Studies on Pharmacokinetics of Ethotoin in Epileptic Children and Adolescents Using a Stable Isotope

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We examined the pharmacokinetics of ethotoin in six children and four adolescents, using a stable-isotope-labeled ethotoin. The parameters obtained after oral administration of labeled ethotoin were not statistically different from those obtained when an unlabeled drug was used, hence we assume that the isotope effect of labeled ethotoin was negligible.

Large inter-individual variations were evident in the serum concentrations of ethotoin. The ratios of serum ethotoin concentrations against the doses of ethotoin were proportional to the half-life values, but did not correlate with the volumes of distribution. The half-lives, which ranged from 4.4 to 19.7 hrs, increased with advancing age of the patients. We conclude from this study that younger children are expected to require twice the dose per body weight of ethotoin, as compared with adolescents and may need ethotoin to be administered three times per day because the half-life of this drug is short.

Key words: ethotoin, stable isotope, pharmacokinetics, pediatrics, epilepsy

Introduction

Ethotoin, 3-ethyl-5-phenylhydantoin (Fig. 1), is effective for the treatment of patients with simple or complex partial seizures and generalized tonic clonic seizures1,2). Ethotoin exhibits minimal adverse reactions as compared with those of phenytoin, such as gingival hyperplasia, hirsutism, and ataxia1,2). Although ethotoin may offer therapeutic advantages over phenytoin, ethotoin has been reported to show lower anticonvulsant activity in epileptic patients than phenytoin, therefore ethotoin is not considered to be a primary antiepileptic agent and little is known of its pharmacokinetic parameters in children2).

Radioactive tracer techniques have been extensively used to investigate the metabolic fate of drugs in laboratory animals. While the administration of a radioisotope-labeled drug to humans is problematic, stable isotope tracer
techniques have advantages of safety, specificity and sensitivity when gas chromatography-mass spectrometry\textsuperscript{3} is used. Another advantage is its ability to determine all pharmacokinetic parameters without interrupting on-going pharmacotherapy. Namely absorption and elimination rate constants, time of peak concentration and volume of distribution in each patient can be obtained during drug treatment without setting up a drug-free period before a pharmacokinetic study. We report here the pharmacokinetics of ethotoin studied by using a stable-isotope-labeled ethotoin given to children and adolescents suffering from epilepsy.

**Methods**

1. **Subjects and sampling**
   
   We studied on ten Japanese epileptic patients (six boys and four girls), aged 3 to 14 years. The characteristics of these patients are listed in Table 1. Patients took other anticonvulsants at the same time. Coadministered drugs are listed below. Cases 1, 2 and 3 were on phenobarbital, case 4 was on phenytoin, phenobarbital, primidone and carbamazepine, case 5 phenobarbital, case 6 phenobarbital, primidone and carbamazepine, case 7 phenytoin, phenobarbital and primidone, cases 8 and 10 phenytoin. Case 9 none (monotherapy with ethotoin). No patient had any documented renal, hepatic, or gastrointestinal disease.

   Ethical aspects of the present study were guided by the Declaration of Helsinki. Prior to this study, a proposed protocol of the study was approved by the institutional review board and informed consents for the studies were obtained from the patients or their guardians.

   In the studies on pharmacokinetics of ethotoin during pharmacotherapy, a daily dose of 250 mg of ethotoin was replaced with the same amount of \textsuperscript{2}H\textsubscript{5}-ethotoin. The dosage form of ethotoin and \textsuperscript{2}H\textsubscript{5}-ethotoin was powder in all patients. Serum samples were obtained at predetermined intervals, 0, 20, 40 min., 1, 1.5, 2, 4, 6, 8 hr, after the simultaneous administration of daily administered unlabeled ethotoin and labeled ethotoin, or a separate administration of 250 mg of labeled ethotoin.

   All samples were stored at \textdegree-20°C until analysis. Ratios of ethotoin concentrations in serum to doses of ethotoin (C/D ratios) were calculated from serum samples obtained just before drug administration after more than two weeks of regular ethotoin administration.

2. **Materials**

   Ethotoin and hexobarbital were purchased from Dainippon Pharmaceutical Co., Osaka, Japan and Tokyo Chemical Industries, Co., Tokyo, Japan, respectively. \textsuperscript{2}H\textsubscript{5}-ethotoin was a generous gift from Dainippon Pharmaceutical Co. (Fig. 1).

   MethElute\textsuperscript{®}, a methylating agent for on-column methylation was purchased from Pierce Chemicals, Rockford, IL, USA. All other chemicals were of reagent grade and were purchased.
Table 1 Patient Characteristics and Pharmacokinetic Parameters of Ethotoin as Determined with $^2$H$_5$-ethotoin

<table>
<thead>
<tr>
<th>Case</th>
<th>Sex</th>
<th>Age yrs</th>
<th>BW kg</th>
<th>C/D (µg/ml)/(mg/kg)</th>
<th>t$_{1/2}$ hr</th>
<th>k$_e$ hr$^{-1}$</th>
<th>V$_f$/f L•kg$^{-1}$</th>
<th>t$_{max}$ hr</th>
<th>k$_a$ hr$^{-1}$</th>
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<tbody>
<tr>
<td>1</td>
<td>M</td>
<td>3</td>
<td>15.0</td>
<td>0.34</td>
<td>4.4</td>
<td>0.157</td>
<td>0.72</td>
<td>1.5</td>
<td>1.51</td>
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<tr>
<td>2</td>
<td>F</td>
<td>5</td>
<td>21.0</td>
<td>0.71</td>
<td>6.8</td>
<td>0.102</td>
<td>0.71</td>
<td>1.5</td>
<td>2.32</td>
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<tr>
<td>3</td>
<td>M</td>
<td>7</td>
<td>22.0</td>
<td>0.51</td>
<td>7.9</td>
<td>0.088</td>
<td>0.69</td>
<td>4.0</td>
<td>0.34</td>
</tr>
<tr>
<td>4</td>
<td>M</td>
<td>10</td>
<td>41.0</td>
<td>0.71</td>
<td>7.7</td>
<td>0.090</td>
<td>0.84</td>
<td>2.0</td>
<td>0.81</td>
</tr>
<tr>
<td>5</td>
<td>F</td>
<td>12</td>
<td>32.0</td>
<td>0.98</td>
<td>10.4</td>
<td>0.067</td>
<td>0.87</td>
<td>1.0</td>
<td>2.61</td>
</tr>
<tr>
<td>6</td>
<td>M</td>
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<td>54.0</td>
<td>1.53</td>
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<td>0.46</td>
<td>4.0</td>
<td>0.28</td>
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<tr>
<td>7</td>
<td>F</td>
<td>13</td>
<td>41.0</td>
<td>0.66</td>
<td>10.0</td>
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<td>0.98</td>
<td>2.0</td>
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<tr>
<td>8</td>
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<td>14</td>
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<td>0.70</td>
<td>10.3</td>
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<td>0.82</td>
<td>3.0</td>
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<tr>
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<td>1.37</td>
<td>12.8</td>
<td>0.054</td>
<td>0.77</td>
<td>1.0</td>
<td>3.17</td>
</tr>
</tbody>
</table>

Mean ± SD

| BW : body weight, C/D : ratio of serum concentration against the dose, t$_{1/2}$ : elimination half-time, k$_e$ : elimination rate constant, f : fraction of the administered dose absorbed, V$_f$ : volume of distribution, t$_{max}$ : time to maximum serum concentration, k$_a$ : absorption rate constant |

from Wako Pure Chemical Industries, Co., Osaka, Japan.

3. Determination of serum ethotoin concentrations

Determination of serum ethotoin and $^2$H$_5$-ethotoin was carried out as described elsewhere$^a$, with a minor modification. To a 100 µl portion of serum in a 10 ml glass test tube, 1 ml of saturated solution of sodium chloride (which was added to increase the partition of the drug to the organic layer by the salting-out effect) and 1 ml of chloroform containing 2 µg of hexobarbital, as an internal standard, were added. The sample was then placed in a vibration mixer for 10 sec, then the upper aqueous phase was aspirated off. The remaining chloroform layer was then transferred to a 2 ml glass sample tube and evaporated to dryness under vacuum at 50°C. The residue was redissolved in 25 µl of MethElute®, and a 1 µl aliquot of the resultant solution was injected into a gas chromatograph-mass spectrometer (GC-MS, QP-1000, Shimadzu, Kyoto, Japan). Inter-day coefficient of variation has been reported to be within 3.6% in the concentration range of 10-100 µg/ml$^b$.

4. Analysis of the data

The serum concentration-time profiles were fitted to a one-compartment open model with a lag time, using a nonlinear regression analysis technique. The linear regression analysis and student’s t test were used for the statistical assessment.

5. Evaluation of clinical effects

The clinical evaluation of effect of anticonvulsant drugs was carried out in the following scheme. No change : no effect, fair : 50% decrease in seizures, good : 75% decrease in seizures and excellent : 100% decrease in seizures. The clinical evaluation was carried out in twenty-one patients, who experienced seizure during phenytoin or phenobarbital therapy (see Table 1, plus additional eleven
Fig. 2 Serum total ethotoin (○) and [2H₅]-ethotoin (●) profiles in a patient (Case No. 5, female, 12 years old) after oral administration of 2,750 mg of unlabeled ethotoin and 250 mg of [2H₅]-ethotoin.

Patients).

Results

Figure 2 shows typical serum concentration profiles of ethotoin and labeled ethotoin in a pediatric patient after the simultaneous oral administration of 2,750 mg of ethotoin and 250 mg of labeled ethotoin. Pharmacokinetic parameters of ethotoin and labeled ethotoin calculated from a non-linear regression analysis did not significantly differ in cases of simultaneous administration, hence there appears to be a negligible effect of deuterium on disposition. Half-life values of labeled and unlabeled ethotoin were: case 1: 4.4 hr and 4.2 hr, case 2: 6.8 hr and 7.8 hr, case 3: 7.9 hr and 7.8 hr, case 4: 7.7 hr and 8.3 hr, case 5: 10.4 hr and 10.4 hr, case 6: 16.8 hr and 16.0 hr, case 7: 10.0 hr and 8.1 hr, case 8: 10.3 hr and 9.7 hr, case 9: 19.7 hr and 22.7 hr, and case 10: 12.8 hr and 14.2 hr.

Pharmacokinetic parameters after oral administration of 250 mg of labeled ethotoin in the patients are summarized in Table 1. The elimination of the drug showed linear kinetics. Half-life (t₁/₂) values (4.4-19.7 hr) in patients of 3-14 years old are plotted against age in Fig. 3. A significant and positive correlation between t₁/₂ values and age was observed. The values of absorption rate constant (kₐ) and volume of distribution (Vₐ) did not differ among children and adolescents.

Although a large variation in serum concentrations of ethotoin/ethotoin dose (C/D) ratios was noted, the C/D ratios were significantly (p<0.01) correlated with the t₁/₂ values as shown in Fig. 4. There was no significant correlation (not shown here) between C/D ratios and volumes of distribution.

In patients who suffered from refractory seizure during phenytoin or phenobarbital therapy, the frequency of seizure was decreased when serum ethotoin levels were over 25 µg/ml (Figs. 5 and 6). Mild ataxia was observed in two patients when serum ethotoin levels exceeded 75 µg/ml. Thus, the therapeutic range
Fig. 4 Correlation between C/D ratios and half-life values of ethotoin after oral administration of 250 mg [2H5]-ethotoin to 10 epileptic patients.

Fig. 5 Clinical evaluation of ethotoin in patients who experienced refractory seizure during phenytoin therapy. Generalized seizure (tonic-clonic, 6 cases, ○), secondary generalized seizure (8 cases, ●), partial seizure (1 case, △), complex partial seizure (2 cases, ▲).

Fig. 6 Clinical evaluation of ethotoin in patients who experienced refractory seizure during phenobarbital therapy. Generalized seizure (tonic-clonic, 2 cases, ○), secondary generalized seizure (2 cases, ●).

for ethotoin in epileptic patients who participated in this study may be estimated to be 25-75 μg/ml.

Discussion

Stable isotopes have been used as tracer materials in pharmacokinetic studies, with the advantages being safety and specificity. The patients are not exposed to radiation, and the use of specific mass/charge (m/z) values for measurement gives a high degree of specificity.

Although the higher molecular weight of the stable-isotope-labeled drug can require a higher activation energy during metabolic reaction and can result in slower metabolic reactions in the stable-isotope-labeled drug than seen in a conventional analog, the present study the isotope effect was negligible. Metabolic pathways involving an ethyl group at position 3 of hydantoin constitute only 24% whereas those involving phenyl and hydrogen at 5 of hydantoin constitutes 76% (as calculated from data reported by Bius et al.). Tritium substitution at ethyl hydrogen increases the mass of the ethyl group only 1.3 fold. These two facts would explain the reason why the pharmacokinetics of ethotoin are different from those of the analog.
kinetic behavior of labeled ethotoin did not apparently differ from that of unlabeled ethotoin.

The primary factor related to the large fluctuation of serum ethotoin concentrations may be attributed to interindividual differences in the half-lives of the drug, but the $V_d$ did not correlate with the C/D ratio of ethotoin. The half-lives were correlated with ages of the patients, hence relatively large doses per body weight are needed to obtain the same serum concentration profiles of ethotoin in children, as compared with doses found in adolescent and adult patients (Fig. 4). Similar findings have been noted with various anticonvulsants\(^{12}\) and theophylline. The greater relative size of the liver to total body weight compared to that in adults is considered to be one of the reasons explaining the greater metabolic activity in younger children.

Carter et al.\(^{2}\) reported that the optimal serum level of ethotoin is 14-34 μg/ml. Younger children required approximately 30-70 mg of ethotoin daily, per kilogram body weight, although adolescents required only one-half of the dose to obtain similar serum levels, as based on the C/D ratio. To avoid a large fluctuation in serum levels of ethotoin, we recommend that ethotoin be given three times a day to children.

Ethotoin is expected to be useful in patients with refractory seizure during therapy with other antiepileptic drugs. Therapeutic drug monitoring is needed in the treatment with ethotoin because of the large inter-patient variation of its pharmacokinetic behavior, but control of its serum levels in patients is easier because such non-linear kinetics as found in phenytoin were not observed in our study with ethotoin.\(^{5}\)

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References


