These genetic variants have been correlated with at least three classes of phenotypes based on the extent of drug metabolism: fast (FM), extensive (EM), and poor (PM) metabolizers; these phenotype classes result in low, normal and high blood levels of the parent drugs, respectively. Whether the polymorphisms of these genes are associated with AED resistance is still not clear; for this reason, this study identified SNPs of *CYP2D6, CYP2C9, CYP2C19 and CYP3A4* that were associated with the metabolism of antiepileptic drugs (AEDs). The aim of this study was to perform a non-inferential exploratory study to identify reported nucleotide changes in a rigorously selected pediatric patients with similar clinical drug-like and seizures characteristics with AED-resistant epilepsy (ADR) and patients with good response to AEDs (CTR).

Methods

Patients and sample collection

An observational study with 23 drug-resistant (cases) and 7 seizure-controlled pediatric epileptic patients (controls) was performed, while avoiding inbreeding between the patient's biological parents.

The inclusion criteria for AED-resistant epileptic patients were: (1) patients had to have demonstrable epileptic focus through EEG and without radiological focal structural lesions, (2) classified as resistant to pharmacological treatment, (3) treated with two or more drugs (Table 1) at appropriate doses, (4) serum levels within therapeutic range for at least six months of continuous treatment and under the supervision a neurologist pediatrician, (5) a frequency of 3 seizures per month, (6) 1 to 16 years of age, and (9) either gender. The asymptomatic control patients had to be seizure-free for at least 6 months before the study.

Table 1
Mean and standard deviation of drug concentrations of patients with AED-resistant epilepsy and patients with good response to AEDs.

Drug	CTR		ADR	
	n	Mean (SD)	n	Mean (SD)
Valproic acid				
Dose (SD), μmol/24h	5	791.66 (62.08)	14	711.80(295.97)
Concentration/saliva (SD), μmol/L	5	2.3 (1.3)	13	0.69(0.069)
Concentration/plasma (SD), μmol/l	3	82.1 (32.5)	11	43.88 (15.97)
Carbamazepine				
Dose (SD),/24h	1	254.23	2	354.33(55.51)
Concentration saliva (SD), μmol/L	1	1.0	2	0.27(0.19)
Concentration/plasma (SD), μmol/L	0	–	1	2.32
Lamotrigine				
Dose (SD), μmol/24h	2	39.06(0.0)		–
Concentration/saliva (SD), μmol/L	1	0.351		–
Concentration/plasma (SD), μmol/L	0	–		–
Phenytoin				
Dose (SD), μmol/24h		–	4	49.20(37.30)
Concentration/saliva (SD), μmol/L		–	2	0.357(0.03)
Concentration/plasma (SD), μmol/L		–	3	9.85(14.88)
Levetiracetam				
Dose (SD), μmol/24h	1	587.2	5	764.7(335.29)
Concentration/saliva (SD), μmol/l		–	4	1(1)
Concentration/plasma (SD), μmol/l		–		–

Amplification and sequencing

Genomic DNA was extracted from leukocytes from patient blood samples using a commercial kit (Genomic DNA Purification kit, Thermo Scientific®, Fremont, California, USA) according to the supplier's recommendations.

The single nucleotide polymorphisms (SNPs) from exons 1 (rs1065852), 3 (rs1058164), 5 (rs35742686), and 6 (rs16947) were located in Gen Bank accession NG_008376.3, corresponding to the *CYP2D6* gene; exons 3 (rs1799853) and 7 (rs1057910) were located in Gen Bank accession NG_008385.1, corresponding to the *CYP2C9* gene; exons 4 (rs4986893) and 5 (rs4244285) were located in Gen Bank accession NG_008384.2, corresponding to the *CYP2C19* gene; and, 5′ UTR region (rs2740574) and exon 6 (rs55901263 and rs113667357) was located in Gen Bank accession NG_008421.1, corresponding to the *CYP3A4* gene. These polymorphisms were selected based on the frequency reported in Hispanic and Mestize-Mexican populations (Pub Med Gene bank). Each amplification reaction was performed with 5 μl (5 ng/μl) of genomic DNA in a 50 μl total reaction volume containing 5 μl of 10× reaction buffer, $MgCl_2$ (concentration depending on the primer set), 1 μl of 10 mM dNTPs (Thermo Scientific®, Fremont California USA), 1 μl of each 10 mM flanking primer (IDT, San Diego, California, USA), and, 1 μl of Taq polymerase (Thermo Scientific®, Fremont, California, USA). PCR was performed in a DNA engine system® thermocycler (Bio-Rad®, Hercules, California, USA) with a cycle program of 94 °C for 5 min, 37 cycles of 94 °C for 60 s, annealing temperature ($T°_h$) for 60 s, 72 °C for 35 s, and one extension cycle of 10 min at 72 °C. The primers, $MgCl_2$ concentration, and $T°_h$ for each amplification reaction are listed in Table 2. The amplification products were purified with the Gene JET Gel Extraction kit (Thermo Scientific®, Fremont, California, USA) and 100 ng of amplicon was sequenced in a 5 μl reaction using a BigDye v 3.1 sequencing Ready Reaction Kit (Applied Biosystems, Foster City, California, USA) according to the manufacturer's recommendations. Sequencing was performed in a DNA engine system® thermocycler (Bio-Rad®, Hercules, California, USA) with a cycle program at 94 °C for 1 min, 30 cycles