Enhancement of the Oral Bioavailability of Phenytoin by N-Acetylation and Absorptive Characteristics

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To improve the absorbability of phenytoin (DPH), a prodrug, N-acetyl-DPH (EDPH), was synthesized, and the absorptive characteristics and pharmacokinetics of the prodrug were evaluated in rats. EDPH was rapidly hydrolyzed to DPH in the intestinal fluid and the mucosa (rate constant, 0.055 and 0.169 min⁻¹, respectively). The plasma concentrations of DPH after intravenous dosing of EDPH declined in a biexponential manner, although two different elimination patterns were observed in these rats. When dosed orally (25 mg/kg, DPH equivalent), the plasma levels of DPH converted from the prodrug were significantly higher and more sustained than those after DPH alone, giving bioavailability 11.4 (rapid decay) and 9.1 times (slow decay) as high, respectively, as that after DPH alone. The concentrations of DPH distributed into the mucosa of the duodenum and jejunum 1 and 5 h after oral dosing of EDPH were significantly higher than those after DPH alone. The prodrug and DPH converted from the prodrug dissolved 2–4 fold more than DPH alone in bile salt solution and bile salt–oleic acid mixed micelles, indicating the increased solubility of the prodrug in the intestinal fluid. It is concluded from the data that such high solubility of EDPH enhanced the intestinal absorption of the prodrug, part of which would be absorbed in the amide form, and thus gave the high bioavailability.

Key words N-acetylnphenytoin; absorption; pharmacokinetics; intestinal distribution; solubility; hydrolysis

Phenytoin (DPH), which is one of the most effective anticonvulsants,\(^1\) is still used extensively in the treatment of epilepsy. Although DPH has strong anticonvulsant activity, it shows great variations in bioavailability following oral administration to patients because of its poor water-solubility.\(^2,3\) Small changes in bioavailability can lead to large changes in steady state plasma DPH concentrations.\(^4\) Thus, extensive improvement of the intestinal absorption of DPH is expected to control seizures. Many water-soluble prodrugs of DPH have been synthesized in an effort to develop potential oral and parenteral applicable forms\(^5–9\); however, their anticonvulsant effects have not been estimated. On the other hand, the bioavailability and anticonvulsant activities of DPH-lipid conjugate prodrugs have been evaluated.\(^10–13\) The area under the plasma concentration–time curves (AUC) of DPH after dosing of these prodrugs were increased by 3–4 fold over that after DPH alone.\(^11,13\)

There have been some reports about the structure–activity relationships of DPH derivatives. It is shown that the presence of substituents at the 1-position of the hydantoin ring decreased the activity,\(^14\) but only DPH derivatives with modifications at the 3-position showed pharmacological activity to central nervous system.\(^15\) These findings led us to develop a new class of amide prodrug with a small acyl molecular substituent at the 3-position, with the intention of improving the poor bioavailability of DPH. The present study was undertaken in order to clarify the effectiveness of the amide prodrug, 5,5-diphenyl-N-acetylhydantoin (EDPH), as an oral applicable form based on absorbability and bioavailability. The plasma concentrations of DPH produced from the prodrug and the amounts of DPH and prodrug distributed into the small intestine were determined after oral dosing of the prodrug to rats, in comparison with those after DPH alone. As DPH was eliminated in two different patterns, slowly and rapidly, in rats,\(^16\) we also analyzed the plasma concentrations in the two elimination patterns. In addition, the solubility in bile salt micelles and the hydrolytic characteristics of the prodrug in various tissues were investigated to clarify the possible mechanism for improved absorption.

MATERIALS AND METHODS

Materials DPH and 5-(4-methylphenyl)-5-phenylhydantoin (5-methyl DPH), an internal standard for HPLC, were obtained from Nacalai Tesque, Inc. (Kyoto, Japan) and Aldrich Chemical Co. (Milwaukee, WI, U.S.A.), respectively. Sodium glycocholate and sodium cholate were purchased from Nacalai Tesque, Inc. and Sigma Chemical Co. (St. Louis, MO, U.S.A.), respectively. All other chemicals were of reagent grade or HPLC quality.

Animals Male Wistar rats (Nippon SLC, Inc., Hamamatsu, Japan), weighing 200–250 g, were used throughout this study. The animals had free access to MF diet (Oriental Yeast Co., Ltd., Tokyo, Japan) for 3–4 d prior to experiment. On the day before the experiment, the rat jugular vein was cannulated with silicon tubing.\(^17\)

Synthesis of EDPH To 2 g of DPH (7.93 mmol), 50 ml (530 mmol) of acetic anhydride was added. The mixture was heated at 150 °C under reflux for 45 min and then evaporated under reduced pressure to give residues. Crude crystal was recrystallized from ether. The yield was 2.04 g (87.6%). A colorless crystalline, mp 133–135 °C. ¹H-NMR (in CDCl₃): δ 2.58 (3H, s, CH₃CO), 7.09 (1H, br, NH), and 7.38 (10H, br, Arom.). Elemental Anal. Calcd for C₃₇H₃₉N₂O₅: C, 69.38; H, 4.79; N, 9.52. Found: C, 69.42; H, 4.90; N, 9.41. (Fig. 1)

Determination of Solubility A suspension of DPH or EDPH was prepared by adding excess solid in water, 0.1 m phosphate buffer (pH 5.5, 6.5 and 7.5), glycocholate and cholate (5–30 mm) dissolved in 0.1 m phosphate buffer (pH 6.5) or the bile salt–oleic acid (5 and 20 mm, respectively) mixed micelles in 0.1 m buffer (pH 6.5), and these suspen-

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sions were shaken for 1.5 h at 25°C. Mixed micellar solutions were prepared by dissolving oleic acid in bile salt solutions, in an equinolar ratio, and by subsequent mixing. The suspension was centrifuged, the supernatant was filtered through a membrane filter (0.45 μm), and the drug in the filtrate was analyzed by the HPLC method as described hereafter.

Enzymatic Hydrolysis in Intestinal Fluid, Intestinal Mucosa, Liver and Plasma Male Wistar rats, weighing 200—250 g, were fasted overnight before being sacrificed by decapitation. The luminal content of the intestine was collected by washing out the whole small intestine with 20 ml of saline. The fluid obtained was used as an intestinal fluid. The upper part of small intestine (a 20 cm length) was removed rapidly from the body, and fat and the omentum were trimmed away. The mucosa, obtained by scraping with a glass slide, was homogenized in 4 volumes of 0.9% NaCl—10 mm phosphate buffer (pH 7.4), and centrifuged at 5000 rpm for 10 min to collect the supernatant. The liver was perfused through the portal vein with physiological saline to remove blood. The minced liver was homogenized in 4 volumes of 1.15% KCl in a Potter–Elvehjem glass homogenizer equipped with a Teflon pestle. The resultant homogenates were centrifuged at 9000×g for 25 min. Blood was collected in heparinized syringes, and the plasma was separated by centrifugation. The resulting supernatants and the intestinal fluid were diluted with the saline—phosphate buffer (pH 7.4) to give 0.1 mg protein/ml (intestinal fluid), 0.05 mg protein/ml (liver), 0.02 mg protein/ml (intestinal mucosa), and 0.01 mg protein/ml (plasma), and were immediately used for the experiment. The protein concentrations were determined by the procedure described by Lowry et al. with bovine serum albumin, fraction V, as a standard.

The reaction mixture consisting of the supernatant or suspension (1.0 ml), the prodrug solution (1.5 mM in ethanol, 0.1 ml) and 0.1 mm phosphate buffer (pH 7.4, 0.5 ml), was incubated at 37°C. At appropriate time intervals, a 50 μl aliquot was withdrawn and immediately added to double volumes of acetonitrile containing the internal standard (5-methyl DPH, 5 μg/ml). After centrifugation, an aliquot (20—40 μl) of the supernatant was loaded onto a HPLC column.

Intravenous (i.v.) Administration DPH and EDPH (20 mg/kg, DPH equivalent) were administered to one group of rats (n=4—6) i.v. as a solution (0.2—0.25 ml/rat) in a mixture of saline—ethanol—propylene glycol (4:1:5, v/v). A 0.2 ml aliquot of blood sample was collected for 8 h after dosing through the cannula for the total time period. The plasma was separated immediately by centrifugation and stored frozen until the time of assay.

Oral Administration DPH and EDPH (25 mg/kg, DPH equivalent) were administered orally as aqueous suspensions (0.4—0.5 ml/rat) in 1.5% carboxymethyl cellulose sodium (CMC) to another group of rats (n=5—6) starved for 12 h before the experiment. A 0.2 ml blood sample was collected for 24 h after dosing through the cannula. The plasma was separated immediately by centrifugation and stored frozen.

Intestinal Tissue Distribution Studies DPH or EDPH (50 mg/kg, DPH equivalent) was administered orally by the same method mentioned above. At the indicated time (1, 3 and 5 h) the rats were killed, and the whole intestine was removed. The duodenum, jejunal and ileum segments (5—20 cm length) were cut off. Intestinal contents were removed by flushing each segment with ice-cold 0.9% NaCl. The mucosa of the segment was scraped off with a glass slide on ice and homogenized in a mixture of one volume of 0.85% NaCl—20 mM citrate buffer (pH 2.5) and 4.0 volumes of methanol containing the internal standard (5-methyl DPH, 5 μg/ml). The homogenates were centrifuged at 3000 rpm for 10 min, and the supernatants were followed by the determination of DPH and EDPH.

Determination of DPH and EDPH A 50 μl aliquot of plasma or supernatant was added to double volumes of acetonitrile containing the internal standard (5-methyl DPH, 5 μg/ml). After centrifugation and filtration through a membrane filter (0.45 μm, Eikkurodisc 3CR, German Sciences, Tokyo, Japan), aliquots of the supernatant were loaded onto a reversed-phase Inertsil ODS column (4.6×150 mm, 5 μm particle size, GL Sciences Inc., Japan) using a Shimadzu liquid chromatograph (model LC-10AS, Kyoto, Japan) equipped with an ultraviolet (UV) detector (model SPD-10A). The mobile phase, methanol—acetonitrile—0.025 M phosphate buffer (pH 7.0) (5:45:50, v/v), was pumped at a flow rate of 1.0 ml/min at 35°C. The detection was at 254 nm. The hydrolysis of EDPH during sample processing and assay was negligible. The sensitivity of the method was 0.5 μg/ml. Linearity of the standard curves were found in the range from 0.2 to 100 μg/ml (r²>0.999). Intra- and interday variabilities were<10%.

Data Analyses Pharmacokinetic parameters were calculated using the nonlinear least squares regression program, MULTI. The plasma concentration—time data after i.v. administration were fitted to the equation:

\[ C_t = A e^{-\alpha t} + B e^{-\beta t} \]

where \( C_t \) is the drug concentration at time \( t \), \( A \), \( \alpha \), \( B \) and \( \beta \) are the biexponential equation constants. The half-life \( (T_{1/2}) \) of the terminal phase was calculated as \( T_{1/2} = \frac{0.693}{\beta} \). Rapid and slow elimination of DPH was separated based on the \( T_{1/2} \) (1.5 h).

The AUC up to the last sampling point was calculated by the trapezoidal method, and the AUC beyond the last observed plasma concentration \( (C_p) \) was extrapolated according to \( C_p/\beta \). The area under the first moment curve \( (AUMC) \) and the mean residence time \( (MRT) \) were calculated by means of moment analysis.

The oral bioavailability \( (F) \) was calculated from the \( AUC_{0 \rightarrow \infty} \) of DPH after i.v. and oral administrations of the parent drug and EDPH. The volume of distribution at steady-state \( (V_{ds}) \) and total clearance \( (CL) \) were calculated by means
of moment analysis.\(^{20}\)

In the hydrolysis experiment, the pseudo first-order rate constant \(k_{obs}\) was calculated by the least squares fit program, MULTI.\(^{19}\) The data obtained were fitted to the following equation:

\[
k_{obs} = \frac{2.303}{t} \log \frac{a}{a-x}
\]

where \(a\) is the initial amount of the compound and \(x\) is the amount of DPH generated in time \(t\).

The means of all data are presented with their standard deviation (mean±S.D.). Statistical analysis was performed using an unpaired Student's \(t\)-test, and the significant level adopted was \(p<0.05\).

RESULTS

**In Vitro Enzymatic Hydrolysis of EDPH** To characterize the tissues or organs capable of hydrolyzing the amide bond of the prodrug, the hydrolytic activity was measured using four tissues involved in hydrolysis and transfer processes: the intestinal fluid, small intestine, liver and plasma. Figure 2 shows semilogarithmic plots of residual prodrug concentrations against time. The plots showed good linearity, indicating that the hydrolysis of EDPH was adequately described by pseudo first-order kinetics. EDPH was rapidly hydrolyzed in the intestinal mucosa and liver compared with that in the intestinal fluid. The plasma hydrolyzed the amide bond too rapidly to estimate the rate constant. No EDHP was detected after 2 min. The pseudo first order rate constant \(k_{obs}\), which subtracted the rate constant in the buffer, was \(0.157±0.027\) min\(^{-1}\) for the liver, \(0.114±0.020\) min\(^{-1}\) for the intestinal mucosa and \(0.003±0.001\) min\(^{-1}\) for the intestinal fluid. The rank order of \(k_{obs}\), corrected with the protein level, was intestinal mucosa > liver > intestinal fluid.

**Plasma Concentration of DPH and EDPH after i.v. Administration of Prodrug** Figure 3 shows the concentration-time profiles after the i.v. administration of DPH and EDPH (20 mg/kg, DPH equivalent). No concentrations of prodrug were detected in the plasma, indicating the rapid hydrolysis of the amide bond, as demonstrated by the in vitro hydrolysis experiment. The plasma levels of DPH after dosing of the prodrug declined in a biexponential manner, similar to those of parent drug dosed. Two different elimination patterns of DPH, slow and rapid elimination, were observed in rats, this also being reported by others.\(^{10}\) The kinetic parameters calculated are listed in Table 1. The \(T_{1/2}\) for the slow elimination group was 3.1 times longer than that for the rapid group, and the \(AUC\) for the former was 3.6 times larger. Large differences were also observed in \(CL\) values between rapid and slow elimination groups. The elimination patterns of DPH converted from the prodrug in the rapid and slow disposition groups were very similar to those after dosing of DPH alone. Consequently, no significant difference between the kinetic parameters of the EDPH and DPH groups was observed in this experiment.

**Plasma Concentration of DPH after Oral Administration of Prodrug** The plasma levels of DPH after oral dosing of EDPH (25 mg/kg, DPH equivalent) are depicted in comparison with those after DPH alone in Fig. 4. On oral dosing, two different elimination patterns were also observed. However, the plasma concentrations of DPH converted from EDPH were significantly higher than those after DPH alone over the time period assayed in both elimination
Fig. 4. Time Profiles of DPH Concentration in Plasma after Oral Administration of DPH and EDPH

Dose of drug was 25 mg/kg, DPH equivalent. Closed and open symbols show the slow and rapid elimination of DPH from plasma, respectively. □ and □, DPH alone; • and ■, DPH produced from prodrug. Each value represents the mean ± S.D. (n = 4–5).

Table 2. Pharmacokinetic Parameters of DPH after Single Oral Administration of DPH and Its Prodrug

<table>
<thead>
<tr>
<th>Parameter</th>
<th>DPH</th>
<th>EDPH</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Fast</td>
<td>Slow</td>
</tr>
<tr>
<td>Cₘ₉ₓ (µg/ml)</td>
<td>1.34±0.24</td>
<td>2.69±0.49</td>
</tr>
<tr>
<td>Tₘ₉ₓ (h)</td>
<td>1.00±0.00</td>
<td>2.00±0.00</td>
</tr>
<tr>
<td>T&lt;sub&gt;1/2&lt;/sub&gt; (h)</td>
<td>0.84±0.35</td>
<td>2.18±0.38</td>
</tr>
<tr>
<td>AUC&lt;sub&gt;0→∞&lt;/sub&gt; (µg·h/ml)</td>
<td>2.43±0.50</td>
<td>14.2±1.27</td>
</tr>
<tr>
<td>MRT (h)&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.64±0.15</td>
<td>4.53±0.53</td>
</tr>
<tr>
<td>F (%)&lt;sup&gt;d&lt;/sup&gt;</td>
<td>6.95±1.73</td>
<td>8.76±0.90</td>
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</table>

Dose of drug was 25 mg/kg, DPH equivalent. Each value represents the mean ± S.D. (n = 4–5).<sup>a</sup> MRT = AUC<sub>0→∞</sub>/AUC. <sup>b</sup> Bioavailability calculated using i.v. AUC. <sup>c</sup> p < 0.05 and <sup>d</sup> p < 0.01, respectively, compared with the corresponding DPH.

groups. The F values (85.7 and 79.4%) for the fast and slow elimination groups dosed with EDPH were much larger than those (8.0 and 8.8%) of DPH alone (p < 0.01), as shown by the larger AUCs than those after DPH alone (Table 2). Consequently, the MRT after dosing of EDPH was also much greater than that after DPH (p < 0.01). Additionally, the initial plasma concentrations were much higher compared with those after DPH alone. The T<sub>max</sub> was relatively delayed in the slow elimination group after dosing of EDPH compared with that after DPH alone. That the T<sub>max</sub> in the slow elimination group was about 6.5 h after dosing suggests the prolonged absorption of the prodrug from the intestine.

The results of oral dosing studies clearly demonstrated that the intestinal absorption of the prodrug was superior to that of DPH alone.

Solubility in Water, Buffer, Bile Salt and Bile Salt-Fatty Acid Micelles

To clarify the mechanism involved in the enhanced absorption of EDPH, the solubility of EDPH in water, buffer, bile salt and bile salt-oleic acid mixed micelles was compared with that of DPH. The solubility was measured after the 1.5 h shaking at 25°C, the time being thought to be close to the time it was transferred in the intestine. The results are shown in Fig. 5. EDPH dissolved in water and 0.1 M phosphate buffer at pH 5.5 to 7.5 more readily than did DPH, although EDPH was partly hydrolyzed in these solutions, with a dominant hydrolysis in the buffer at pH 7.5. Consequently, the sum (DPH equivalent) of EDPH and converted DPH which were dissolved in the solutions was about 3.5 times larger than DPH alone. EDPH plus converted DPH also dissolved in bile salt solutions much more readily than DPH alone, being 3–4 fold greater than DPH alone. The prodrug plus converted DPH dissolved in the bile salt–oleic acid mixed micelles in the highest levels of all the solutions tested, giving a solubility of 2–4 fold larger than that of DPH alone; however, a large part of the drug in the micelles was presented as DPH after the 1.5 h shaking experiment. Thus, the higher solubilities of EDPH in bile salt solutions and bile salt–fatty acid mixed micelles would result in increased absorption after oral administration of the prodrug.

Distribution to Intestinal Mucosa

To further confirm the increased intestinal absorption of the prodrug, concentrations of parent drug and prodrug were measured in the intestinal mucosa after a single oral dose of 50 mg/kg (DPH equivalent). No prodrug was detected in the mucosa, suggesting the rapid hydrolysis of the N-acetyl bond in the tissue. The concentrations (µg/g wet tissue) of DPH which were distributed into the duodenum, jejunum and ileum are shown in Fig. 6, along with those of DPH alone.

Administration of EDPH significantly enhanced the concentrations of DPH in the duodenal mucosa at 1 and 5 h after dosing compared with DPH dosing. The concentration in the duodenum at 1 h after dosing of EDPH was 6.6 times higher than that after DPH alone. A similar phenomenon was also
observed with the concentrations of DPH in the jejunum after dosing of EDPH. However, in the ileum the concentration after DPH alone was high, but not significant, compared with that after EDPH. These data clearly demonstrated that EDPH was more easily uptaken into the intestinal mucosa, especially into the duodenal mucosa, than DPH. This was probably due to the absorption, at least partly, in the amide form, as demonstrated by the solubility experiment (Fig. 5). Of particular interest was the existence of DPH in the mucosa in the appropriate amount 5 h after oral dosing of EDPH, which would probably be related to the prolonged plasma concentrations after dosing of the prodrug.

DISCUSSION

The prodrug, EDPH, was patented in the U.S. 30 years ago, but no pharmacokinetics or characterization of the prodrug have been reported. To improve the absorbability of DPH, EDPH was synthesized and the absorption and pharmacokinetics were evaluated in rats. An essential requisite for prodrug effectiveness is its ability to readily release the parent drug after oral and i.v. administrations. This prodrug (EDPH) was rapidly hydrolyzed to DPH in the intestinal mucosa, liver (Fig. 2) and plasma, this being probably due to the prodrug having a short chain acyl substituent which is readily cleaved by esterases. Our previous study on N-carboxyamido DPH showed that a prodrug with a short chain was hydrolyzed more rapidly than that with a longer chain in these tissues. Thus, EDPH is compatible with the essential requisites for a prodrug.

When EDPH was orally administered to rats, the plasma levels of DPH converted from the prodrug were much higher and more sustained than those after DPH alone, demonstrating the extensive absorption of both DPH and converted DPH (Fig. 3). Consequently, higher bioavailabilities (85.7 and 79.4% for rapid and slow elimination groups, respectively) were observed compared with those of DPH alone. These results clearly revealed that the intestinal absorption of this prodrug was much superior to that of DPH alone, although EDPH was partly hydrolyzed to DPH in the buffer (pH 5.5–7.5) and bile salt solution.

To clarify the mechanism involved in the enhanced absorption of EDPH, the solubility was compared with that of DPH. The total amounts of EDPH and DPH, derived from the prodrug, dissolved in bile salt solutions and bile salt–oleic acid micelles were 2–4 times more than those of DPH alone. Interestingly, the total amounts dissolved were highest in the micelles among the various solvents used in this experiment. Since the critical micelle concentration (CMC) of taurodeoxycholate is 1.5 mM at 20°C, the concentrations of bile salts used in this experiment are over the CMC value. Thus, the prodrug was judged to be incorporated in the micelles formed. Such high solubilization of EDPH in the bile salt micelles would facilitate diffusion through the unstirred water layer (UWL) that constitutes a barrier to absorption, leading to increased absorption of the prodrug and converted DPH at the upper small intestine. Consequently, bile and mixed micelles might improve the bioavailability of EDPH by enhancing its rate of dissolution and/or solubility.

The partial residence of prodrug in the mixed micelles and bile salt solution 1.5 h after incubation (Fig. 5) suggests that part of prodrug dosed was absorbed through the intestinal mucosa in an amide form, followed by rapid hydrolysis in the tissue. Our concept of the enhanced absorption of EDPH was supported by a theory that the constituents of the mixed micellar phase have an impact on the intestinal permeability of poorly water-soluble drugs via three major processes: 1) changing the intrinsic permeability of the intestinal membrane, 2) facilitating diffusion through the UWL, and 3) decreasing the intermicellar “free” fraction of a drug. The reason both EDPH and converted DPH were extensively dissolved in the bile salt–oleic acid micelles may be explicable as follows: hydantoin derivatives dissociate in water, as shown in Fig. 7 (A). On the other hand, EDPH which does not have a dissociable proton is neutral, as depicted in (B). However, the amide bond is easily hydrolyzed, and the acetic acid produced from the prodrug would lower the pH of the solution gradually. In fact, the pH of a saturated solution of EDPH was 7.60±0.2 immediately after, but the pH was 4.76±0.03 after 30 min, whereas the pH of DPH was 5.96±0.03. Judging from the solubility experiments of EDPH and DPH, a small amount of acetic acid and acidifying by the acid would increase the solubility of DPH and EDPH in the solutions, especially in the bile salt and mixed micelles.

The data on the distribution to intestinal mucosa after oral
dosing indicated that the prodrug penetrated more easily through the mucosal membrane than DPH, and that the prodrug was sustainedly absorbed over a relatively prolonged period, probably due to the absorption in the amide form partly, and to the high solubility of EDPH in the bile salt micelles and bile salt solutions, while DPH with poor solubility was partly absorbed. Consequently, these results clearly verified the high bioavailability and sustained plasma levels of DPH after oral dosing with EDPH. The higher concentrations of EDPH in the duodenum 1 h after dosing, compared with those after DPH alone, provide an evidence that the plasma levels of DPH at early time periods after dosing of EDPH were much higher than those after DPH alone. The lower polarity of EDPH compared with DPH would also contribute to the easy and rapid absorption of the prodrug at the intestine.

Several prodrugs of DPH have been evaluated to overcome the drug’s limitations due to its poor aqueous solubility and great variations in bioavailability. A prodrug, DPH-bis-hydroxyisobutyrate resulted in an approximate 2.5-fold increase of $C_{\text{max}}$ and a higher bioavailability of DPH (a 3-fold increase in $AUC$) after oral administration in rats. On the other hand, the $AUC$ of DPH after oral dosing of 3-(hydroxymethyl)-DPH $N,N$-dimethylaminoethyl carbonate methanesulfonate was 5-fold more than that after sodium DPH in dogs. Our results were equivalent to or superior to the data reported above, in respect to the sustained plasma concentrations ($MRT$, 7.4 and 12.8 h in the rapid and slow elimination groups, respectively) and high bioavailabilities (85.7 and 79.4%, respectively) after oral administration to rats.

In conclusion, an amide prodrug of DPH, EDPH, was synthesized. When EDPH was administered orally, the plasma levels of DPH converted from the prodrug were significantly higher and more sustained than those after DPH alone, with delayed $T_{\text{max}}$. In addition, the concentrations of DPH in the duodenal and jejunal mucosa after oral dosing of EDPH were also much higher than those after DPH alone. The underlying mechanism is due to that the dissolution of the prodrug and DPH in bile salt solution and in bile salt–oleic acid mixed micelles was greater than that of DPH alone. Consequently, the high solubilization of EDPH would facilitate diffusion through the UWL and absorption from the intestine. Therefore, EDPH will be expected to be used as a prodrug for the desired delivery of DPH.