CYP2C19 polymorphism effect on phenobarbitone
Pharmacokinetics in Japanese patients with epilepsy: analysis by population pharmacokinetics

Abstract  Objective: The aim of this study was to clarify the effect of genetic polymorphisms of CYP2C19 on the pharmacokinetics of phenobarbitone (PB) using a non-linear mixed-effects model (NONMEM) analysis in Japanese adults with epilepsy.

Methods: A total of 144 serum PB concentrations were obtained from 74 subjects treated with both PB and phenytoin but without valproic acid. All patients were classified into three groups by CYP2C19 genotyping: G1, G2 and G3 were homozygous for the wild type of CYP2C19 (*1/*1), heterozygous extensive metabolizers (EMs), (*1/*2 or *1/*3), and poor metabolizers (PMs), (*2/*2, *2/*3), respectively. All data were analyzed using NONMEM to estimate pharmacokinetic parameters of PB with respect to the CYP2C19 genotype.

Results: Thirty-three patients belonged to G1 (44.6%), 35 to G2 (47.3%), and 6 to G3 (8.1%). The total clearance (CL) of PB significantly decreased by 18.8% in PMs (G3) relative to EMs (G1 and G2). The CL tended to be lower in G2 than in G1.

Conclusion: In this study, we first demonstrated the effect of the CYP2C19 polymorphism on pharmacokinetics of PB by genotyping. The contribution of other metabolic enzymes in the metabolism of PB in humans remains to be elucidated; however, it appears that the disposition of PB is mediated in part by this enzyme. The estimated population clearance values in the three genotype groups can be used to predict the PB dose required to achieve an appropriate serum concentration in an individual patient.

Key words  Phenobarbitone · CYP2C19 · Genetic polymorphism · Pharmacokinetics · NONMEM

Introduction

Phenobarbitone (PB) is the oldest and one of the most widely used of the modern antiepileptic drugs (AEDs). The optimal use of PB in patients with epilepsy requires information regarding pharmacokinetics, for which there is widespread inter-individual variability [1]. We had investigated the factors that influenced the total body clearance (CL) of PB using population pharmacokinetics and clarified that age, total body weight, daily dose, and concomitant AEDs were some of these factors [2, 3]. Even taking these factors into consideration, there is still much inter-individual variability in the pharmacokinetics of PB.

PB is eliminated both by hepatic metabolism and renal excretion. The main metabolic pathway is aromatic hydroxylation to form p-hydroxyphenobarbitone by cytochrome P450 (CYP) isoenzymes. Another important metabolic pathway is N-glucosidation to form phenobarbitone glucoside [4]. A few reports suggested that CYP2C9, CYP2C19, and CYP2E1 play a role in the formation of p-hydroxy-phenobarbitone [5, 6, 7]. Of these CYPs, CYP2C9 and CYP2C19 are known to indicate genetic polymorphism resulting in altered pharmacokinetic and, thereby, pharmacodynamic properties of certain drugs. The genetic polymorphisms of CYP2C19 have been reported to be caused by point mutations of G to A (CYP2C19*2) in exon 5 and G to A
(CYP2C19*3) in exon 4 [8, 9]. Subjects with the genetic mutations CYP2C19*2/*2, *3/*3, and *2/*3 are called poor metabolizers (PMs) of CYP2C19 because they have no CYP2C19 enzyme activity. However, several amino acid variants of CYP2C9 have been reported: Arg144/Cys (CYP2C9*2) and Ile359/Leu (CYP2C9*3) [10]. We recently reported that phenytoin (PHT) metabolism is disturbed in patients with epilepsy who possess the CYP2C19*2, 2C19*3, or 2C9*3 allele [11]. The frequency of mutant alleles of CYP2C19 is much higher in oriental subjects than in white subjects [12]. Thus, we intended to clarify using population pharmacokinetic analysis whether CYP2C19 polymorphism affects PB pharmacokinetics.

Materials and methods

Data sources
A total of 144 serum PB concentrations were obtained from 74 adult patients with epilepsy at the Department of Neuropsychiatry of Kyushu University Hospital. All of the patients were treated with both PB and PHT but without valproic acid (VPA). The other concomitant AEDs were carbamazepine (31 patients), zonisamide (3), diazepam (5), clonazepam (4), nitrazepam (4), acetazolamide (6), sulthiame (2), and acetylsalicylic acid (4). The details of these patients are summarized in Table 1. It was confirmed that the patients had not changed their PB and PHT doses or their co-medications for at least 1 month prior to the study. No patients had hepatic or renal failure. There were three patients who had body weights of only 35–40 kg; however, all of them were elderly women (82 years old). In this study, as described in the clinical records. To evaluate the effect of genetic polymorphism of CYP2C19 on the population estimates of PB CL, patients were separated into three groups based on their genotypes; G1, G2, and G3 were homozygous for the wild type of CYP2C19, heterozygous EMs of CYP2C19, and PMs of CYP2C19, respectively. There were no significant differences in PB daily doses (mg/kg/day) and in serum PT concentrations (μg/ml) among the three genotype groups. Each patient was informed about this study by medical doctors and gave written consent to participate in the study which was approved by the local ethics committee.

Genotype procedures for CYP2C19 and CYP2C9
Blood samples (10 ml) were obtained from all patients, and genomic DNA was isolated from peripheral lymphocytes using an extraction kit (GENOMIX, Talent, Italy). The CYP2C19*1 gene and two mutant alleles associated with the PM of (S)-mephentoin, CYP2C19*2 in exon 5 and CYP2C19*3 in exon 4, were identified according to the methods of de Morais et al. [8, 9], with minor modifications [12]. The CYP2C9*1 (Arg144 Ile359) gene and two mutant alleles, CYP2C9*2 (Cys144) in exon 3 and CYP2C9*3 (Leu359) in exon 7, were identified according to the methods of Wang et al. [13], with minor modifications [12]. For the detection of mutant alleles (CYP2C19*2*3 and CYP2C9*2*3), the polymerase chain reaction (PCR) was carried out using specific primers for each mutant DNA sequence. The PCR products were then digested with restriction enzymes which can discriminate between wild and mutant alleles. The DNA fragments digested by restriction enzymes were separated electrophoretically on an agarose gel and visualized by means of irradiation, using ethidium bromide staining. The genotyping of CYP2C19 and CYP2C9 was performed by analysis of electrophoretic bands patterns.

Population pharmacokinetics of PB
Modelling was carried out using the NONMEM program (version IV, level 1.1) developed by Beal and Sheiner [14] on a Hewlett Packard computer (HP Apollo 9000 model 712/60; Palo Alto, Calif.). The statistical model used in this program was based on the premise that particular pharmacokinetic parameters of a patient population rise from a distribution that could be described by the population mean and interpatient variation.

The pharmacokinetics of PB were described using the following steady-state pharmacokinetic model:

\[
C_{ss} = \frac{D}{(CL \times \tau)}
\]

where \(C_{ss}\) is the steady-state serum concentration (μg/ml); \(D\) the dose per body weight (μg/kg); CL the total body clearance per body weight (ml/kg/h) and \(\tau\) the dosing interval (h).

In this model, the effects of bioavailability (F) and CL cannot be separated, as only their ratio (CL/F) is estimated. As the half-life of PB is the longest among the frequently used AEDs [15], the fluctuation in the serum concentration within a day is small. Therefore, differences in the sampling time of the blood within a day may not influence an estimate in the steady state.

In a previous study [2], total body weight was the fixed effect that had the most significant influence on the PB CL. Thus, the CL models tested for individual groups were:

Model 1: \(CLij = 01\) (2)

### Table 1 Summary of patients’ data expressed as mean with range (SD). PB phenobarbitalone; PHT phenytoin

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>G1</th>
<th>G2</th>
<th>G3</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients</td>
<td>33</td>
<td>35</td>
<td>6</td>
<td>74</td>
</tr>
<tr>
<td>Number of observations</td>
<td>62</td>
<td>68</td>
<td>14</td>
<td>144</td>
</tr>
<tr>
<td>Male/female</td>
<td>20/13</td>
<td>19/16</td>
<td>3/3</td>
<td>42/32</td>
</tr>
<tr>
<td>Age (years)</td>
<td>52.6 (11.6)</td>
<td>50.3 (13.7)</td>
<td>42.8 (17.6)</td>
<td>50.5 (13.4)</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>58.5 (12.8)</td>
<td>60.0 (9.1)</td>
<td>57.2 (11.0)</td>
<td>59.0 (11.0)</td>
</tr>
<tr>
<td>PB daily dose (mg/kg/day)</td>
<td>1.15 (0.56)</td>
<td>1.03 (0.52)</td>
<td>0.97 (0.56)</td>
<td>1.07 (0.54)</td>
</tr>
<tr>
<td>Steady-state concentration of PB (μg/ml)</td>
<td>9.90 (4.3)</td>
<td>10.1 (5.30)</td>
<td>(6.93)</td>
<td>(10.0 (5.06)</td>
</tr>
<tr>
<td>Steady-state concentration of PHT (μg/ml)</td>
<td>2.3–19.6</td>
<td>1.3–21.3</td>
<td>1.8–23.7</td>
<td>1.3–23.7</td>
</tr>
<tr>
<td>Steady-state concentration of PHT (μg/ml)</td>
<td>6.6 (4.5)</td>
<td>6.7 (4.2)</td>
<td>6.0 (3.1)</td>
<td>6.6 (4.2)</td>
</tr>
</tbody>
</table>