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Enhancement of oral bioavailability of phenytoin by esterification, and in vitro hydrolytic characteristics of prodrugs

Tadatoshi Tanino, Taro Ogiso *, Masahiro Iwaki, Genzou Tanabe, Osamu Muraoka

Faculty of Pharmaceutical Sciences, Kinki University, 3-4-1 Kowakae, Higashi-Osaka, Osaka 577, Japan

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Abstract

To improve the oral absorbability of phenytoin (DPH), prodrugs of DPH with a small acyl substituent, *N*-carboethoxy- and *N*-carboisopropoxy-DPH (PT-1 and PT-2, respectively), were synthesized and bioavailabilities of them were evaluated after oral administration in rats, compared to that of DPH dosed. The prodrugs were rapidly hydrolyzed in the intestinal fluid, intestinal mucosa, liver homogenates and plasma of rats, the plasma giving the highest hydrolytic activity. Two different eliminations of DPH, slow and rapid, were observed after intravenous and oral administrations of prodrugs. The bioavailabilities of DPH after oral administration at a dose of 25 mg/kg of PT-1 and PT-2 (DPH equivalent), increased to approximately 8.5- and 6.0-fold for PT-1 and PT-2 (rapid elimination group) or 3.0- and 3.0-fold (slow elimination group), respectively, compared to those after dosing of DPH. The plasma levels of DPH converted from PT-2 dosed were lower, but more sustained in slow elimination groups than those from PT-1. The normalized AUC values after oral dosing of prodrugs at a dose of 50 mg/kg were increased dramatically, compared to those at a dose of 25 mg/kg, suggesting non-linear clearance at a high dose. In order to clarify the mechanism for preponderance of intestinal absorption of the prodrugs, concentrations of parent drug and prodrug were measured in intestinal mucosa after a single oral dosing of 50 mg/kg (DPH equivalent). Upon the administration of PT-1 and PT-2, greater amounts of DPH, in comparison with those after dosing of DPH, and small amounts of intact prodrugs were detected in the duodenal and jejunal mucosa. These data indicated that these prodrugs was subjected to the extensive intestinal absorption compared to DPH, giving comparatively high plasma levels. Therefore, PT-1 and PT-2 will be useful prodrugs as an orally applicable form. In particular, PT-2 seems to serve as a benign prodrug with the intention of improving the absorption of DPH.

Keywords: Phenytoin; Oral bioavailability; Esteric prodrug; Enzymatic hydrolysis; Rat

* Corresponding author. Tel.: +81 6 7212332, ext. 3819; fax: +81 6 7301394; e-mail: tanino@phar.kindai.ac.jp

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1. Introduction

Phenytoin (DPH), which is one of the most effective anti-convulsants (Merritt and Putnam, 1938), is still used extensively in the therapy of epilepsy. However, DPH shows great variations in bioavailability following oral administration to patients, because of its poor water-solubility (Buchthal, 1972; Stella et al., 1985). Thus, improvement of intestinal absorption of DPH is needed to control seizures adequately. Many water-soluble prodrugs of DPH have been synthesized to develop the potential oral and parenteral applicable forms (Arnold et al., 1970; Bansal et al., 1981a,b; Varia et al., 1984) and it is shown that DPH levels from intramuscular administration of the prodrug were far superior to those generated from sodium DPH similarly administered (Varia and Stella, 1984). On the other hand, DPH-lipid conjugates as the prodrugs have been evaluated on the bioavailability and anti-convulsant activity compared with the parent drug (Scriba et al., 1995a,b,c). Administration of these conjugates resulted in a 4-fold increase of the area under the plasma concentration-time curves (AUC) of DPH with no changes in the half-life and the effective anti-convulsant activity (Scriba et al., 1995b). On the other hand, manufacturing attempts have been also made to improve the bioavailability of DPH based on enhancing its absorption with surfactants such as deoxycholate and glycholate (Sekikawa et al., 1978; Yakou et al., 1986; Suzuki et al., 1990), in which DPH bioavailability and the dissolution rate in intestinal lumens were greater than those for the water-soluble prodrugs and DPH sodium (Arnold and Gerber, 1970; Varia et al., 1984; Suzuki et al., 1990). However, Sugiyama et al. (1997) demonstrated that bile salts and fatty acids (20 mM) caused remarkable damage to the small and/or large intestinal membranes. Thus, it has often been difficult to overcome the drawbacks in intestinal tracts as an oral dosage form.

There are some reports about structure–activity relationships of DPH derivatives. Nakamura et al. (1970) published that the presence of substituents at 1-position of the hydantoin ring decreased the original activity and that only DPH derivatives with modifications at the 3-position could be easily synthesized without significantly affecting the area that is relevant for the pharmacological activity of central nervous system (Nakamura et al., 1965). In our previous studies, the derivatives with high lipophilic (2-propyl-pentanoyl and pentanoyl) groups were found to reduce the permeability through intestine when compared to DPH and to rapidly clear from plasma when administered intravenously and orally to rats (Ogiso et al., 1993). The findings mentioned above led us to develop a class of ester prodrugs with a small acyl molecular substituent at the 3-position, in attempt to improve the poor bioavailability of DPH. A prodrug, 5,5-diphenyl-*N*-carboethoxyhydantoin (PT-1), was already synthesized by Nakamura et al. (1970), but no pharmacokinetics has been reported.

The purpose of the present study is to assess: (1) the effectiveness of the prodrugs as an oral applicable form based on the bioavailability; (2) the amounts of DPH and prodrugs distributed into the small intestine, with the intention of improving the absorbability of DPH; and (3) hydrolytic characteristics of the prodrugs to DPH in various rat tissues, the plasma, liver, intestinal mucosa and intestinal fluid.

2. Materials and methods

2.1. Materials

DPH was purchased from Nacalai Tesque (Kyoto, Japan). Ethoxycarbonyl chloride and isopropyl chloroformate were obtained from Tokyo Chemical Industry (Tokyo, Japan) and Aldrich (Milwaukee, WI), respectively. Acemethacin and flunitrazepam as internal standards for high performance liquid chromatography (HPLC), were generous gifts of Kowa (Nagoya, Japan) and Eisai Pharmaceutical (Tokyo, Japan), respectively. All other chemicals used were of reagent grade or HPLC quality.

2.2. Organic synthesis of prodrugs

The chemical structures of DPH and its prodrugs are shown in Fig. 1.

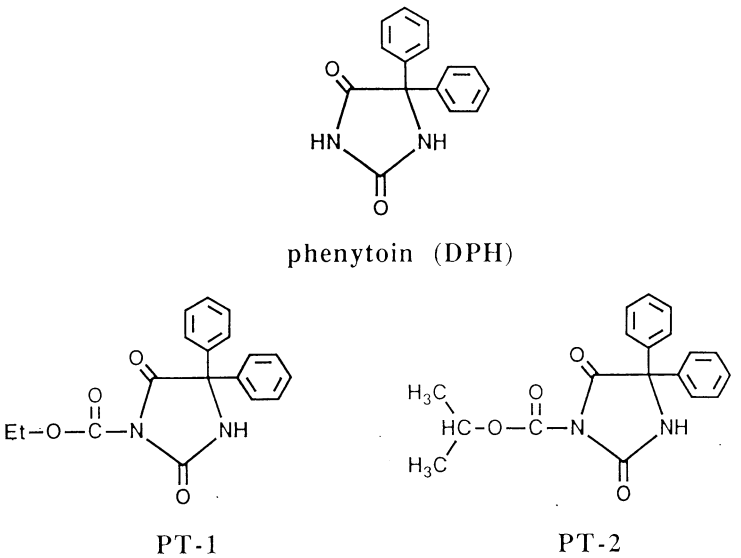

Fig. 1. Structures of DPH and its prodrugs.

2.2.1. 5,5-Diphenyl-N-carboethoxyhydantoin (PT-1)

A 13.6-ml amount of ethoxycarbonyl chloride (143 mmol) was slowly added to 30 g of DPH (119 mmol) dissolved in the mixture of dichloromethane (300 ml) and triethylamine (82.5 ml, 595 mmol) on an ice bath and stirred for 1 h under chilling. The mixture was added to 10% HCl and then the resulting product was extracted with dichloromethane. The organic phase was washed three times with saturated NaCl solution. After dryness and evaporation, crude crystal was recrystallized from ethanol. The yield was 35.5 g (92%). Colorless prisms, mp 137–138°C. ^{1}H-NMR (in $CDCl_3$): δ 1.39(3H, t, $J = 7.0$ Hz), 4.43 (2H, q, $J = 7.0$ Hz), 6.85 (1H, br) and 7.37 (10H, s). Elemental analysis: calcd for $C_{18}H_{16}N_2O_4$: C, 66.66; H, 4.97; N, 8.64. Found: C, 66.74; H, 5.08; N, 8.66.

2.2.2. 5,5-Diphenyl-N-carboisopropoxyhydantoin (PT-2)

PT-2 was synthesized using 19.8 ml of isopropyl chloroformate (143 mmol) by the same method as the synthesis of PT-1. The yield was 38.2 g (95%). Colorless prisms, mp 129–130°C. ^{1}H-NMR (in $CDCl_3$): δ 1.39 (6H, d, $J = 6.3$ Hz), 5.18 (1H, sept., $J = 6.3$ Hz), 6.93 (1H, br) and 7.37 (10H, s). Elemental analysis: calcd for $C_{19}H_{18}N_2O_4$: C, 67.45; H, 5.36; N, 8.26. Found: C, 67.59; H, 5.38; N, 8.54.

2.3. In vitro enzymatic and chemical hydrolysis

Male Wistar rats (Nippon SLC, Hamamatu, Japan), weighing 220–260 g, were fasted overnight before sacrificed by decapitation. Luminal content of intestine was collected by washing out 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 trimmed away fat and omentum. The mucosa was obtained by scraping with a slide glass. The mucosa obtained 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 were homogenized in 4 volumes of 1.15% KCl with a Potter-Elvehjem glass homogenizer equipped with a Teflon pestle. The resultant homogenates were centrifuged at $9000 \times g$ for 25 min. Blood was collected in heparinized syringes and the plasma was separated by centrifugation. The resulted supernatants and the intestinal fluid were diluted with the saline-phosphate buffer (pH 7.4) to give 0.1 mg protein/ml, except for plasma (0.05 mg protein/ml) and were immediately used for experiment. The protein concentrations were determined by the procedure reported by Lowry et al. (1951) 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 M 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 standards (2 μg/ml). After centrifugation, an aliquot (20–40 μl) of the supernatant was loaded onto a HPLC column.

The chemical stability of the prodrugs was assessed in 0.1 M phosphate buffer (pH 7.4) or 0.1 N HCl, at 37°C for 24 h.

2.4. Animal experiments

Male Wistar rats, weighing 220–260 g, were used throughout this study. On the day before the experiment, the rat jugular vein was cannulated with a silicone tubing (Upton, 1975). For a single oral administration, one group of the animals were starved overnight and during the experiment allowed water at libitum. DPH and prodrugs were sifted by a 100-mesh sieve. The fine grains of DPH or its prodrugs (25 and 50 mg/kg, DPH equivalent) was administered as suspensions in 1.5% aqueous carboxymethyl cellulose in a volume of 2 ml/kg. On the other hand, DPH or its prodrugs (20 mg/kg, DPH equivalent) was administered intravenously to another group as a solution in a mixture of saline, ethanol and propylene glycol (4:1:5, v/v). A 0.2-ml blood sample was collected periodically after dosing with a heparinized syringe through the cannula and centrifuged at 12 000 rpm for 1 min. A 50-μl aliquot of plasma was added to double volumes of acetonitrile containing the internal standard (acemethacin, 2 μg/ml). After centrifugation and filtration, aliquots of the supernatant were loaded onto a HPLC column.

2.5. Intestinal tissue distribution studies

DPH or prodrugs (50 mg/kg, DPH equivalent) was administered orally by the same method as described above. At the indicated time (1, 3 and 7 h), rats were killed and the whole intestine was removed. The duodenum segment was obtained from the first 5-cm portion of the intestine close to the stomach. The upper two-fifths from the ligament of Treitz to the ileo-cecal junction was utilized as the jejunal section and the remainder was designated as the ileum segment. Intestinal contents were removed by flushing each segment with ice-cold 0.9% NaCl. The mucosa of segments was scraped off with a slide glass on ice and homogenized in a mixture of one half volume of 0.1 M KH_2PO_4 and 3.5 volumes of acetonitrile including the internal standard (acemethacin, 2 μg/ml). The homogenates were centrifuged at 3000 rpm for 10 min and the supernatants were collected. DPH and prodrugs in the supernatant were analyzed by the HPLC method after filtration.

2.6. Analytical methodology

HPLC systems were used for quantitative analysis of DPH and its prodrugs. The analyses were performed on an Inertsil ODS C18 column ($4.6 \times$ 150 mm, 5 μm particle size, GL Sciences, Japan). The mobile phase (methanol, acetonitrile and 0.025 M phosphate buffer (pH 7.0) (2:3:5, v/v)) was pumped at a flow rate of 1.0 ml/min at 35°C. The detection was at 254 nm. In the hydrolytic experiment using intestinal fluid, the mobile phase (1:4:5, v/v) modified, was used to separate several contaminants.

Table 1
In vitro stability and hydrolysis of PT-1 and PT-2

Mediums[a]	PT-1		PT-2	
	Rate constant (min^{-1})	Half-life (min)	Rate constant (min^{-1})	Half-life (min)
Plasma	0.278 ± 0.006	2.49 ± 0.06	0.161 ± 0.003[b]	4.32 ± 0.09[b]
Liver homogenates	0.091 ± 0.007	7.67 ± 0.60	0.056 ± 0.017[b]	13.5 ± 3.65[b]
Intestine homogenates	0.064 ± 0.009	11.0 ± 1.68	0.020 ± 0.004[b]	36.7 ± 6.63[b]
Intestinal fluid	0.056 ± 0.018	14.3 ± 5.95	0.014 ± 0.004[b]	52.4 ± 15.4[b]
0.1 N HCl	0[c]		0[c]	
pH 7.4 buffer	0[c]		0[c]	

The results represent the mean ± S.D. of three to four rats.
[a] The concentration of protein used was 0.1 mg/ml except for plasma (0.05 mg/ml).
[b] $p < 0.01$ compared with PT-1.
[c] No DPH was produced in this experiment (at 37°C for 24 h).

2.7. Data analysis

In the hydrolysis experiment, the pseudo first-order rate constant (k_{obs}) was calculated by the least squares fit program, MULTI (Yamaoka et al., 1981). The data obtained were fitted to the following equation:

$$k_{obs} = \frac{2.303}{t} \cdot \log \frac{a}{a-x}$$

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

Pharmacokinetic parameters were calculated using the non-linear least squares regression program, MULTI (Yamaoka et al., 1981). The plasma concentration-time data after intravenous (i.v.) administration were fitted to the two compartment open model that can be described by the following equation:

$$C_p = A\ e^{-\alpha t} + B\ e^{-\beta t}$$

where C_p is the drug concentration at time t and A, α, B and β are the biexponential equation constants. The AUC up to the last sampling point was calculated by the trapezoidal method and the AUC beyond the last observed concentration ($C_p(t)$) was extrapolated according to $C_p(t)/\beta$. The oral bioavailability (F) was calculated from $AUC_{0-\infty}$ of DPH after i.v. and oral administrations of the parent drug and prodrug. The volume of distribution at steady-state (Vd_{ss}) and total-body clearance (CL_{tot}) were calculated using means of moment analysis (Gibaldi and Perrier, 1982). The terminal half-life ($T_{1/2}$) of DPH and its prodrugs was calculated as $T_{1/2} = 0.693/\beta$. The area under the first moment curve (AUMC) and the mean residence time (MRT) were calculated by the following equations:

$$AUMC = \int_0^\infty tC\ dt$$

$$MRT = \frac{AUMC}{AUC}$$

The means of all data are presented with their S.D. Statistical analysis was performed by the unpaired Student's t-test, and a p value of 0.05 or less was considered to be significant.

3. Results

3.1. In vitro enzymatic and chemical hydrolysis of the prodrugs

An essential requisite for prodrug effectiveness is its ability to readily release the parent drug after oral administration. To characterize the tissues or organs capable of hydrolyzing the ester bond of the prodrugs, the hydrolytic activity was estimated using intestinal fluid, small intestine, liver and plasma, which are involved in the hydrolysis. The semi-logarithmic plots of residual prodrug concentrations against time revealed good linear-

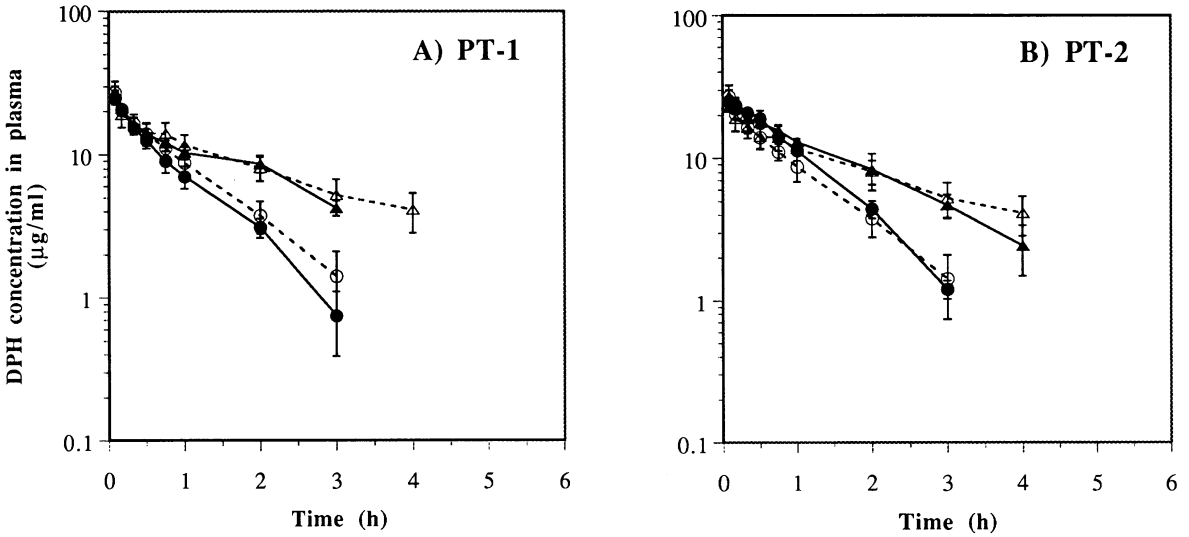

Fig. 2. Time profiles of DPH concentration in plasma after i.v. administration of DPH and its prodrugs to rats. Dose: 20 mg/kg, DPH equivalent. Triangular and circle symbols show the slow and rapid disposition of DPH from plasma, respectively; (○) and (△), DPH alone; (●) and (▲), DPH converted from prodrugs. Each value represents the mean ± S.D. ($n = 3$–4).

ity, indicating that the hydrolysis of both prodrugs was adequately described by pseudo first-order kinetics. PT-1 was relatively rapidly hydrolyzed in all samples compared with PT-2. The plasma gave the highest hydrolytic activity for both prodrugs when expressed by DPH μg/min per mg protein. The pseudo first-order rate constants calculated are summarized in Table 1. The rank order of degradative constants (k_{obs}) were plasma > liver > intestinal mucosa > intestinal fluid. The k_{obs} values for PT-1 in the intestinal mucosa and fluid were approximately 3- or 4-fold greater than those of PT-2. On the other hand, PT-1 and PT-2 were perfectly stable in acidic and neutral solutions without tissues up to 24 h, as shown in Table 1.

3.2. Plasma concentration after i.v. administration

Fig. 2 shows the concentration-time profiles after i.v. administration of DPH and its prodrugs. No measurable concentrations of prodrugs could be detected in the plasma, demonstrating the rapid hydrolysis of the ester bond in plasma, as shown in the in vitro study (Table 1). The plasma levels of DPH after prodrug dosing declined in a bi-exponential manner, as well as those of parent drug. Two different eliminations of DPH, slow and rapid, were observed in rats, as reported by others (Colburn and Gibaldi, 1977). The kinetic parameters calculated are summarized in Table 2. The elimination rate (β) of terminal phase for the rapid elimination group was two times greater than that for the slow group, although the difference in Vd_{ss} was not observed between both groups. The elimination patterns were similar in both groups administered DPH and prodrugs. Consequently, no significant difference in the kinetic parameters of parent drug and DPH converted from prodrugs was seen after i.v. dosing.

3.3. Plasma concentration after oral administration

The plasma levels of DPH after oral administration of PT-1 and PT-2 at a dose of 25 mg/kg (DPH equivalent), are depicted in comparison with those after dosing of DPH in Fig. 3. The two different eliminations were also observed after oral administration. Plasma concentrations of DPH converted from PT-1 and PT-2 were significantly higher than those after dosing of DPH over

Table 2
Pharmacokinetic parameters obtained from DPH plasma levels after i.v. administration of DPH, PT-1 and PT-2 to rats

Parameter	DPH		PT-1		PT-2	
	Rapid	Slow	Rapid	Slow	Rapid	Slow
k_{12} (h)	3.60 ± 1.56	9.44 ± 5.12	1.64 ± 0.86	3.72 ± 1.70	2.92 ± 1.12	3.99 ± 2.79
k_{21} (h)	6.95 ± 2.34	5.08 ± 1.66	4.28 ± 2.96	3.80 ± 1.47	5.29 ± 2.53	3.30 ± 0.92
k_{10} (h)	1.43 ± 0.41	1.15 ± 0.15	1.42 ± 0.07	0.92 ± 0.18	1.48 ± 0.05	0.89 ± 0.21
$T_{1/2}^{a}$ (h)	0.81 ± 0.15	2.02 ± 0.38	0.80 ± 0.10	1.60 ± 0.17	0.81 ± 0.07	1.74 ± 0.10
$AUC_{0-\infty}$ (μg/h per ml)	25.2 ± 3.59	46.7 ± 10.9	22.1 ± 2.20	40.9 ± 2.79	23.9 ± 2.45	40.1 ± 2.00
CLtot[b] (l/h per kg)	0.81 ± 0.13	0.45 ± 0.10	0.91 ± 0.10	0.49 ± 0.03	0.84 ± 0.08	0.50 ± 0.02
Vdss[c] (l/kg)	0.89 ± 0.11	1.09 ± 0.19	0.81 ± 0.12	0.95 ± 0.09	0.84 ± 0.12	1.03 ± 0.08

Dose: 20 mg/kg, DPH equivalent. The values are expressed as the mean $\pm$ S.D. ($n = 3$–4).
[a] The elimination half-life ($T_{1/2}$) was estimated from the terminal phase of the curve.
[b,c] The parameters were obtained using the moment analysis.

the time period assayed. F values for the rapid and slow elimination groups were much greater than those after DPH, as shown by 3–8-fold differences in AUCs between prodrugs and DPH dosed (Table 3).

The results of oral dosing studies clearly revealed that the intestinal absorption of these prodrugs was much superior to that of DPH itself. Interestingly, PT-2 apparently gave sustained plasma levels of DPH in the slow elimination group, but the peak levels of DPH were lower than those after PT-1.

When dosed at a 50 mg/kg (DPH equivalent), the plasma levels of DPH after administration of prodrugs were also significantly higher than those after dosing of DPH (Fig. 4). In the slow elimination group, PT-1 and PT-2 gave high steady-state levels of DPH over the time period of 3–12 h. The normalized AUC values after dosing of DPH and the prodrugs at this dose were increased significantly in both rapid and slow elimination groups in comparison with those at a dose of 25 mg/kg (Table 4), suggesting non-linear clearance of DPH at a high dose due to saturable oxidative biotransformation. AUCs after administration of PT-1 and PT-2 were consistency two times larger than those after dosing of DPH.

3.4. Distribution to intestinal tissues

To clarify the mechanism for preponderance of intestinal absorption of the prodrugs, concentrations of parent drug and prodrug were measured in the intestinal mucosa after a single oral dose of 50 mg/kg (DPH equivalent). The concentrations of DPH and prodrug distributed into the duodenum, jejunum and ileum are shown in Fig. 5, with those after administration of DPH.

Administration of PT-1 and PT-2 significantly enhanced the concentrations of DPH in the duodenum and jejunum 1–3 h after dosing. The particular interest was that small amounts of the prodrugs were present in the tissues in the intact form, indicating the partial absorption of the ester prodrug itself. The concentrations of DPH converted from PT-1 were slightly higher than those from PT-2, but not significant. On the other hand, the relatively higher levels of PT-2 in all segments were observed compared with those of PT-1. PT-2 was recognized in the jejunum even after 3 h of dosing. In the ileum, there was no significant difference in the concentrations between DPH and the prodrugs dosed from 1 to 3 h, although the amount of DPH generated from PT-2 was significantly larger than that after PT-1 and DPH at 7 h.

4. Discussion

Since DPH shows the strong pharmacological activity and much lower side-effects than other anti-convulsant drugs, the development of DPH prodrugs is of particular significance for the effec-

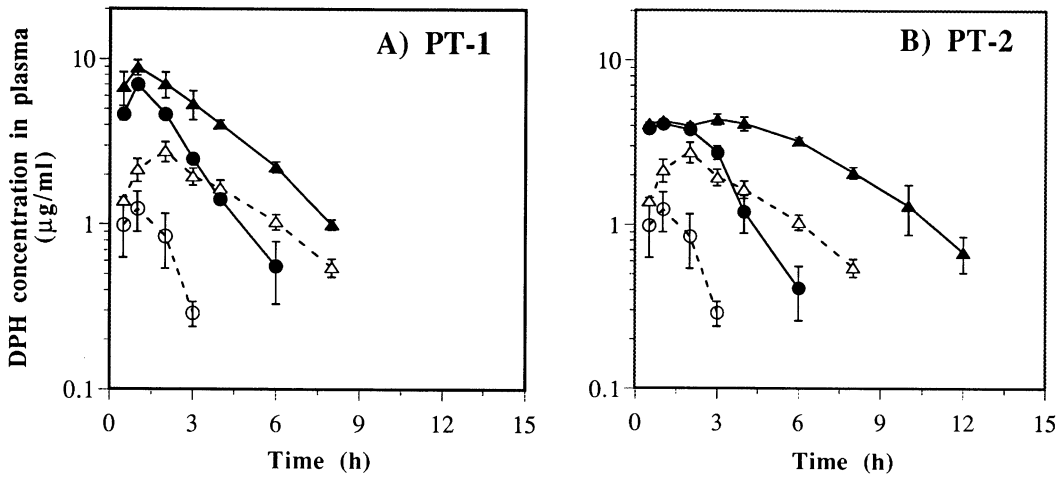

Fig. 3. Time profiles of DPH concentration in plasma after oral administration of DPH and its prodrugs. Dose: 25 mg/kg, DPH equivalent. Triangular and circle symbols show the slow and rapid disposition of DPH from plasma, respectively; (○) and (△), DPH alone; (●) and (▲), DPH converted from prodrugs. Each value represents the mean ± S.D. ($n = 3–6$).

tive drug therapy. We previously reported the pharmacokinetics (Ogiso et al., 1993) and physicochemical characteristics (Ogiso et al., 1994) of novel derivatives of DPH. Disposition of the derivatives from plasma was 1.5–2.0-fold faster than that of DPH when intravenously administered to rats, the fast disposition being partly ascribed to high binding (more than 98%) of derivatives to rat plasma (Ogiso et al., 1994). Additionally, we also found that extents absorbed of the derivatives through gastrointestine were much less than those after dosing of DPH (unpublished data). Because of the lipophilic nature of gastrointestinal mucosal cells, suitable balance between aqueous solubility and lipophilicity is requisites as desirable characteristics of prodrugs.

The purpose of this study is to develop the prodrugs of DPH capable of readily absorbing through the intestine and hydrolyzing in the body. Ester prodrugs, PT-1 and PT-2, used in the present studies could be simply synthesized with higher yields compared with other published prodrugs of DPH (Varia et al., 1984; Pop et al., 1986; Scriba, 1994; Lambert et al., 1996). No intact prodrugs were found in plasma after intravenous administration of them (Fig. 2). This result demonstrated the rapid conversion of the prodrugs to DPH in plasma (Table 1).

The present ester prodrugs showed the plasma behavior similar to DPH itself when intravenously administered to rats. Namely, the two different dispositions were clearly observed in plasma as shown in Fig. 2. In general, pharmacokinetic parameters for DPH have been estimated without dividing into the slow and rapid elimination groups. However, Colburn and Gibaldi (1977) have demonstrated that an endogenous inhibitor is responsible for the difference in binding capability of DPH to plasma protein, resulting in two different disappearance from plasma. As shown in Table 2, our data was in good agreement with the pharmacokinetic parameters ($T_{1/2}$: 0.53 ± 0.07 and 2.70 ± 1.20 h) published by them. The rats were separated according to the elimination rate constants into the rapid and slow elimination groups, which existed at the ratio of 3:2 in our study. Thus, rapid and slow elimination groups were separately analyzed to obtain more detailed information on ester prodrugs as an oral applicable form.

After a single oral administration of prodrugs at a dose of 25 mg/kg, higher level and rapid appearance of DPH in plasma were observed (Fig. 3). High bioavailabilities obtained in this study might be ascribed to increased absorption of prodrugs. On the other hand, plasma concen-

Table 3
Pharmacokinetic parameters obtained from DPH plasma levels after single oral administration of DPH, PT-1 and PT-2 to rats

Parameter	DPH		PT-1		PT-2	
	Rapid	Slow	Rapid	Slow	Rapid	Slow
$T_{1/2}^{a}$ (h)	0.80 ± 0.09	2.18 ± 0.38	0.71 ± 0.02	2.17 ± 0.32	0.93 ± 0.14	2.49 ± 0.39
C_{max}^{b} (μg/ml)	1.34 ± 0.24	2.69 ± 0.49	7.00 ± 0.02^{f}	8.89 ± 0.92^{f}	4.10 ± 0.11	4.37 ± 0.34
T_{max}^{c} (μg/h per ml)	1.00 ± 0	2.00 ± 0	1.00 ± 0	1.00 ± 0^{f}	0.83 ± 0.24	2.25 ± 1.30
$AUC_{0-\infty}$ (μg/h per ml)	2.43 ± 0.50	14.2 ± 1.27	18.7 ± 1.02^{f}	37.1 ± 3.34^{f}	14.4 ± 0.47^{f}	36.4 ± 2.19^{f}
AUCcorr.[d] (μg/h per ml)	0.10 ± 0.02	0.57 ± 0.05	0.75 ± 0.04^{f}	1.49 ± 0.13^{f}	0.58 ± 0.02^{f}	1.45 ± 0.09^{f}
MRT (h)	1.64 ± 0.15	4.55 ± 0.53	2.45 ± 0.39^{f}	3.80 ± 0.27	2.40 ± 0.05^{f}	5.42 ± 0.53
F^{e} (%)	7.71 ± 1.57	24.3 ± 2.15	67.7 ± 3.56^{f}	72.6 ± 6.56^{f}	48.2 ± 1.65^{f}	72.6 ± 4.38^{f}

Dose: 25 mg/kg, DPH equivalent. The values are expressed as the mean $\pm$ S.D. ($n = 3$–6).
[a] The elimination half-life ($T_{1/2}$) was estimated from the terminal phase of the curve.
[b] C_{max} and [c] T_{max} were obtained from the plasma concentration-time profiles.
[d] Normalized AUC by dose (AUC/dose).
[e] F was calculated using the corresponding i.v. AUC.
[f] $p < 0.01$ compared with DPH data.

tration profiles of the corresponding DPH obviously differed between PT-1 and PT-2 in slow elimination groups. At the initial time stage, the peak plasma levels after dosing of PT-2 were much lower than those after PT-1, suggesting apparently slow absorption of PT-2 compared to PT-1 (Fig. 3). This is probably due to the slow hydrolysis of this prodrug in the intestinal membranes (Table 1 and Fig. 5). At present, a prodrug showing slow biotransformation has been often used to obtain sustained plasma levels of the drug (Bialer et al., 1985; Stella et al., 1985; Badir et al., 1991; Hadad et al., 1992). Additionally, continuous absorption of drugs from intestinal tract is necessary to obtain prolonged plasma levels (Baldessarini et al., 1977). In hydrolysis study, both intestinal mucosa and fluids exhibited considerable difference in the hydrolytic activity between PT-1 and PT-2, in which a relatively slow hydrolyzing capacity of PT-2 was seen (Table 1). This is explained by the difference in the structure of the esters. PT-2 has a branched chain and the activities of hydrolytic enzymes would be partly affected by the steric hindrance.

The normalized AUC values were increased dramatically, when a high dose (50 mg/kg) of DPH and its prodrugs was orally administered, compared with those at a 25 mg/kg dose available in usual experiments (Tables 3 and 4). In human, there are many reports that the elimination of DPH shows non-linearly disposition kinetics (Arnold and Gerber, 1970). The non-linearity is mainly due to a limited capacity for drug metabolism and inhibitory effect of main metabolite, 5-(*p*-hydroxyphenyl)-5-phenylhydantoin, on the oxidative metabolism of DPH in liver (Gerber et al., 1971; Borondy et al., 1972). Based on such evidence, the ratios of AUCs at a dose of 50 mg/kg were used as an enhancement index instead of bioavailability in Table 4. Our data indicated that the indices of prodrugs at a 50 mg/kg dose were 2-fold larger than those after dosing of DPH. The data of drug distribution showed greater amounts of DPH in intestinal tissues after prodrug, compared with those after DPH and small amounts of intact prodrugs in the deodenum and jejunum, although the amount of prodrug in the tissues was generally higher in PT-2. These results demonstrated that the prodrugs dosed orally were absorbed through the intestine much more than the parent drug itself. However, the appreciable parts of prodrugs were converted to DPH in the lumen and the tissues. Judging from the in vitro hydrolysis data, prodrugs absorbed into the intestinal mucosa would be much more than the concentrations determined. Consequently, the intestinal absorption of prodrug in the ester form is thought to be an effective mean

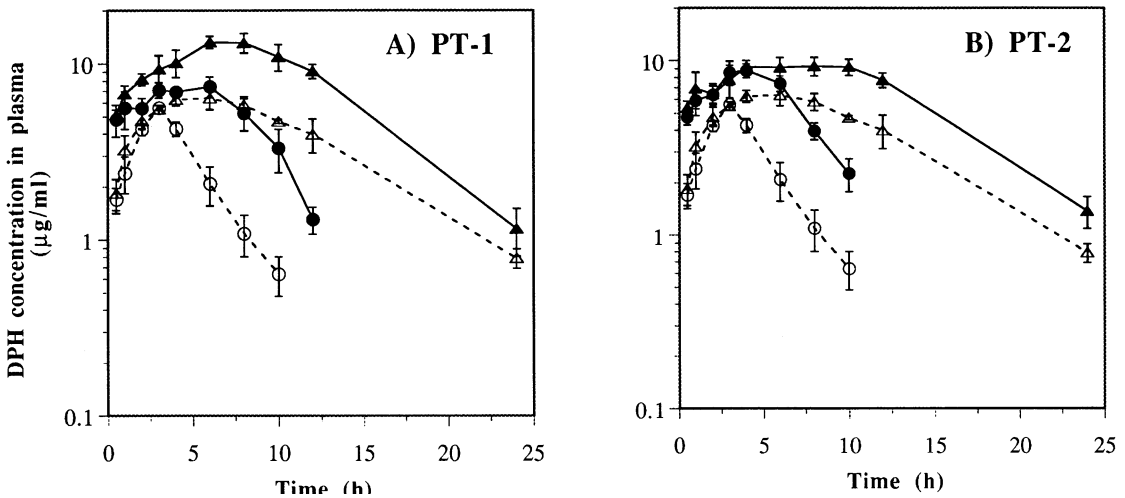

Fig. 4. Time profiles of DPH concentration in plasma after oral administration of DPH and its prodrugs. Dose: 50 mg/kg, DPH equivalent. Triangular and circle symbols show the slow and rapid disposition of DPH from plasma, respectively; (○) and (△), DPH alone; (●) and (▲), DPH converted from prodrugs. Each value represents the mean ± S.D. ($n = 4$–5).

for enhancement of absorption. The relatively longer residence of intact PT-2 compared with PT-1 in the intestine was due to the slow hydrolysis in intestinal mucosa and fluid mentioned above. Additionally, the amounts in the tissues would be apparent values, because the distributed amounts of DPH and/or prodrug are derived from their efflux and influx at the duodenum and jejunum. But, this is not denying the fact that the prodrugs were more effectively absorbed from the intestine than DPH itself. Consequently, PT-2 will be a superior prodrug more than PT-1 in respect of the high and continuous absorption.

The anti-convulsant activity and toxicological properties of PT-1 were already published by Nakamura et al. (1970), without pharmacokinet-

Table 4
Pharmacokinetic parameters obtained from DPH plasma levels after single oral administration of DPH, PT-1 and PT-2 to rats

Parameter	DPH		PT-1		PT-2	
	Rapid	Slow	Rapid	Slow	Rapid	Slow
$T_{1/2}^{a}$ (h)	2.13 ± 0.61	6.28 ± 0.78	2.39 ± 0.31	5.06 ± 0.89	2.29 ± 0.19	5.47 ± 0.52
C_{max}^{b} (μg/ml)	5.61 ± 0.24	6.34 ± 0.65	7.38 ± 0.78[f]	13.2 ± 1.21[f]	8.77 ± 0.87[f]	9.30 ± 1.02[f]
T_{max}^{c} (h)	3.00 ± 0	4.50 ± 0.87	4.33 ± 1.25	6.67 ± 0.94	3.75 ± 0.43	6.50 ± 1.66
$AUC_{0-\infty}$ (μg/h per ml)	28.0 ± 1.55	96.3 ± 6.01	67.1 ± 4.18[f]	193.0 ± 8.19[f]	67.5 ± 4.95[f]	163.4 ± 17.7[f]
AUCcorr.[d] (μg/h per ml)	0.56 ± 0.03[g]	1.93 ± 0.12[g]	1.34 ± 0.08[f,g]	3.86 ± 0.16[f,g]	1.35 ± 0.10[f,g]	3.27 ± 0.35[f,g]
MRT (h)	4.63 ± 0.28	10.3 ± 0.40	5.92 ± 0.33[f]	9.72 ± 0.42	5.53 ± 0.24[f]	10.4 ± 0.42
Index[e]	—	—	2.40 ± 0.15	2.00 ± 0.09	2.41 ± 0.18	1.70 ± 0.18

Dose: 50 mg/kg, DPH equivalent. The values are expressed as the mean ± S.D. ($n = 4$–5).
[a] The elimination half-life ($T_{1/2}$) was estimated from the terminal phase of the curve.
[b,c] C_{max} and T_{max} were obtained from the plasma concentration-time profiles.
[d] Normalized AUC by dose (AUC/dose).
[e] The enhancement index was obtained from the ratio of $AUC_{0-\infty}$, DPH to $AUC_{0-\infty}$, DPH produced from prodrug.
[f] $p < 0.01$ compared with DPH data.
[g] $p < 0.01$ compared with DPH data in Table 3.

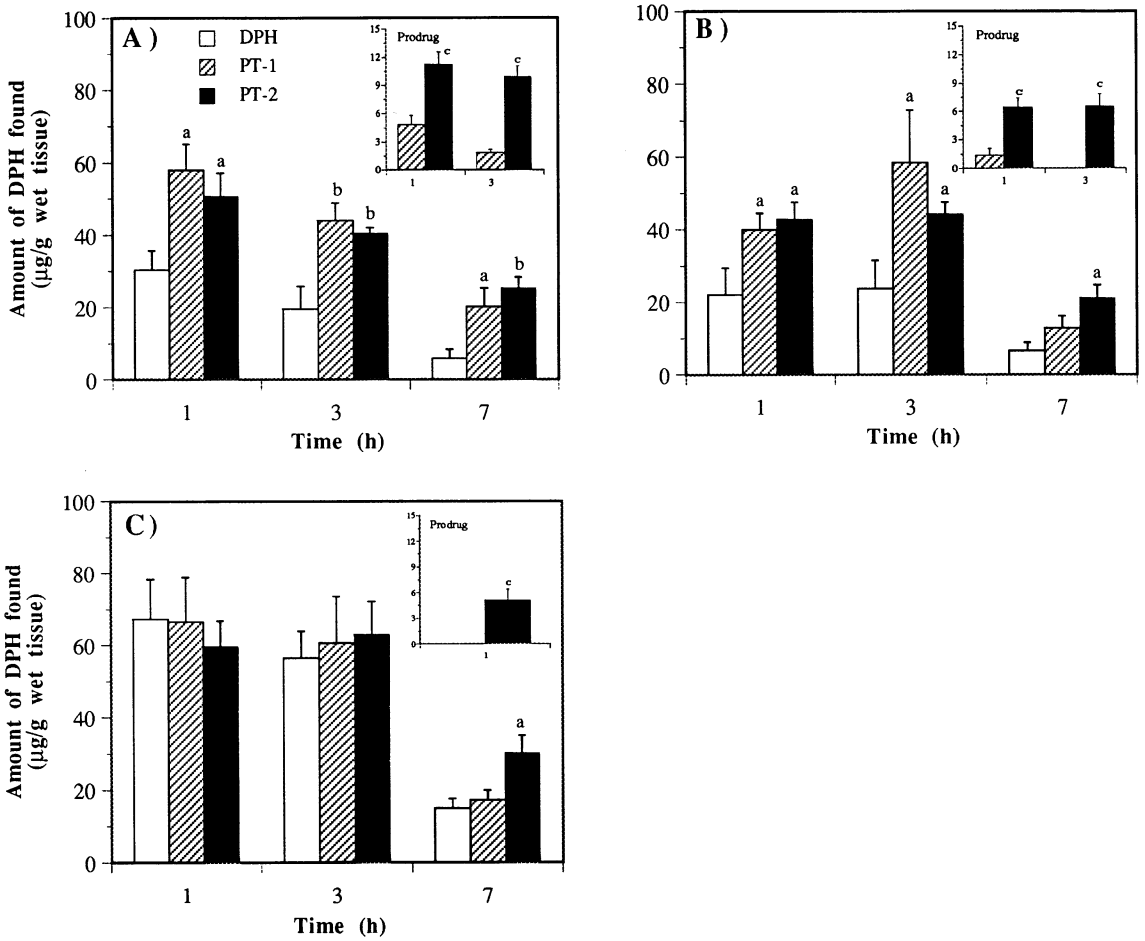

Fig. 5. Distribution of DPH and intact prodrug to (A) duodenum (B) jejunum and (C) ileum after oral dosing of DPH, PT-1 and PT-2. Dose: 50 mg/kg, DPH equivalent. Each value represents the mean ± S.D. ($n = 3$–4). The small insert shows the distribution of PT-1 and PT-2 themselves. [a] $p < 0.05$ and [b] $p < 0.01$ compared with DPH alone. [c] $p < 0.01$ compared with PT-1 in intact form.

ics and plasma concentrations. Therefore, it is clear that these prodrugs are pharmacologically effective.

In conclusion, the ester prodrugs were hydrolyzed rapidly in rat plasma, liver, intestinal mucosa and contents. The hydrolysis rates of PT-2 were relatively slower than those of PT-1 in the intestinal mucosa and fluids. After oral administration of the prodrugs, the plasma concentrations of DPH converted were much higher and partly sustained compared with those after dosing of DPH, giving high bioavailabilities. A single oral dose of PT-1 and PT-2 resulted in higher levels of DPH in the small intestine than that after DPH. Therefore, both PT-1 and PT-2 will be more useful prodrugs as an oral applicable form. In particular, PT-2 will be expected as a benign prodrug capable of preventing the excessive high plasma levels.

References

Arnold, K., Gerber, N., 1970. The rate of decline of diphenylhydantoin in human plasma. Clin. Pharmacol. Ther. 11, 121–134.

Arnold, K., Gerber, N., Levy, G., 1970. Absorption and dissolution studies on sodium diphenylhydantoin capsules. Can. J. Pharm. Sci. 5, 89–92.

Badir, K., Haj-Yehia, A., Vree, T.B., van der Kleijn, E., Bialer, M., 1991. Pharmacokinetics and anticonvulsant activity of three monoesteric prodrugs of valproic acid. Pharm. Res. 8 (6), 750–753.

Baldessarini, R.J., Kula, N.S., Walton, K.G., Borgman, R., 1977. Hydrolysis of diester prodrugs of apomorphine. J. Biochem. Pharmacol. 26, 1749–1756.

Bansal, P.C., Pitman, I.H., Tam, J.N.S., Mertes, M., Kaminski, J.J., 1981a. *N*-hydroxymethyl derivatives of nitrogen heterocycles as possible prodrugs I: *N*-hydroxymethylation of uracils. J. Pharm. Sci. 70, 850–854.

Bansal, P.C., Pitman, I.H., Higuchi, T., 1981b. *N*-hydroxymethyl derivatives of nitrogen heterocycles as possible prodrugs II: possible prodrugs of allopurinol, glutethimide and phenobarbital. J. Pharm. Sci. 70, 855–857.

Bialer, M., Friedman, M., Dubrovsky, J., Raz, I., Abramsky, O., 1985. Pharmacokinetic evaluation of novel sustained-release dosage forms of valproic acid in humans. Biopharm. Drug Dispos. 6, 401–411.

Borondy, P., Chang, T., Glazko, A., 1972. Inhibitor of DPH hydroxylation by 5-(*p*-hydroxyphenyl)-5-phenylhydantoin (*p*-HPPH). J. Fed. Proc. 31, 582.

Buchthal, F., 1972. M.A. Lennox-Buchthal in antiepileptic drugs. In: Woodbury, D.M., Penry, J.K., Schmidt, R.P. (Eds.), Raven, New York, pp. 193–209.

Colburn, W.A., Gibaldi, M., 1977. Plasma protein binding and metabolic clearance of DPH in the rat. J. Pharmacol. Exp. Ther. 203 (3), 500–506.

Gerber, N., Weller, W.L., Lynn, R., Rangno, R.E., Sweetman, B.J., Bush, M.T., 1971. Study of dose-dependent metabolism of 5,5- diphenylhydantoin in the rat using new methodology for isolation and quantitation of metabolites in vivo and in vitro. J. Pharmacol. Exp. Ther. 178, 567–579.

Gibaldi, M., Perrier, D., 1982. Pharmacokinetics, 2nd ed. Marcel Dekker, New York, pp. 84; idem, ibid, p. 409.

Hadad, S., Vree, T.B., van der Kleijn, E., Bialer, M., 1992. Pharmacokinetic analysis of ester prodrugs of valproic acid. J. Pharm. Sci. 81 (10), 1047–1050.

Lambert, D.M., Masereel, B., Gallez, B., Geurts, M., Scriba, G.K.E., 1996. Bioavailability and anticonvulsant activity of 2-cyanoguanidino DPH, a structural analogue of DPH. J. Pharm. Sci. 85 (10), 1077–1081.

Lowry, O.H., Rosebrough, N.J., Farr, A.L., Randall, R.J., 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193, 265–275.

Merritt, M.H., Putnam, J.T., 1938. Sodium diphenylhydantoinate in treatment of convulsive disorders. J. Am. Med. Assoc. 111, 1068–1073.

Nakamura, K., O'Hashi, K., Nakatsuji, K., Hiroka, T., Fujimoto, K., Ose, S., 1965. The anticonvulsant activity of 3-ethoxycarbonyl-5,5-diphenylhydantoin (P-6127) in animals. Arch. Int. Pharmacodyn. 156 (2), 261–270.

Nakamura, H., Nakatsuji, K., Kadokawa, T., Nakamura, K., 1970. Pharmacological studies of a new anti-inflammatory drug, 1-phenylsulfonyl-5,5-diphenylhydantoin (PC-796) in experimental animals. Arzneim-Forsch. 20, 1032–1046.

Ogiso, T., Iwaki, M., Tanino, T., Muraoka, O., Tanabe, G., 1993. Pharmacokinetic analysis of DPH and its derivatives in plasma and brain in rats. Biol. Pharm. Bull. 16 (10), 1025–1030.

Ogiso, T., Tanino, T., Iwaki, M., Muraoka, O., Tanabe, G., 1994. Physicochemical and hydrolytic characteristics of DPH derivatives. Biol. Pharm. Bull. 17 (10), 1425–1429.

Pop, E., Shek, E., Murakami, T., Bodor, N., 1986. Improved anticonvulsant activity of DPH by a redox brain delivery system I: synthesis and some properties of the diphydropyridine derivatives. J. Pharm. Sci. 78 (8), 609–616.

Scriba, G.K.E., 1994. DPH-lipid conjugates as potential prodrugs of DPH. Arch. Pharm. 327, 347–348.

Scriba, G.K.A., Lambert, D.M., Poupaert, J.H., 1995a. Anticonvulsant activity of DPH-lipid conjugates, a new class of DPH prodrugs. J. Pharm. Pharmacol. 47, 197–203.

Scriba, G.K.A., Lambert, D.M., Poupaert, J.H., 1995b. Bioavailability and anticonvulsant activity of a monoglyceride-derived prodrug of DPH after oral administration to rats. J. Pharm. Sci. 84, 300–302.

Scriba, G.K.A., Lambert, D.M., Poupaert, J.H., 1995c. Bioavailability of DPH following oral administration of DPH-lipid conjugations to rats. J. Pharm. Pharmacol. 47, 945–948.

Sekikawa, H., Fujiwara, J., Naganuma, T., Nakano, M., Arita, T., 1978. Dissolution behaviors and gastrointestinal absorption of DPH in DPH-polyvinylpyrrolidone coprecipitate. Chem. Pharm. Bull. 26, 3033–3039.

Stella, V.J., Charman, W.N.A., Naringrekar, V.H., 1985. Prodrugs: do they have advantages in clinical practice? Drugs 29, 455–473.

Suzuki, S., Miyazaki, Y., Sakurai, S., Yakou, S., Sugihara, M., 1990. Dissolution behavior of DPH solid dispersion. Yakuzaigaku 50, 273–278.

Sugiyama, T., Yamamoto, A., Kawabe, K., Uchiyama, T., Quan, Ying-Shu, Muranishi, S., 1997. Effect of various absorption enhancers on the intestinal absorption of water-soluble drugs by in vitro using chamber method: correlation with an in situ absorption experiment. Biol. Pharm. Bull. 20 (7), 812–814.

Upton, R.A., 1975. Simple and reliable method for serial sampling of blood from rats. J. Pharm. Sci. 64, 112–114.

Varia, S.A., Stella, V.J., 1984. DPH prodrugs VI: in vivo evaluation of a phosphate ester prodrug of phenytoin after parenteral administration to rats. J. Pharm. Sci. 73 (8), 1087–1090.

Varia, S.A., Schuller, S., Sloan, K.B., Stella, V.J., 1984. DPH prodrugs III: water-soluble prodrugs for oral and/or parenteral use. J. Pharm. Sci. 73 (8), 1068–1073.

Yakou, S., Yamazaki, S., Sonobe, T., Nagai, T., Sugihara, M., 1986. Dissolution and bioavailability of DPH in DPH-polyvinylpyrrolidone-sodium deoxycholate coprecipitate. Chem. Pharm. Bull. 34, 3408–3414.

Yamaoka, K., Tanigawara, T., Nakagawa, T., Uno, T., 1981. Pharmacokinetic analysis program (MULTI) for microcomputer. J. Pharmacobio-Dyn. 4, 879–885.