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Influence of Bile on the Gastrointestinal Absorption of Phenytoin in Rats

DENJI SHINKUMA,*,[a] TSUNEO HAMAGUCHI,[a] YOU YAMANAKA,[a] NOBUYASU MIZUNO,[b] and NOBORU YATA[c]

Department of Pharmacy, The Hospital of Hyogo College of Medicine,[a] 1–1, Mukogawa-cho, Nishinomiya-shi, Hyogo 663, Japan, Faculty of Pharmaceutical Sciences, Mukogawa Women's University,[b] 4–16, Edagawa-cho, Nishinomiya-shi, Hyogo 663, Japan and Institute of Pharmaceutical Sciences, Hiroshima University School of Medicine,[c] 1–2–3, Kasumi, Minami-ku, Hiroshima 737, Japan

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The influence of endogeneous bile on the gastrointestinal absorption of phenytoin (DPH) was studied in rats after oral administration of DPH as an aqueous or oil suspension. A bile salt such as sodium taurocholate considerably enhanced DPH dissolution and solubility. The bioavailability of DPH from aqueous suspension was significantly lower in bile duct-ligated rats than in control or sham-operated animals. When DPH was administered to bile duct-ligated rats as a sesame oil suspension, its maximum blood concentration was significantly lower than that in sham-operated rats. These results suggest that endogenous bile plays an important role in the dissolution of DPH and the emulsification or dispersion of oil in the gastrointestinal tract.

After laparotomy, DPH administered with vehicles of high viscosity, such as sesame oil, showed significantly delayed absorption but increased bioavailability.

Keywords——phenytoin; gastrointestinal absorption; endogenous bile effect; dissolution profile; vehicle viscosity; laparotomy; rat

Introduction

Fluctuations in gastrointestinal absorption of phenytoin (DPH), which has long been used as an anticonvulsant, among preparations have been attributed to its very slight solubility in water.[1] Recent papers have reported that its bioavailability can be significantly improved by suspending it in oil or by formulating it into an emulsion.[2,3]

It was reported that bile salts such as sodium deoxycholate and sodium cholate markedly increase the solubility and dissolution rate of poorly soluble drugs in water.[4–6] Bates *et al.*[4,5,7] suggested that one of the steps in gastrointestinal absorption of relatively insoluble drugs is the preliminary solubilization of the drugs by bile salts. The important role of these physiological surfactants in the digestion and absorption of dietary fats in the small intestine is well known.[8] However, little work has been done to study the influence of endogenous bile on gastrointestinal absorption of DPH.

The present study was conducted to determine the influence of bile salts on the dissolution profile of DPH. The influence of endogenous bile on the gastrointestinal absorption of DPH suspended in aqueous solution or sesame oil was also investigated in rats.

Experimental

Materials——A fine powder of DPH with a mean particle size of 4.1 μm was prepared by passing commercially available DPH (JP X grade) from Fujinaga Pharmaceutical Co., Ltd. through a 200-mesh sieve. Methylcellulose (MC) was purchased from Wako Pure Chemical Industries, Ltd. and sesame oil (JP X grade) from Maruishi Pharmaceutical Co., Ltd. All other compounds used in this study were of reagent grade.

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Preparation of Suspensions——An aqueous suspension of DPH was prepared by suspending fine powder of DPH in 0.1% MC aqueous solution to make a 2.5% DPH suspension. A sesame oil suspension was prepared by suspending DPH in the oil to make a 2.5% DPH suspension. These suspensions were used after incubation for 24 h at 37 °C.

Procedure for Dissolution Studies——The dissolution profile of DPH was determined by the paddle method in JP X, using 1 ml of 0.1% MC suspension (containing 25 mg of DPH). The dissolution media were 0.1 M acetate buffer of pH 5.0 and the 2nd fluid (pH 6.8) of the JP X disintegration test fluids with or without 10 mM sodium taurocholate. The dissolution test was performed using 500 ml of the medium at 37 °C at a stirring rate of 100 rpm. The amount of DPH dissolved was measured by gas liquid chromatography (GLC) as in the previous study.[9]

Animal Study——Male Wistar rats weighing 300±20 g were randomly divided into three groups: control, bile duct-ligated and sham-operated animals.

a) Control Rats: As in the previous study,[9] the animals were fasted overnight prior to the experiments. A DPH suspension was administered orally with a metallic catheter at a dose of 1 ml/kg (corresponding to 25 mg DPH/kg) and the animals were not allowed to take water for 3 h thereafter. Blood samples of 0.5 ml were collected from a jugular vein at appropriate time intervals after administration. Plasma samples obtained by centrifugation were stored at −20 °C until analysis. The concentration of DPH in plasma was determined by GLC.[9]

b) Bile Duct-Ligated Rats: A rat was anesthetized with pentobarbital Na and then its bile duct was exposed by abdominal incision. The proximal bile duct was ligated. The incision was discontinously sutured. The animals were fasted overnight after the operation and then treated in the same way as the controls.

c) Sham-Operated Rats: Sham-operated rats were fasted overnight after the operation and then treated in the same way as the controls.

Mean Absorption Time (*MAT*)——*MAT* was determined by the statistical moment analysis method described by Riegelman and Collier.[10] Intravenous administration of DPH was done by the method of Ashley and Levy.[11]

Data Analysis——The maximum blood concentration (C_{max}) and the time required to reach the maximum blood concentration (T_{max}) were obtained from the individual blood concentration–time curves. The area under the blood concentration–time curve (*AUC*) was calculated by means of the trapezoidal rule. The statistical significance of pharmacokinetic parameters for DPH was analyzed by using Student's *t*-test.

Results and Discussion

Figure 1 shows the influence of sodium taurocholate on the dissolution profile of DPH from 0.1% MC suspension in acetate buffer of pH 5.0. A markedly accelerated dissolution and increased solubility were observed in the presence of 10 mM sodium taurocholate. Similar results have been reported for indomethacin[6] and griseofulvin[4] which are also slightly soluble drugs. Micellar solubilizing and wetting effects of the bile salt seem to contribute to the observed acceleration of dissolution of DPH.[12] An orally administered drug is absorbed after

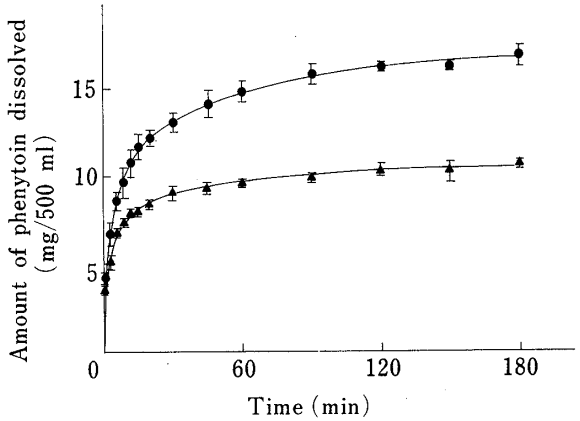

Fig. 1. Effect of Bile Salt on the Dissolution Profile of Phenytoin from 0.1% MC Suspension in pH 5.0 Acetate Buffer

With (●) or without (▲) sodium taurocholate. Each point represents the mean±S.D. of three determinations.

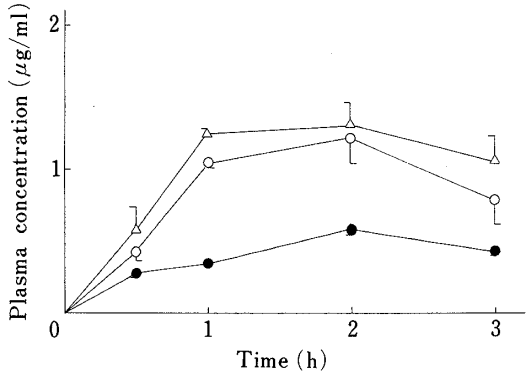

Fig. 2. Effect of Bile Duct Ligation on the Gastrointestinal Absorption after Oral Administration of 25 mg/kg Phenytoin as a 0.1% MC Suspension in Rats

Each point represents the mean±S.E. of four rats. △, control rats; ○, sham-operated rats; ●, bile duct-ligated rats.

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TABLE I. Pharmacokinetic Parameters and Plasma Concentrations of Phenytoin after Oral Administration of 25 mg/kg Phenytoin as a 0.1% MC Suspension

Parameter	Control rats A	Bile duct-ligated rats B	Sham-operated rats C	Statistical analysis[a)]
AUC_{0-3} (h μg/ml)	3.08±0.53	1.21±0.14	2.61±0.48	A, C>B
C_{max} (μg/ml)	1.44±0.18	0.58±0.07	1.31±0.22	A, C>B
T_{max} (h)	1.50±0.50	2.00±0.00	1.75±0.43	NS

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

dissolution in the digestive tract, and the concentration of sodium taurocholate used in this study (10 mM) was comparable to the concentration of bile salts being excreted in the human small intestine: 2 to 10 mM.[13)] Therefore, an increase of dissolution of DPH in the small intestine due to endogenous bile and resultant increased absorption of DPH *in vivo* may be expected. The dissolution profile of DPH in the 2nd fluid of JP X with or without sodium taurocholate was approximately similar to that obtained with pH 5.0 acetate buffer.

To examine the influence of endogenous bile on the gastrointestinal absorption of DPH in suspension, a DPH suspension was given to control, sham-operated and bile duct-ligated rats and blood concentrations of the drug were determined.

Figure 2 and Table I show the influence of endogenous bile on the gastrointestinal absorption of DPH which was orally administered to rats in 0.1% MC suspension. *AUC* up to 3 h after administration (AUC_{0-3}) and C_{max} in bile duct-ligated rats were significantly decreased to about a half of those in control and sham-operated rats. Mean values of T_{max} in the bile duct-ligated rats were larger than those in control or sham-operated rats, though there were no significant differences among them. These findings suggest that endogenous bile plays a role in accelerating the dissolution of DPH in the gastrointestinal tract. Miyazaki *et al.*[14)] and Moriyama *et al.*[15)] report a similar effect of endogenous bile on the absorption of indomethacin and phenylbutazone.

Bile salts are known to play a very important role in the digestion and absorption of oil.[8)] Figure 3 and Table II show the influence of endogenous bile on the gastrointestinal absorption of DPH that was orally administered as a sesame oil suspension. C_{max} was lower in the bile duct-ligated rats than in the control or the sham-operated rats. The mean value of *AUC* up to 24 h after administration (AUC_{0-24}) of DPH in the bile duct-ligated rats was lower than that in the sham-operated rats, though no significant differences were observed among them. The bile duct-ligated rats showed a longer T_{max}, which was confirmed by determining the *MAT* (Table II). *MAT* includes the transit rates of DPH in suspensions from the stomach to the intestinal tract where DPH is absorbed and also the dissolution rate of DPH from the suspensions in the gastrointestinal tract. These results agree with the findings in the 0.1% MC suspension (Table I). These results also suggest that endogenous bile participated in the absorption of DPH from aqueous and oily suspensions. The lower C_{max}, the longer T_{max} and the lower *AUC* in the bile duct-ligated rats compared with the sham-operated rats may be explained by a suppressed emulsification of oil due to the absence of bile in the intestinal tract in the bile duct-ligated rats. Our previous studies using normal rats or dogs[3,9)] demonstrated an increasing tendency in *AUC* and C_{max}, and a decreasing tendency in T_{max} when DPH was orally administered as a sesame oil emulsion rather than as an oil suspension. Endogenous bile may therefore promote the emulsification and dispersion of sesame oil, resulting in accelerated DPH absorption.

In the absorption study of DPH from an oily suspension, an increase in *AUC* was

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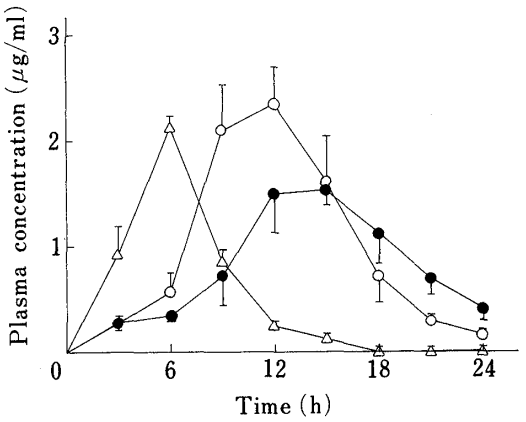

Fig. 3. Effect of Bile Duct Ligation on the Gastrointestinal Absorption after Oral Administration of 25 mg/kg Phenytoin as a Sesame Oil Suspension in Rats

Each point represents the mean ± S.E. of four rats. △, control rats; ○, sham-operated rats; ●, bile duct-ligated rats.

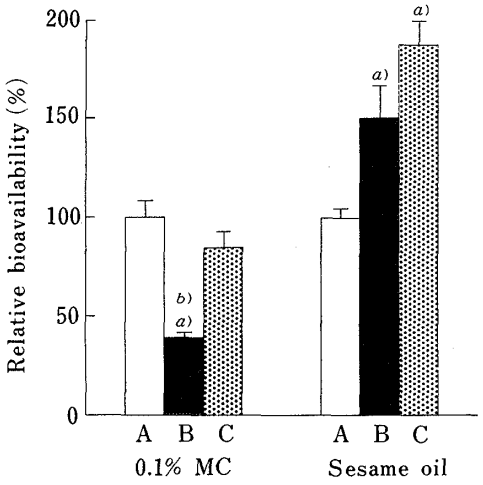

Fig. 4. Relative Bioavailability of Phenytoin after Oral Administration of 25 mg/kg Phenytoin as Various Suspensions

Each column represents the mean ± S.E. of four rats.

A, control rats; B, bile duct-ligated rats; C, sham-operated rats.

Significance level set at $p<0.05$: *a*), significantly different from control rats; *b*) significantly different from sham-operated rats.

TABLE II. Pharmacokinetic Parameters and Plasma Concentrations of Phenytoin after Oral Administration of 25 mg/kg Phenytoin as a Sesame Oil Suspension

Parameter	Control rats A	Bile duct-ligated rats B	Sham-operated rats C	Statistical analysis[a)]
AUC_{0-24} (h μg/ml)	12.69 ± 1.16	19.10 ± 4.23	23.87 ± 3.05	A<B, C
C_{max} (μg/ml)	2.11 ± 0.24	1.80 ± 0.59	2.81 ± 0.36	A, B<C
T_{max} (h)	6.00 ± 0.00	15.00 ± 2.12	12.00 ± 2.12	A<B, C
MAT (h)	4.44 ± 1.57	12.01 ± 0.51	9.26 ± 1.63	A<C<B

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

observed in the sham-operated rats compared to that of the control group. This phenomenon was not observed after the administration of an aqueous suspension. Therefore, the influence of laparotomy should be considered. The influence of laparotomy on the gastrointestinal absorption of DPH from suspensions is shown in Fig. 4 in terms of relative bioavailability (ratio of *AUC* to the *AUC* of control rats). In the case of suspension in 0.1% MC, *AUC* and T_{max} were not significantly affected by laparotomy (Table I). However, in laparotomized rats given the sesame oil suspension, *AUC* approximately doubled and T_{max} increased considerably (Table II). Previously we reported[9] that a delay in the gastric emptying time when DPH was administered with a viscous vehicle resulted in an increase of *AUC*, C_{max} and T_{max}. We also suggested that a delay in the gastric emptying time will play a role in detaining the drug at the absorption site. The viscosity of the suspension vehicle used in the present study at 25 °C was 1.5 cP for 0.1% MC and 63.2 cP for sesame oil. It was also reported that a viscous vehicle will delay the intraintestinal transfer of drugs.[16] Ware *et al.*[17] demonstrated that the intestinal tract could not completely recover its normal physiological function (such as intestinal motility) in 3 d after an operation. Therefore, increased relative bioavailability of

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DPH in sham-operated rats after administration as a viscous sesame oil suspension can be explained in terms of the high viscosity of sesame oil and a delay in gastric emptying time and/or the suppression of intestinal motility due to laparotomy. An increase of bioavailability of DPH from the oil suspension in the bile duct-ligated rats as compared to the control suggests that the effect of laparotomy is greater than that of endogenous bile.

We concluded that endogenous bile probably facilitates the dissolution of DPH and the emulsification and dispersion of sesame oil, consequently facilitating the gastrointestinal absorption of DPH in aqueous or sesame oil suspension. Further, laparotomy prior to the administration of DPH formulated with highly viscous vehicles (such as sesame oil) results in a significant delay of drug absorption and an increase in bioavailability.

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