Influence of Vehicle on Gastrointestinal Absorption of Phenytoin in Rats

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(Received January 31, 1985)

The relationship between the bioavailability of various phenytoin (DPH) suspensions and the physicochemical properties of the vehicle as well as the physiological factors influencing the bioavailability were studied in rats. The vehicles used were aqueous solutions of methylcellulose (0.1, 0.5 and 1%), 1% polysorbate 80 aqueous solution, sesame oil and sesame oil emulsion. The gastric emptying time was determined from the amount of phenol red remaining in the stomach after oral administration.

As the gastric emptying time increased, the area under the blood concentration–time curve, the maximum blood concentration and the time required to reach the maximum blood concentration increased. The gastric emptying time became smaller as the fluidity of the vehicle increased. Clearly, the viscosity is an important factor affecting the bioavailability of DPH.

As the gastric emptying and the dissolution rate slowed down, the apparent absorption rate constant (k_a) became small. However, k_a obtained from an oily suspension was only one-third of the value obtained from an aqueous suspension. Thus, the mechanism by which DPH is absorbed from the digestive tract after administration as an oily suspension appears to be considerably different from that after administration as an aqueous suspension.

The value of k_a obtained from the *in vivo* absorption study was about half of that found *in situ*. These results suggest that k_a obtained from the *in vivo* absorption study includes the transit rate of suspension from the stomach to the intestinal tract.

Keywords——phenytoin; aqueous suspension; oily suspension; dissolution rate; phenytoin solubility; vehicle viscosity; bioavailability; rat

Introduction

The bioavailability of a slightly soluble drug is affected by its dissolution rate in the digestive tract.[1] This often poses problems as to efficacy and safety.[2] Phenytoin (DPH) is a weakly acidic and sparingly soluble drug. Many reports have shown that its bioavailability differs greatly among individuals[3] and effective blood concentrations are difficult to attain.[4,5] Thus, various attempts have been made to increase its solubility and improve its bioavailability.[6] Recent papers have reported that the bioavailability of DPH is significantly increased by oral administration of oily suspensions or emulsions instead of powder or an aqueous suspension.[7,8] This suggests that the physicochemical interaction between DPH and the vehicle as well as the physiological interaction between the vehicle and the gastrointestinal tract can affect the solubility and absorption of DPH. However, the reported studies did not include experiments capable of concretely explaining the differences in DPH bioavailability from different dosage forms.

The present study was conducted to find how the physicochemical properties of the vehicle affect the bioavailability of DPH.

Experimental

Materials——DPH, a commercially available product (Fujinaga Pharmaceutical Co.), was passed through a 200-mesh sieve to obtain a fine powder (average particle size: 4.1 μm). Methylcellulose (MC) and polysorbate 80 (PS-80) were purchased from Wako Pure Chemical Industries Ltd. and sesame oil (JP X) from Maruishi Pharmaceutical Co. Commercially available sodium pentobarbital (Abbott Laboratories, Nembutal® Sodium solution) were used. All other reagents used were of reagent grade.

Preparation of Suspensions——To prepare an aqueous suspension of DPH, DPH was suspended in an aqueous solution of MC (0.1, 0.5 and 1.0%) or 1% PS-80 aqueous solution to make a DPH suspension of 2.5%. To prepare a sesame oil suspension, DPH was suspended in the oil to make a 2.5% DPH suspension. To prepare an emulsion, 10 g sesame oil and 250 mg PS-80 were added to 625 mg of DPH, and the mixture was heated to 70 °C. Purified water heated to 70 °C was then added to the mixture to make a total volume of 25 ml and the resultant mixture was subjected to ultrasonic treatment (Nihon Seiki Works, type US-50) for 30 min. A part of the DPH still exists in suspension in the emulsion. All preparations were used in the absorption study after incubation for 24 h at 37 °C.

Measurement of Specific Gravity and Viscosity of the Vehicle——The specific gravity was measured in accordance with method 3 in JP X. The viscosity was measured with a Ubbelohde-type viscometer at 25 °C in accordance with JP X.

Measurement of Solubility——Various suspensions were prepared as described above. They were shaken at 37 °C for 24 h, then filtered through a 0.22-μm membrane filter. The DPH concentration in the filtrate was determined by gas liquid chromatography (GLC).[5]

Measurement of Dissolution Rate——The dissolution rate of DPH was measured in accordance with the paddle method in JP X. The dissolution test was performed using 900 ml of pH 5.0 acetate buffer (0.1 M) at 37 °C at a stirring rate of 25 rpm. After introducing 1 ml of suspension (containing 25 mg of DPH), 5 ml of sample solution was withdrawn periodically and filtered through a 0.22-μm membrane filter. In determining the dissolution rate of DPH from an emulsion, 3—4 ml of sample solution was put into Visking cellulose tubing, 21/32 inches in diameter, and ultracentrifuged at 3000 rpm for 1—2 h. The drug concentration in the clear filtrate (aqueous phase) was determined. The amount of DPH dissolved was measured by GLC.[5]

Animal Experiments——Male Wistar rats weighing 300 ± 20 g were used. They were randomly divided into three groups consisting of 3 or 4 rats each.

a) Oral Administration: Four rats were used. After being anesthetized with pentobarbital Na (Nembutal® 40 mg/kg, i.p.), the jugular vein was cannulated with silicon tubing (Dow Corning Co., 0.02 inch i.d., 0.037 inch o.d.) filled with heparinized saline (1000 U/ml).[9] The tubing was anchored securely and then brought subcutaneously to the back of the neck. The tubing was terminated with a needle plug. Rats were housed in individual cages and fasted overnight after operation. A suspension was orally administered through a metal tube at a dose of 1 ml/kg (equivalent to 25 mg DPH/kg). The animals were not allowed to take water for 3 h after drug administration.

b) Intraduodenal Administration: Three rats were fasted overnight prior to the experiments. The rats were anesthetized with pentobarbital Na (Nembutal® 40 mg/kg, i.p.), then the jugular vein was cannulated with silicon tubing, and the pylorus was exposed by an abdominal incision and ligated. A suspension was injected into the duodenum through a metal tube from a small opening made just below the ligature at a dose of 1 ml/kg. The small opening was ligated and the incision was discontinuously sutured. The body temperature was kept at 37 °C during the experiments with a heater.

c) Intravenous Administration: Three rats were used. The jugular vein was cannulated with silicon tubing as described in the case of oral administration. Rats were fasted overnight after operation. Rat was anesthetized lightly with ether and then DPH injection (25 mg/ml as DPH), prepared according to the method of Ashley and Levy,[10] was injected at a dose of 1 ml/kg into the jugular vein for 30 s. Just after the administration of DPH, a small amount of saline (about 0.3—0.5 ml) was flushed through to avoid contamination with injected drug solution remaining in the cannula.

After administration of DPH suspension or injection, 0.5 ml of blood was collected periodically via the silicon tubing inserted in the jugular vein. Just after the blood sampling, 0.03 ml of heparinized saline (100 U/ml) was flushed through to remove the blood remaining in the tubing. The blood sample was immediately centrifuged to obtain the plasma. The plasma samples were stored at −20 °C until analysis. The stored plasma samples were analyzed within a week.

Determination of DPH in Plasma——Plasma concentrations of DPH were determined by a modification of the method of Utsui et al.[11] A half ml of 0.1 N NaOH solution containing 1.0 μg of 5-(p-tolyl)-5-phenylhydantoin as an internal standard and 0.5 ml of 6 N HCl were mixed with 0.2 ml of the plasma sample. The mixture was extracted with 7.5 ml of CHCl$_3$–iso-BuOH (4 : 1) for 10 min. After centrifugation at 3000 rpm for 5 min, 7 ml of the organic layer was

transferred to a 20-ml glass-stoppered centrifuge tube containing 7.5 ml of 1 N NaOH. The mixture was shaken for 10 min and centrifuged at 3000 rpm for 5 min. Then 7 ml of the aqueous layer was placed in another centrifuge tube, to which 3 ml of 6 N HCl and 4.5 ml of $CHCl_3$–iso-BuOH (4 : 1) were added. After being shaken vigorously for 10 min, the mixture was centrifuged at 3000 rpm for 5 min. The aqueous layer was discarded, and 4 ml of the organic layer was evaporated to dryness in a 10-ml glass-stoppered centrifuge tube. The residue was dissolved in 30 μl of methanol solution containing 0.1 M phenyl trimethylammonium hydroxide and 3 μl of the solution was injected into a GLC column.

GLC was performed with a Shimadzu model GC-6 AM equipped with a hydrogen flame ionization detector. The column used was a 1 m × 3 mm i.d. glass column packed with 3% OV-17 on 80/100 mesh Chromosorb W AW DMCS. The temperatures of the column, the injection port and the detector were 225, 300 and 300 °C, respectively. The flow rate of carrier gas (N_2) was 40 ml/min. The retention times for internal standard and DPH were 8.7 and 6.2 min, respectively.

The linearity of the assay in the 0.2—6 μg/ml range was determined by spiking known amounts of DPH in rat plasma. There was a linear relationship between the peak area ratio and the plasma concentration of DPH ($r = 0.997$).

Measurement of the Apparent Absorption Rate Constant (k_a)——Percent absorbed of DPH was obtained by deconvolution from the plasma concentration-time data after intravenous and oral or intraduodenal administration using Yamaoka's program on a Sharp MZ-2200 personal computer.[12] The value of k_a was calculated from a semilogarithmic plot of percent unabsorbed of DPH against time.

Measurement of Gastric Emptying Time——The gastric emptying time was determined from the amount of phenol red remaining in the stomach in accordance with the method of Reynell and Spray.[13] To prepare the phenol red suspension, each vehicle was added to 62.5 mg of phenol red, which had been passed through a 200-mesh sieve, to make a total volume of 25 ml. After incubation for 24 h at 37 °C, this suspension was orally administered at a dose of 1 ml/kg to rats which had been fasted overnight. After 2 h, the animals were sacrified. After ligation of the pylorus and cardiac orifice, the stomach was removed, then cut into pieces with scissors. The pieces were homogenized with 15 ml of 0.1 N NaOH. The homogenate was transferred to a volumetric flask and made up to 25 ml by addition of 0.1 N NaOH. This homogenate was centrifuged for 10 min. One milliliter of the supernatant was diluted with 9 ml of 0.1 N NaOH. The concentration of phenol red in the solution was determined spectrophotometrically at 558 nm.

Data Analysis——The apparent elimination rate constant (β) was calculated from the slope of a semilogarithmic plot of DPH plasma concentration against time, from 7 to 9 h after administration. The total area under the blood concentration–time curve after administration ($AUC_{0-\infty}$) was calculated as the sum of the area obtained by the trapezoidal rule from $t = 0$ to $t = 9$ (AUC_{0-9}) and the area calculated as C_9/β from $t = 9$ to $t = \infty$ ($AUC_{9-\infty}$). The significance of differences in pharmacokinetic parameters of DPH was assessed by means of Student's t-test.

Results and Discussion

The specific gravity, viscosity and fluidity (the reciprocal of viscosity) of the vehicles used in this experiment and the solubility of DPH are summarized in Table I. While the differences in specific gravity were small, the differences in viscosity were large. An increase of the MC concentration from 0.1% to 1% had little influence on the DPH solubility.

Figure 1 shows the dissolution profiles of DPH from the suspensions. The dissolution rates of the aqueous suspensions were clearly faster than those of the sesame oil suspension and the emulsion. A lag time was found only with the sesame oil suspension. This is probably

TABLE I. Physicochemical Characteristics of Various Vehicles and Solubility of Phenytoin

Vehicle	Specific gravity	Viscosity (cP)	Fluidity (cP^{-1})	Phenytoin solubility (μg/ml)
0.1% MC	1.001	1.459	0.685	35.0
0.5% MC	1.003	8.435	0.119	35.0
1.0% MC	1.004	22.329	0.045	35.0
1.0% PS-80	1.002	1.048	0.954	134.7
Sesame oil	0.917	63.173	0.016	332.7
Emulsion	0.967	4.105	0.244	342.4

All measurements were performed at 25 °C. Each value represents the mean of three experiments.
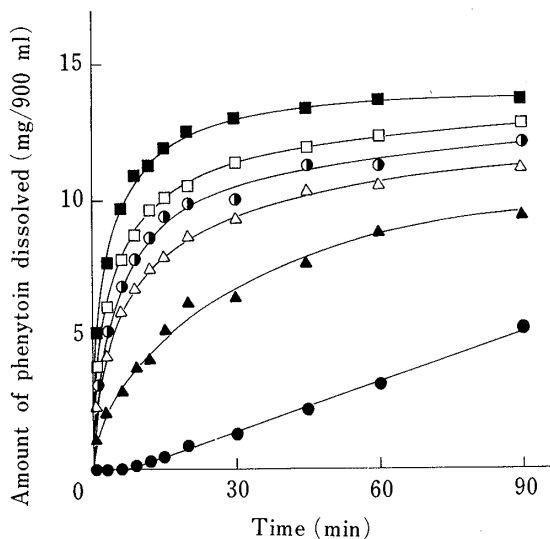
Fig. 1. Dissolution Profiles of Phenytoin from Suspensions

□, 0.1% MC; ◐, 0.5% MC; △, 1% MC; ■, 1% PS-80; ●, sesame oil; ▲, emulsion.
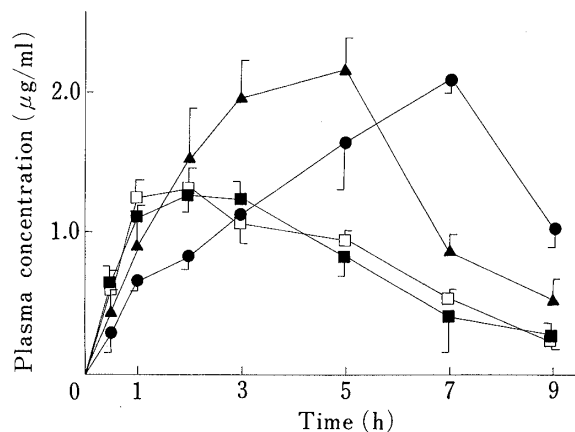Each point represents the mean of three determinations.

Fig. 2. Representative Plasma Concentrations of Phenytoin after Oral Administration of 25 mg/kg Phenytoin as Suspensions

□, 0.1% MC; ■, 1% PS-80; ●, sesame oil; ▲, emulsion.
Each point represents the mean ± S.E. of four rats.

TABLE II. Pharmacokinetic Parameters after Oral Administration of Phenytoin as Suspensions at a Dose of 25 mg/kg

Parameter	0.1% MC A	1.0% PS-80 B	Sesame oil C	Emulsion D	Statistical analysis[a]
$AUC_{0-\infty}$ (h μg/ml)	8.56 ± 0.90	8.64 ± 2.32	15.62 ± 3.62	15.45 ± 3.25	A, B < C, D
C_{max} (μg/ml)	1.44 ± 0.18	1.53 ± 0.21	2.30 ± 0.30	2.53 ± 0.29	A, B < C, D
T_{max} (h)	1.50 ± 0.50	1.75 ± 0.83	6.50 ± 0.87	3.25 ± 1.09	A, B < C; A < D < C
k_a (h^{-1})	0.87 ± 0.10	0.94 ± 0.17	0.20 ± 0.03	0.26 ± 0.09	C, D < A, B

a) Significance level set at $p < 0.05$. Each value represents the mean ± S.D. of four rats.

because the transfer rate of DPH from the oil phase to the water phase is relatively slow.

Figure 2 illustrates the blood concentration–time curves after oral administration of various DPH suspensions. Table II shows the pharmacokinetic parameters obtained from these curves. The values of AUC and the maximum blood concentration (C_{max}) after oral administration of the sesame oil suspension and emulsion were increased significantly (approx. 1.5 times) as compared with those obtained after the 0.1% MC suspension (used as the control). The differences in AUC and C_{max} were not significant between the sesame oil suspension and the emulsion, in agreement with some results reported previously,[8] but not with those of experiments using corn oil reported by Chakrabarti and Belpaire.[7] The latter authors found a significant difference among the dosage forms in the following order of AUC: emulsion > corn oil suspension > aqueous suspension. These conflicting results probably arose because they did not use a constant dose of vehicle. It has been reported that when the dose of vehicle is increased with a fixed amount of drug, for instance, in an emulsion of griseofulvin[14] or sesame oil suspension of DPH,[8] the bioavailability of the drug is increased.

The solubility of DPH in 1% PS-80 was about four times higher than that in 0.1% MC (Table II). However, the AUC and C_{max} after administration of 1% PS-80 showed no significant difference from those in the case of 0.1% MC. This suggests that the bioavailability of an aqueous suspension is not much affected by the solubility of DPH in the vehicle.[7]

TABLE III.   Effect of Vehicle Viscosity on Bioavailability Parameters
after Oral Administration of 25 mg/kg Phenytoin

Parameter	0.1% MC A	0.5% MC B	1.0% MC C	Statistical analysis[a]
$AUC_{0-\infty}$ (h μg/ml)	8.56 ± 0.90	16.63 ± 5.35	12.61 ± 0.73	A < B, C
C_{max} (μg/ml)	1.44 ± 0.18	2.11 ± 0.53	2.06 ± 0.13	A < B, C
T_{max} (h)	1.50 ± 0.50	3.00 ± 2.00	3.25 ± 2.28	NS
k_a (h^{-1})	0.87 ± 0.10	0.70 ± 0.31	0.77 ± 0.35	NS

a) Significance level set at $p < 0.05$. Each value represents the mean $\pm$ S.D. of four rats. NS, not significant.
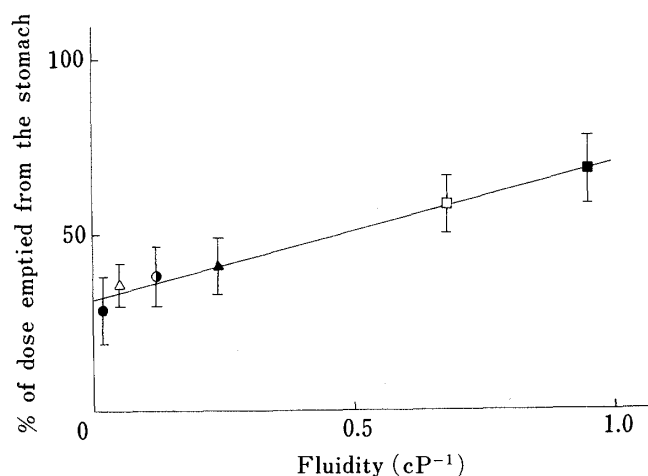

Fig. 3.   Relationship between Fluidity of Various Vehicles and Extent of Gastric Emptying in 2 h after Oral Administration

□, 0.1% MC; ◑, 0.5% MC; △, 1% MC; ■, 1% PS-80; ●, sesame oil; ▲, emulsion. Each point represents the mean $\pm$ S.E. of four rats. $Y = 38.9532X + 31.6661$ ($r = 0.9893$, $p < 0.01$).

Similar phenomena have been reported for griseofulvin, a slightly soluble drug.[15] Our present study demonstrated a decreasing tendency in T_{max} and an increasing tendency in k_a when DPH was orally administered as an aqueous suspension rather than an oily suspension.

T_{max} tended to be delayed as the viscosity of the vehicle (Table I) became higher. While the viscosities of the vehicles for the 0.5 and 1% MC suspensions are very different, the solubility of DPH in each vehicle was the same (ca. 35 μg/ml). Table III shows the pharmacokinetic parameters of DPH after oral administration of MC suspensions of different viscosities. AUC and C_{max} obtained with 0.5% MC were significantly greater than those with 0.1% MC, but the magnitude of the AUC and C_{max} did not increase further with higher MC concentration. On the other hand, the mean value of T_{max} was delayed with increase in the viscosity, but no significant difference was obtained because of the large variations among individuals.

The gastric emptying time is a very important factor affecting the absorption of drugs. Many parameters, including the volume,[16] specific gravity,[17] viscosity[18] and osmotic pressure[19] of the gastric content, are known to alter the gastric emptying time. We therefore examined the relationship between the fluidity of various vehicles used in this experiment and the gastric emptying rate. As shown in Fig. 3, the rate was linearly related to the fluidity of the vehicle. No correlation was found between the specific gravity and the gastric emptying rate. Thus, differences in the gastric emptying time of the vehicles used in the present study are considered to be mainly due to the differences of viscosity.

Figure 4 illustrates the relationship between the gastric emptying rate and each parameter of bioavailability. The AUC and C_{max} increased as the gastric emptying was delayed. A good correlation was found (AUC: $r = 0.8664$, $p < 0.05$; C_{max}: $r = 0.8284$, $p < 0.05$). The increase of bioavailability of DPH in the present experiment was probably due to a delay in the gastric
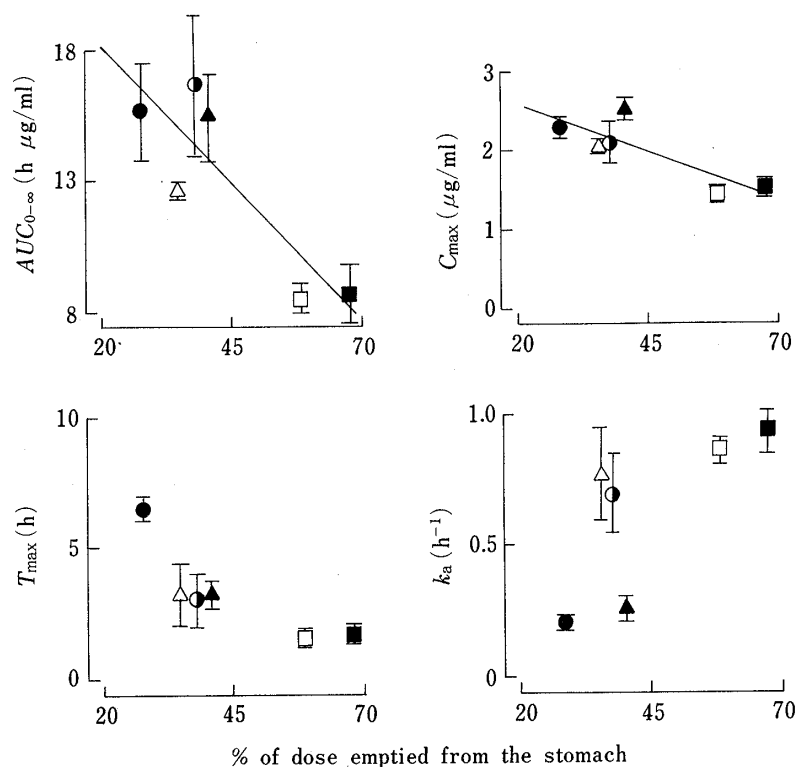
Fig. 4. Relationship between Bioavailability Parameters and Extent of Gastric Emptying in 2 h after Oral Administration

□, 0.1% MC; ◑, 0.5% MC; △, 1% MC; ■, 1% PS-80; ●, sesame oil; ▲, emulsion.
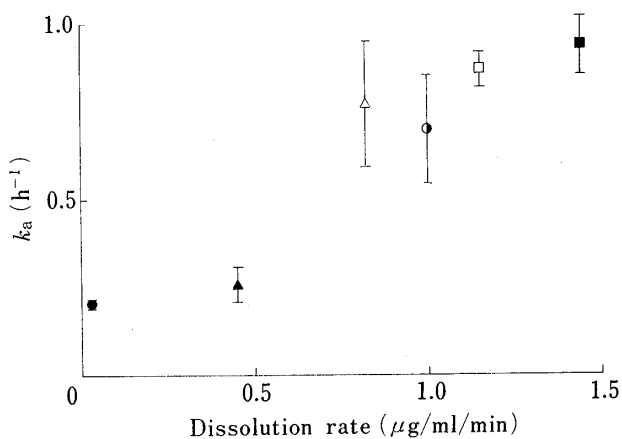Each point represents the mean ± S.E. of four rats.

Fig. 5. Relationship between Dissolution Rate and Absorption Rate after Oral Administration of 25 mg/kg Phenytoin as Suspensions

□, 0.1% MC; ◑, 0.5% MC; △, 1% MC; ■, 1% PS-80; ●, sesame oil; ▲, emulsion.
Each point represents the mean ± S.E. of four rats.

emptying time, prolonging the retention time of the drug at the absorption site, and resulting in improved absorption. This phenomenon may occur with many drugs, including griseoful-vin[20] and digoxin.[21] Accordingly, the gastric emptying time is clearly an important factor affecting the bioavailability of DPH. On the other hand, there was a tendency for T_{max} to be prolonged and for k_a to become small when the gastric emptying was delayed. However, the values of k_a of the oil suspension and emulsion are only about 1/3 of that of the aqueous suspension, despite the similar gastric emptying times. Therefore, the small k_a value obtained from an oily suspension suggests that many factors other than the gastric emptying, such as the oil–water partition coefficient of a drug,[22] the lipid metabolism in the epithelial cells of the small intestine[23] and the adsorption of oil droplets at the surface of the absorbing membrane,[24] are involved in the absorption of DPH from an oily suspension.
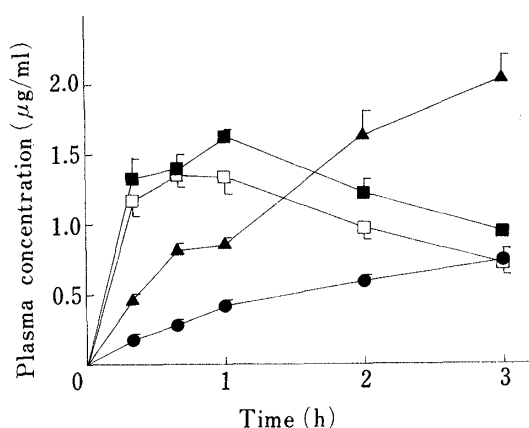
Fig. 6. Representative Plasma Concentrations of Phenytoin after Intraduodenal Administration of 25 mg/kg Phenytoin as Suspensions

□, 0.1% MC; ■, 1% PS-80; ●, sesame oil; ▲, emulsion.

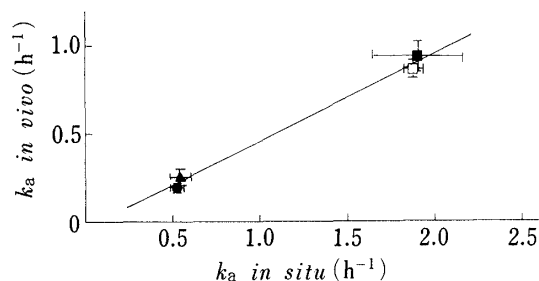Each point represents the mean ± S.E. of three rats.

Fig. 7. Correlation of Apparent *in Situ* and *in Vivo* Absorption Rate Constants

□, 0.1% MC; ■, 1% PS-80; ●, sesame oil; ▲, emulsion.

Each point represents the mean ± S.E. of at least three rats.

As DPH is sparingly soluble and its absorption is known to be restricted by the dissolution rate, the relationship between the rate and k_a was examined. The mean dissolution rates were calculated from the dissolution curve up to 15 min (Fig. 1). As shown in Fig. 5, the k_a values tended to increase with increase in the dissolution rate.

The k_a value also includes the transit rate of suspensions from the stomach to the intestinal tract where DPH will be absorbed. We therefore examined the influence of gastric emptying time on k_a obtained from the *in vivo* absorption study. In order to exclude the participation of gastric emptying, the pylorus was ligated and the suspension was directly injected into the duodenum. Figure 6 illustrates the blood concentration–time curves after intraduodenal administration of various DPH suspensions, and Fig. 7 shows a good correlation between k_a found from the *in situ* absorption study and k_a obtained *in vivo* ($y = 0.493 x - 0.034$: $r = 0.9970$, $p < 0.001$). The value of k_a obtained *in vivo* was about half of that found *in situ*. Accordingly, the large difference between k_a values *in vivo* and *in situ* is attributable to delayed gastric emptying of the vehicle.

We concluded that the viscosity of the vehicle is an important factor in the bioavailability of DPH after oral administration in suspension form. The mechanism by which DPH is absorbed from the digestive tract after administration as an oily suspension appears to be considerably different from that after administration as an aqueous suspension.

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