[Chem. Pharm. Bull. 33(11)4989—4994(1985)]

Comparison of the Effects of Sesame Oil and Oleic Acid as Suspension Vehicles on Gastrointestinal Absorption of Phenytoin in Rats

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(Received January 31, 1985)

The bioavailabilities of phenytoin (DPH) from oil suspensions (sesame oil and oleic acid) were studied in rats in relation to the physicochemical properties and gastric emptying time of the vehicles.

The maximum blood concentration (C_{max}) of DPH after oral administration of sesame oil suspension was about twice that from the oleic acid suspension. DPH in oleic acid suspension was absorbed more slowly; this was consistent with the dissolution rate data. Oleic acid delayed gastric emptying to a significant degree in comparison with sesame oil. The area under the blood concentration–time curve (AUC) for the oleic acid suspension was significantly higher than that for the sesame oil suspension. On the other hand, AUC and blood concentrations of DPH after intraduodenal administration of oleic acid suspension were approximately twice those obtained with the sesame oil suspension up to one hour after administration.

It was concluded that the lower C_{max} of DPH, the higher AUC and the longer T_{max} (the time required to reach the maximum blood concentration) after oral administration of oleic acid suspension as compared with those in the case of sesame oil suspension could be attributed to the delayed gastric emptying.

Keywords——phenytoin; sesame oil; oleic acid; oily suspension; dissolution rate; phenytoin solubility; vehicle viscosity; bioavailability; rat; gastric emptying time

Introduction

Since phenytoin (DPH) is sparingly soluble in water, its low bioavailability, depending on the dissolution rate in the gastrointestinal tract, often causes problems as to its efficacy and safety.[1] Recently, attempts have been made to improve the bioavailability by suspending DPH in oil. Oral administration of an oily suspension of DPH resulted in significant increases of bioavailability in comparison with that of powder form[2] or aqueous suspension.[3] However, the bioavailability of an oil suspension is influenced by the viscosity of the vehicle.[4] In a comparison of the bioavailability of DPH from sesame oil with that from oleic acid, a principal fatty acid constituent of sesame oil, it was found that the maximum blood concentration (C_{max}) of DPH after administration as an oleic acid suspension was about a half of that in the case of sesame oil suspension, while the time required to reach the maximum blood concentration (T_{max}) tended to be prolonged with the suspension in oleic acid.[2]

In the present study, the bioavailability of DPH was studied in relation to the physicochemical properties of sesame oil and oleic acid and their influence on gastric emptying time.

Experimental

Materials——A fine powder of DPH with a mean particle size of 4.1 μm was prepared by passing commercial DPH (Fujinaga Pharmaceutical Co., Ltd.) through a 200-mesh sieve. Sesame oil (JP X grade) manufactured by Maruishi Pharmaceutical Co., oleic acid from Wako Pure Chemical Industries Ltd., and commercially available sodium pentobarbital (Abbott Laboratories, Nembutal® Sodium solution) were used. All other compounds used in this study were of reagent grade.

Preparation of Suspensions——DPH (625 mg) was suspended in a sufficient amount of sesame oil or oleic acid to make 25 ml of suspension. The suspensions were used after incubation for 24 h at 37 °C.

Determination of Specific Gravity and Viscosity——Specific gravity was determined in accordance with method 3 in JP X and viscosity was determined at 25 °C with a Ubbelohde-type viscometer in accordance with JP X.

Determination of Solubility——Each suspension prepared was continuously shaken at 37 °C for 24 h and then filtered through a membrane filter of 0.22-μm pore diameter. The concentration of DPH in the filtrate was determined by gas liquid chromatography (GLC).[5]

Determination of Dissolution Rate——The dissolution rate of DPH was determined 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 stirring rates of 25 and 100 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. The amount of DPH dissolved was measured by GLC.[5]

Determination of Oil–Water Partition Coefficient——To 5 ml of an oil solution of DPH at a concentration of 50 μg/ml, 5 ml of 0.1 M acetate buffer (pH 5.0) or 5 ml of distilled water was added. The mixture was shaken at 37 °C until distribution equilibrium was achieved. After centrifugation, the concentration of DPH in the aqueous phase was determined by GLC[5] and the partition coefficient was calculated.

Animal Study——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. The rats were anesthetized with pentobarbital Na (Nembutal® 40 mg/kg, *i.p.*), and 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).[6] 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 of 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.*), the jugular vein was cannulated with silicon tubing, and then 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 ligation 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. Each rat was anesthetized lightly with ether and then DPH injection (25 mg/ml as DPH), prepared according to the method of Ashley and Levy,[7] was injected at a dose of 1 ml/kg into the jugular vein for 30 s. Just after 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* 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 rinse out 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——Determination of DPH in plasma was carried out as previously described.[4] The plasma concentration of DPH was determined by GLC after solvent extraction, and formation of trimethylsilyl derivatives. 5-(*p*-Tolyl)-5-phenylhydantoin was used as an internal standard in the assay of DPH.

Measurement of Gastric Emptying Time——The gastric emptying time was determined from an amount of phenol red remaining in the stomach in accordance with the method of Reynell and Spray.[8] 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 6 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 homogenates were 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.

Mean Absorption Time (*MAT*)——The application of statistical moment analysis to pharmacokinetics was described by Riegelman *et al.*[9] and Yamaoka *et al.*[10] This method is model-independent. *MAT* was defined as follows, $MAT = MRT_{p.o.} - MRT_{i.v.}$, where $MRT_{p.o.}$ is the mean residence time (*MRT*) after oral administration, and $MRT_{i.v.}$ is the *MRT* after intravenous administration. *MRT* was calculated from the plasma concentration–time data using Yamaoka's program on a Sharp MZ-2200 personal computer.[11]

Apparent Absorption Rate Constant (k_a)——Percent absorbed of DPH was obtained by deconvolution from plasma concentration–time data after intravenous and 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.

Data Analysis——The apparent elimination rate constant (β) was calculated from the slope of a semilogarithmic plot of DPH plasma concentration against time, from 18 to 24 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 $t=24$ (AUC_{0-24}) and the area calculated as C_{24}/β from $t=24$ to $t=\infty$ ($AUC_{24-\infty}$). The significance of differences in pharmacokinetic parameters of DPH was assessed by means of Student's *t*-test.

Results and Discussion

The physicochemical characteristics of oils and the solubility of DPH are summarized in Table I.

Figure 1 shows the dissolution profiles of DPH from the oily suspensions. The dissolution rate obtained from the sesame oil suspension was greater than that from the oleic acid suspension. The dissolution rates of the drug from the oily suspensions became smaller with an increase of the stirring rate; this phenomenon may be due to a decrease in the interface between oil and water, because larger droplets were found around the paddle shaft at a high stirring rate (100 rpm).

At 37 °C, the partition coefficient of DPH was 11.48 between sesame oil and pH 5.0 buffer and 48.37 between oleic acid and the buffer. The values between sesame oil or oleic acid

TABLE I. Physicochemical Characteristics of the Oils and Solubility of Phenytoin

Oil	Specific gravity	Viscosity (cP)	Fluidity (cP^{-1})	Phenytoin solubility (μg/ml)
Sesame oil	0.917	63.173	0.016	332.7
Oleic acid	0.890	31.079	0.032	745.2

All measurements were performed at 25 °C. Each value represents the mean of three experiments.

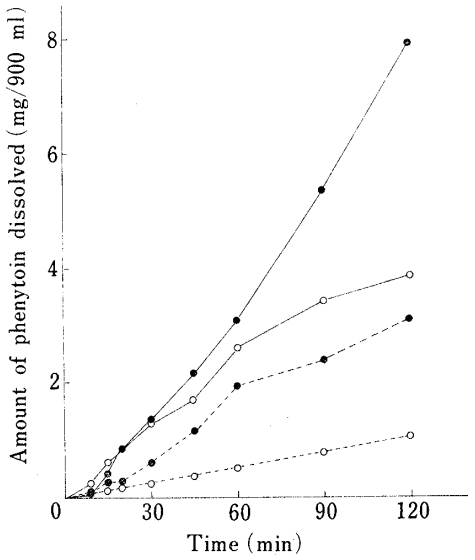

Fig. 1. Dissolution Profiles of Phenytoin from Sesame Oil (●) and Oleic Acid Suspensions (○)

Stirring rate: ——, 25 rpm; – – – –, 100 rpm.
Each point represents the mean of three determinations.

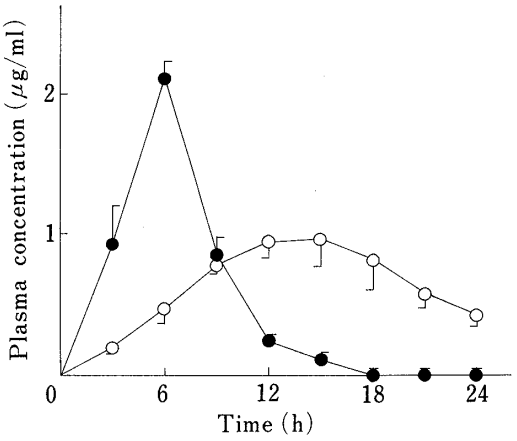

Fig. 2. Representative Plasma Concentrations of Phenytoin after Oral Administration of 25 mg/kg Phenytoin as Oily Suspensions

●, sesame oil; ○, oleic acid.
Each point represents the mean ± S.E. of four rats.

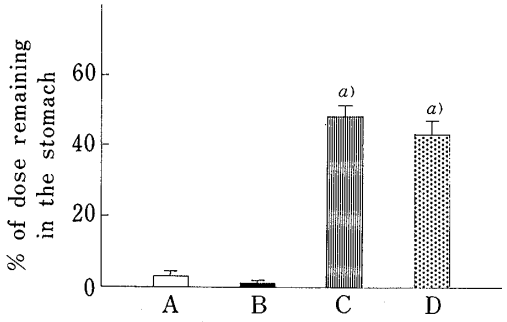

Fig. 3. Gastric Emptying of Phenol Red in 6 h after Oral Administration in Various Oils

A, sesame oil; B, triolein; C, oleic acid; D, linoleic acid.
a) $p < 0.001$ *vs.* sesame oil.
Each column represents the mean ± S.E. of three rats.

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

Parameter	Oil		Student's *t*-test
	Sesame oil	Oleic acid	
$AUC_{0-\infty}$ (h μg/ml)	12.69 ± 1.16	18.90 ± 3.79	$p < 0.05$
C_{max} (μg/ml)	2.11 ± 0.24	1.08 ± 0.27	$p < 0.01$
T_{max} (h)	6.00 ± 0.00	14.25 ± 1.30	$p < 0.001$
MAT (h)	3.67 ± 0.61	10.79 ± 1.20	$p < 0.001$

Each value represents the mean ± S.D. of four rats.

and distilled water were 11.27 and 42.23, respectively (mean of three experiments). The large difference of partition coefficient of DPH between sesame oil and oleic acid is considered to be reflected in the delayed dissolution of DPH from oleic acid suspension.

The blood concentration–time curves after oral administration of DPH in the suspensions are shown in Fig. 2, and the parameters of bioavailability in Table II. The C_{max} of DPH from sesame oil suspension was about twice that of DPH from oleic acid suspension. However, the *AUC* after administration of oleic acid suspension was significantly higher than that in the case of sesame oil suspension. Oleic acid prolonged T_{max} significantly, approximately twice as much as with sesame oil, and this is consistent with the *MAT* value. The absorption rate of DPH (estimated from T_{max} and *MAT*) tended to increase with increase in the dissolution rate of the drug (Fig. 1). In the previous paper,[4] we also found a correlation between the absorption rate of DPH and the dissolution rate of the drug from an aqueous suspension, sesame oil suspension and sesame oil emulsion.

As DPH is absorbed in the small intestinal tract, its absorption rate may be influenced by the gastric emptying of the vehicle. To study the behavior of an oily suspension in the gastrointestinal tract, gastric emptying rates of sesame oil and oleic acid after oral administration were compared in rats. Figure 3 shows the proportion of phenol red remaining in the stomach 6 h after oral administration of sesame oil or oleic acid suspension. The remaining percentage of the dose for the oleic acid suspension was about 15 times higher than

TABLE III. Pharmacokinetic Parameters after Intraduodenal Administration of Phenytoin as Oily Suspensions at a Dose of 25 mg/kg

Parameter	Time (min)	Oil		Student's *t*-test
		Sesame oil	Oleic acid	
Plasma concentration (μg/ml)	20	0.18±0.03	0.39±0.04	$p<0.01$
	40	0.28±0.01	0.56±0.08	$p<0.01$
	60	0.42±0.02	0.61±0.12	NS
AUC_{0-1} (h μg/ml)		0.22±0.01	0.42±0.05	$p<0.01$
k_a (h^{-1})		1.41±0.03	1.56±0.11	NS

Each value represents the mean±S.D. of three rats. NS, not significant.

the value of 3% found with sesame oil. Linoleic acid also delayed gastric emptying, while the effect of triolein was similar to that of sesame oil (Fig. 3). Stewart *et al.*[13] reported that oleic acid considerably depressed the digestive contractile activity of the intestinal tract but that such depression was not found with triolein. Miller *et al.*[14] also suggested that the depression occurs at the pyloric area. Factors affecting gastric emptying include volume,[15] viscosity[16] and osmotic pressure[17] of the gastric contents and physiological factors, such as posture and disease state.[18] The significantly delayed absorption of DPH after oral administration of oleic acid suspension should be attributable to the delayed gastric emptying caused by the acid. Bioavailability of DPH from oleic acid suspension was larger than that from sesame oil suspension (Table II). The increase of bioavailability of DPH in the oleic acid suspension may be due to a delay in the gastric emptying, prolonging the retention time of the drug at the absorption site, and resulting in improved absorption.

In order to examine the direct influence of the oil on the intestinal absorption of DPH, the pylorus was ligated and the suspension was directly injected into the duodenum (Table III). The *AUC* of DPH obtained from oleic acid suspension up to one hour after administration was about twice that from sesame oil suspension. Oleic acid suspension tended to give higher DPH blood concentrations at each sampling time after administration and larger k_a values than sesame oil suspension. The *in situ* experiment demonstrated better absorption of DPH from oleic acid suspension. This offers further support for the conclusion that the delayed absorption of DPH from oleic acid suspension after oral administration is attributable to delayed gastric emptying. Furthermore, oleic acid itself seems to be more absorbable than sesame oil, since chylification of the blood was marked after the administration of oleic acid suspension into the duodenum. Muranushi *et al.*[19] reported that a lipid having a polar head could accelerate drug absorption. In the case of oleic acid, the presence of a polar head and the higher solubility of DPH in the oil seem to contribute largely to the increased intestinal absorption of DPH under the *in situ* experimental conditions.

We concluded that the delayed gastric emptying caused by oleic acid leads to a lower C_{max}, a higher *AUC* and a longer T_{max} than those in the case of sesame oil after oral administration.

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