Effect of Enteral Formulations on the Intestinal Absorption of Phenytoin in Rats

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The plasma concentration of phenytoin, when coadministered orally with an enteral formulation, is markedly decreased compared with that when phenytoin is administered alone. However, the mechanism behind this interaction has yet to be clarified. Here we tried to clarify the mechanism by rat intestinal loop perfusion method. Drug concentrations were measured by HPLC. Enteral formulations affected the intestinal absorption of phenytoin, but their inhibitory effects varied. Moreover, a good correlation was observed between the inhibitory effect and the osmotic pressure of the formulations. However, the lipid components in the formulations did not affect the absorption of phenytoin, and no effect on perfusate pH was recognized. These results indicate that the osmotic pressure of the enteral formulations affected the intestinal absorption of phenytoin. A defined formula containing various peptides had a greater inhibitory effect than that predicted from its osmotic pressure. The contribution of peptide transporters to the intestinal absorption of phenytoin was studied. Cephalexin and cephradine, as typical substrates of peptide transporters, markedly inhibited the absorption. These results indicate that osmotic pressure and peptides in the perfusate were the major factors responsible for the inhibitory effects of the enteral formulations on the intestinal absorption of phenytoin.

Key words —— phenytoin, enteral formulation, drug interaction, osmotic pressure, peptide transporter, intestinal absorption

Introduction

Phenytoin, frequently used as an anticonvulsant, has a molecular weight of 252.27 and pKa of 8.3. It is essential to maintain an appropriate blood level of phenytoin to prevent an epileptic seizure. However, the therapeutic concentration range of phenytoin is narrow, and its pharmacokinetics shows nonlinearity in that range. The monitoring of its blood concentration is necessary for clinical treatment. Enteral formulations are preparations given directly to the intestines for patients who cannot ingest orally though their gastrointestinal function is maintained. The preparations contain various nutrients (including glucides, nitrogen sources, lipids, electrolytes, vitamins, etc.) and are generally administered by nasal intubation. Enteral formulations are produced in a form easily digested, and often used together with a therapeutic drug, so it is easily predicted whether they affect drug absorption as compared with a normal diet. There are numerous reports of the blood concentration of phenytoin markedly decreasing when an enteral formulation was coadministered. Namely, Bauer reported that the level of phenytoin in blood decreased approximately 73%, when Isocal® and phenytoin were given at the same time via nasal intubation, as compared with phenytoin alone. Hatton found that the phenytoin’s bioavailability decreased when used with Osmolite®. On the other

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hand, Nishimura et al.® reported that the blood level of phenytoin was not influenced by Ensure® after a single dose of phenytoin in capsule form among healthy subjects. In addition, Kitada et al.® reported that steady-state plasma phenytoin concentrations remained low after normal doses of phenytoin were given orally with an enteral formulation in patients, while they were unchanged when phenytoin was administered intravenously. These findings suggest the reductions in the blood concentration of phenytoin to have been caused by interaction with enteral formulations. However, there is no report on the inhibition of intestinal absorption of phenytoin by enteral formulations in detail. Therefore, to clarify the inhibitory mechanism, we examined the absorptive properties of phenytoin administered with enteral formulations using the rat small intestinal loop perfusion method.

Methods

Materials — Phenytoin (Wako Pure Chemical Industries, Ltd.) and the enteral formulations Hepan ED® (Ajinomoto Pharmaceuticals Co., Ltd.), Elental® (Ajinomoto Pharmaceuticals Co., Ltd.), and Clinimeal® (Eisai Co., Ltd.) were purchased from commercial sources. All other materials were of analytical grade.

Animals — Male Wistar rats weighing about 200 g (7 weeks of age) were purchased from Japan Laboratory Animals Inc. They were housed in rooms with a controlled environment (temperature: 23 ± 1°C, humidity: 55 ± 5%) and allowed access to water and food 15 hours before the drug absorption experiment, and to water only after that.

Perfusion experiment — Rats were anesthetized with pentobarbital (50 mg/kg, intraperitoneally) and their abdomen cut along the midline. Glass tubes (3.0 mm in inner diameter, 5.0 mm in outer diameter, 50 mm in length) were inserted into the upper part of the intestine and about 20 cm of the lower part, and fixed in place with surgical suture. After the small intestinal loop was made, 20 mL of warm isotonic saline was perfused to remove residual substances in the loop. The loop was perfused with 25 mL of various mediums containing 0.1 mM phenytoin at a rate of 4 mL/min with a peristaltic pump for 120 min at 37 degrees Celsius. The perfusion medium was prepared with saline or various enteral formulations just prior to use. Perfusate (0.5 mL each) was collected at 0, 30, 60, and 120 min from the reservoir and blood (0.1 mL) was collected at 0 and 120 min from the carotid. The samples were used to assay drug concentrations. Also, the volume of the perfusate was monitored during the perfusion period. After the perfusion experiment, the perfused intestine loop was extracted and washed immediately with cold saline (20 mL). The tissue was tapped on filtration paper and weighed to assay the drug concentration. Furthermore, the pH and osmotic pressure in the perfusate were monitored before and after perfusion. After the experiment, a large quantity of pentobarbital was given intravenously to euthanize the rats. Percent remaining and % removal were calculated as follows; ((drug concentration × perfusate volume)/(initial concentration × 25 mL)) × 100 and (100- (% remaining)).

Removal rate constants were calculated by least squares regression analysis.

Analytical method — The intestinal loop was minced with scissors, added to distilled water at 4 times the tissue weight, and homogenized with a Teflon homogenizer in an ice bath. This homogenate was used to assay the drug concentration in tissue.

The phenytoin in each sample (perfusate, intes-
intestinal homogenate and blood) was extracted with chloroform under acidic conditions, and the organic layer was removed and evaporated using nitrogen gas at 40 degrees Celsius. Phenytoin concentrations were assayed by the modified method of Tanaka et al. and Susan et al. Briefly, HPLC was performed with a LC-10A (SHIMADZU Company) and ODS reverse phase column (4.6 mm inside diameter × 150 mm in length, particle diameter 5 μm, capillary diameter 120 Å), and with a mixture of 8 mM phosphate buffer (pH6.0) and CH₃CN(65:35, V/V) as the mobile phase, at 0.8 mL/min, and 254 nm.

Osmometry — Osmotic pressure was measured with the freezing point depression degree method using an Osmometer OM801 (Vogel Company).

Preparation of ether treated Hepan ED — Diethyl ether (100 mL) was added to Hepan ED (80 g) and shaked slowly, and the mixture was left to stand. After Hepar particles were precipitated, the supernatant (the ether layer) was removed. These procedures were repeated four times. After the final procedure, the residue was warmed at 37 degrees Celsius to remove diethyl ether. The final sediment was used as ether-treated Hepan ED (namely, treated-Hepan). The lipid component in Hepan ED is occupied mainly triglycerides derived from bean oil, about 90% of triglycerides in it were removed by the treatment with diethyl ether.

Statistical analysis — The results are given as means ± standard deviations. Tukey’s HSD test, Dunnett’s pairwise multiple comparison t-test and ANOVAs were used. The level of significance was 5% (p<0.05). In addition, the statistical analysis was performed using Microsoft Excel 2003, Statcel, and SPSS (ver.10.1).

Results

1. Effect of enteral formulations on the intestinal absorption of phenytoin

We used the rat small intestine loop perfusion method to determine the effect of enteral formulations on the absorption of phenytoin. Phenytoin disappeared immediately from the perfusate when administered alone, with 89.3% removed after 120 min of perfusion. However, when perfused with an enteral formulation (Hepan ED, Elental, or Clinimeal), it disappeared immediately from the perfusate, the rate of removal varying significantly (p<0.01) with each formulation (Table 1). Moreover, the extent of inhibition decreased in the following order: Elental, Hepan ED, and Clinimeal. When the amount of phenytoin remaining in the perfusate was plotted as a semilogarithm vs. time, each experiment’s plots showed a straight line. Namely, phenytoin was absorbed from the perfusate by first-order kinetics regardless of the enteral formulation. (Fig. 1A) The removal rate constants in each experiment calculated from Fig. 1A are shown in Table 1. The rate constant in the presence of the enteral formulations was significantly lower than that for phenytoin alone. The drug concentrations in the intestine after 120 min perfusion were monitored, and 3~7% of the initially administered dose was recovered in the tissues. Conversely, phenytoin could not be detected in the blood at any time point. (Table 1)

2. Effect of lipid components on absorption of phenytoin from the intestine

To clarify the mechanism by which the enteral formulations inhibited the intestinal absorption of phenytoin, the effect of lipid components was studied. Namely, the amount of phenytoin removed from the intestine loop with Hepan ED or
Table 1  Effect of enteral formulations on the removal of phenytoin from the perfusate

<table>
<thead>
<tr>
<th></th>
<th>control</th>
<th>enteral formulation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Saline</td>
<td>Hepar ED</td>
</tr>
<tr>
<td></td>
<td>0 min</td>
<td>(n = 4)</td>
</tr>
<tr>
<td>% removed from</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>perfusate</td>
<td>30 min</td>
<td>51.4 ± 8.6</td>
</tr>
<tr>
<td></td>
<td>60 min</td>
<td>71.2 ± 7.4</td>
</tr>
<tr>
<td></td>
<td>120 min</td>
<td>89.3 ± 7.9</td>
</tr>
<tr>
<td>absorption rate constant (1/h)</td>
<td>1.23 ± 0.47</td>
<td>0.38 ± 0.05 **</td>
</tr>
<tr>
<td>% remaining in perfused tissue</td>
<td>2.0 ± 1.3</td>
<td>3.9 ± 1.4 **</td>
</tr>
</tbody>
</table>

Each value represents the mean ± S.D. for four experiments.

* p<0.05, ** p<0.01 (vs. isotonic saline)
treated-Hepan was compared. Treated-Hepan affected phenytoin’s absorption from the loop. \( (p < 0.05) \) The inhibition was similar to that by Hepan ED, with no difference between the two formulations. \( (p = 0.984) \) (Fig. 1B)

3. Effect of enteral formulations on perfusate pH

Intestinal absorption depends on the molecular form of a drug. To clarify the effect of enteral formulations on perfusate pH, the pH was monitored before and after perfusion. As shown in Table 2, the pH was slightly varied after as compared with before perfusion, while percent molecular form of phenytoin did not change markedly. The pH was shown as the mean before and after perfusion, and the relationship between the percentage of phenytoin removed and percent molecular form for each enteral formulation was monitored. No correlation was found. (Fig. 2)

4. Effect of osmotic pressure on intestinal absorption of phenytoin

As most enteral formulations are a hypertonic liquid, we studied the effect of osmotic pressure on the intestinal absorption of phenytoin. We compared % removal of phenytoin at various concentrations of saline, namely hypertonic (1.8%), hypotonic (0.45%), and isotonic (0.9%) solutions. As shown in Fig. 1C, phenytoin was quickly removed from each solution. The amount remaining in the perfusate was plotted as a semi-logarithm vs. time, and removal rate constants for each perfusate were obtained. As shown in Fig. 3

### Table 2 Effect of enteral formulations on perfusate pH

<table>
<thead>
<tr>
<th>Perfusion</th>
<th>pH before</th>
<th>pH after</th>
<th>Mean pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isotonic saline</td>
<td>6.69 ± 0.01</td>
<td>6.44 ± 0.01</td>
<td>6.57 ± 0.01</td>
</tr>
<tr>
<td>Hypertonic saline</td>
<td>6.56 ± 0.02</td>
<td>6.72 ± 0.09</td>
<td>6.64 ± 0.05</td>
</tr>
<tr>
<td>Hypotonic saline</td>
<td>7.12 ± 0.15</td>
<td>6.26 ± 0.38</td>
<td>6.69 ± 0.19</td>
</tr>
<tr>
<td>Hepan ED</td>
<td>6.21 ± 0.01</td>
<td>5.48 ± 0.47</td>
<td>5.84 ± 0.24</td>
</tr>
<tr>
<td>Elental</td>
<td>5.99 ± 0.04</td>
<td>6.49 ± 0.16</td>
<td>6.24 ± 0.06</td>
</tr>
<tr>
<td>Clinimeal</td>
<td>6.76 ± 0.01</td>
<td>6.15 ± 0.18</td>
<td>6.45 ± 0.07</td>
</tr>
<tr>
<td>Treated Hepan</td>
<td>6.75 ± 0.19</td>
<td>5.55 ± 0.84</td>
<td>6.15 ± 0.33</td>
</tr>
</tbody>
</table>

Each value represents the mean ± S.D. for four experiments.

Mean pH = (pH before perfusion + pH after perfusion) / 2

Fig. 2 Relationship between perfusate pH and % removed from the perfusate

Each point represents the mean ± S.D. for four experiments.
Percent of molecular form was calculated from \( 100 \times (1/(1 + 10^{pH - pKa})) \)

Fig. 3 Relationship between the osmotic pressure of the perfusate and rate constant of phenytoin’s removal from the intestinal loop

Each point represents the mean ± S.D. for four experiments.
 Straight line was calculated from the rate constants and the osmotic pressures obtained from three kind of saline solutions by least squares regression analysis.
(circles), the rate constants decreased as osmotic pressure increased, and a good correlation was recognized between the two for each solution. So, a relationship between the osmotic pressure of the enteral formulation and the removal rate constant was observed. As shown in Fig. 3, the more hypertonic formulation had a greater depressing effect on the removal rate constant. The point for each enteral formulation was slightly outside of the regression line (straight line in Fig. 3) obtained from various concentrations of saline.

5. Effect of substrates of peptide transporters on intestinal absorption of phenytoin

Clinimeal is a formula that includes various peptides, while Elental and Hepan ED are elemental diets that do not contain peptides. To clarify the contribution of peptide transporters to the intestinal absorption of phenytoin, the drug concentration in perfusate containing cephradine, cephalixin or dipeptide, known substrates of peptide transporters, was monitored and compared with that of phenytoin alone. Phenytoin disappeared immediately from the perfusate in each experiment, but its rate of removal decreased with each substrate. The degree of inhibition by the substrates differed markedly and decreased in the order of cephradine, cephalixin, glycylosarcosine, glycylglycine, and glycylleucine. (Fig. 1D) The intestinal absorption of phenytoin was inhibited significantly ($p<0.01$) by cephradine and cephalixin but not by the dipeptides.

Discussion

The plasma concentration of phenytoin, when coadministered orally with an enteral formulation, is markedly decreased compared with that when phenytoin is administered alone. However, there has been no detailed report on the mechanism inhibiting the absorption of phenytoin. Here we tried to clarify this mechanism with the intestine loop perfusion method in rat.

The enteral formulations affected the intestinal absorption of phenytoin, and their inhibitory effects differed. Moreover, a good correlation was found between the inhibition and osmotic pressure. (Fig. 3) These results indicated that the osmotic pressure of the perfusate affected the rate of absorption of phenytoin from the intestine. However, some other mechanism may have been involved because the inhibition by certain enteral formulations was greater than that predicted from the regression line obtained with various concentrations of saline.

Iseki and others \cite{Iseki11} reported that Hepan ED had the greatest inhibitory effect on the intestinal absorption of ceftibuten, as a cephem antibiotic, among Twinline®, Enterued®, and Hepan ED. So, we compared the composition of these three products, and found that Hepan ED contained a lot more bean oil than the others. Therefore, we examined the participation of the transport system for fatty acids and intestinal absorption of phenytoin using Hepan ED and treated-Hepan. The profile of phenytoin’s removal from perfusate did not differ between treated-Hepan and Hepan ED. These results suggested that the fatty acid transport system did not contribute to the intestinal absorption of phenytoin.

In addition, there was no significant difference in the pH of the perfusate among the enteral formulations, and no correlation between the molecular form and removal rate constant of phenytoin. Therefore, the effect of enteral formulations on the perfusate’s pH is extremely low as a inhibitory factor for the intestinal absorption of phenytoin.
As shown in Fig. 3, the inhibitory effect of Clinimeal predicted from its osmotic pressure was greater than that of Elental or Hepan ED. We speculate that Clinimeal affects factors other than osmotic pressure to depress the intestinal absorption of phenytoin. Elental and Hepan ED are elemental diets containing multi amino-acids, but Clinimeal is a defined formula and contains various peptides. The contribution of peptide transporters to the intestinal absorption of phenytoin was examined. The absorption of phenytoin was inhibited by cephradine and cephalixin. In preliminary experiments using Caco-2 cell monolayer grown in Transwell chambers, we recognized that dipeptides and cephem antibiotics inhibited the phenytoin transport from apical side to basal side in transwell. So, we predicted that peptide transporters contributed to the effect of Clinimeal. Glycylsarcosine, glycylglycine, and glycylleucine did not inhibit the intestinal absorption of phenytoin, perhaps because these peptides were hydrophilic compared with cephem antibiotics and had low affinity for the transporters.

In this study, we clarified that osmotic pressure and peptides in the perfusate were the major factors responsible for the enteral formulation’s effect on the intestinal absorption of phenytoin. These results provide useful suggestions for pharmaceutical use in accomplishing effective and safe medical treatment.

Acknowledgements

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References