Arch. Pharm. Res. Vol.20, No.5, pp. 480-485, 1997

Gastrointestinal Absorption of Phenytoin from an Oil-in-water Microemulsion

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(Received July 1, 1997)

The absorption profile of phenytoin Na emulsion were examined compared to that of phenytoin suspension after oral administration in the rat. The corn oil-in-water emulsion, particle size of 184±57.8 nm, was prepared using a microfludizer, and phenytoin Na added by shaft homogenizer. The phenytoin emulsion or suspension, 100 mg/kg, were intubated intragastrically using oral dosing needle and blood samples were withdrawn via an indwelling cannula from the conscious rat. Plasma concentrations of phenytoin were measured with HPLC using phenacetin as an internal standard. The plasma concentration versus time data were fitted to a one compartment open model and the pharmacokinetic parameters were calculated using the computer program, Boomer. The phenytoin plasma concentrations from the emulsion at each observed time were about 1.5-2 times higher than those from the suspension, significantly at time of 5, 6 and 7 hr after administration. The absorption (k_a) and elimination rate constant (k_e) were not altered significantly, however the AUC increased from 65.6 to 106.7 μg · hr/ml after phenytoin suspension or emulsion oral administration, respectively. From an equilibrium dialysis study, the diffusion rate constant (k_{if}) was considerably higher from the phenytoin Na emulsion (0.0439 hr^{-1}) than phenytoin suspension (0.0014 hr^{-1}).

Key words : phenytoin, micro-emulsion, suspension, pharmacokinetic, bioavailability, equilibrium dialysis, Boomer, catheterized rat

INTRODUCTION

Phenytoin, a poorly water soluble drug shows problematic bioavailability and requires the examination of new improved formulations (Savio *et al.*, 1994; Macheras *et al.*, 1991; Savio *et al.*, 1991). In addition, phenytoin is also one of the most common drugs subjected to therapeutic drug monitoring (TDM) because of it's narrow therapeutic range (10~20 μg/ml) and many of the dose related adverse effects on the central nervous system and cardiovascular system (Macheras *et al.*, 1991; Young *et al.*, 1996; Physicians GenR. Mosby. 1996). There have been many attempts to improve phenytoin bioavailability from conventional solid dosage forms including a change in crystal size, use of prodrugs, and the use of drug-milk freeze dried formulations (Macheras *et al.*, 1991; Savio *et al.*, 1991; Alvarez *et al.*, 1989; Khalil *et al.*, 1990). As the gastric emptying time and small intestine transit time can be varied also by the droplet size of the emulsion, the digestion and absorption of emulsions may differ with droplet sizes (Borel *et al.*, 1994). Some water insoluble, liphophilic drugs including griseofulvin, amphotericin B and mitomycin C were formulated successfully in an oil-in-water emulsion (Carrigan *et al.*, 1973; Kirsh *et al.*, 1988; Kararli *et al.*, 1992). This paper describes the formulation of a phenytoin Na microemulsion (droplet size of about 200 nm in diameter) with various oils such as corn oil, sunflower oil and peanut oil. Drug release via membrane dialysis and GI absorption of the prepared phenytoin Na microemulsion in the rat were compared to phenytoin suspension.

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MATERIALS AND METHODS

Preparation of emulsions

Each of fine emulsion 500 ml were prepared from corn oil, sunflower oil, peanut oil or canula oil. One hundred milliliters of each oil was added to 25 ml of Tween 80 and 375 ml of distilled water. After hand shaking to produce a coarse mix, the mixture were emulsified using a microfludizer (Microfluidics Co. Model M110F) for 10 to 30 min (Carrigan *et al.*, 1973; Kararli *et al.*, 1992). After particle size determination over 12 weeks, corn oil emulsion emulsified for 30

min, was selected for the final absorption studies. Phenytoin or phenytoin Na (Sigma) emulsion for the micro-dialysis study were prepared by incorporating 250 mg drug into 10 ml emulsion with or without 1 ml ethanol. The emulsion and drug mixture were homogenized again by shaft homogenizer (Kinematica) for 1 min. Phenytoin Na emulsion (25 mg/ml) for the drug absorption study were prepared by mixing 1 g of powdered phenytoin Na with 38 ml emulsion and 2 ml ethanol (2.5%) by shaft homogenizer for 2 min (Savio *et al.*, 1994; Corvari *et al.*, 1991).

Particle size determination of emulsion

The distribution of the emulsion particle size was determined using a particle size analyser (NICOMP model 720 submicron particle sizer, Pacific Scientific). Measurements were obtained using diluted emulsion at a count rate of 350~450 kHz for 10 min. Mean diameter and the distribution of the size of the emulsion were calculated by the volume weighted Gaussian analysis and volume weighted NICOM distribution analysis with the particle sizer software (Borel *et al.*, 1994).

Equilibrium dialysis

The equilibrium dialysis of phenytoin Na emulsion and phenytoin suspension were evaluated using a Spectra/Por (MWCO 12000~14000, 23 mm) molecularporous dialysis membrane. Each dialysis procedure was performed for 24 hr in continuously stirred beakers. 2 ml (phenytoin, 50 mg) of phenytoin Na emulsion and phenytoin suspension was added to the dialysis bag with the external phase of 500 ml 0.05 M phosphate buffer (pH 7.2). The phenytoin concentrations in the solution were measured immediately by HPLC. The diffusion rate constant (k_{IE}) was calculated from the slope of semi-log A.R.D. (amount remaining to be dialyzied) plot (Gibaldi *et al.*, 1982).

HPLC assay

HPLC was performed with a Shimadzu LC-6A pump system with SPD 6A UV spectrophotometric detector, SCL 6B system controller, SIL-6B/9A automatic sample injector and C-R3A Chromatopac integrator. The chromatographic system included an octadecyl C-18 reverse phase column (5 μm, 4.6×150 mm, J.T. Baker) and 20 μl of the sample was injected into the HPLC. The mobile phase consisted of pH 5.5 phosphate buffer solution and acetonitrile (68/32). The phosphate buffer used in the mobile phase was prepared by adding 0.4 ml of 1.0 M monobasic potassium phosphate to 1 L of distilled water and adjusting pH to 5.5 with 1.0 M dibasic potassium phosphate. The detector wavelength was set at 254 nm and flow rate was maintained at 1 ml/min (Borel *et al.*, 1994; Carrigan *et al.*, 1973; Kirsh *et al.*, 1988; Vila *et al.*, 1985).

Extraction of plasma samples

To 100 μl of plasma in a polypropylene microcentrifuge tube (1.5 ml), 15 μl of 2 μg/ml phenacetin was added as an internal standard. 0.5 ml of ethyl acetate 96% and isopropyl alcohol 4% was added and the tubes were placed on a reciprocating shaker (Nutator Model 1105, Clay Adams) for 10 min. After centrifugation, the organic layer was transferred into a polypropylene tube and evaporated to dryness under clean air in an N-EVAP evaporator (Organomation Mode 112). The residue was then re-dissolved with 100 μl mobile phase. After centrifugation, about 70 μl was transferred to the autosampler vial (Macheras *et al.*, 1991; Khalil *et al.*, 1990; Stout *et al.*, 1984).

Animal and Catheterization

Male Sprague Dawley (SD) rats weighing 300±15 g were catheterized under anesthesia (Ketamine 120 mg/kg, I.P.) into a carotid artery one day prior to oral administration. The left carotid artery was surgically exposed and ligated with cotton thread at one end and clamped with an artery clip at the other. A half incision was made into the carotid artery and one end of cannula, flexible polyethylene tube, i.d. 1.0 mm, was inserted deep into the artery. The other end of cannula was connected to a syringe with a 25 G needle containing heparin 50 I.U/ml in saline to confirm the function of cannula, and a wire plug was pulled under the skin with a long needle to emerge at the back of the neck and the site was sutured (Khalil *et al.*, 1990).

Dose and administration

The prepared phenytoin Na emulsion or phenytoin suspension (UDL Laboratories Inc.), dose of 100 mg/kg (30 mg/300 g rat) were intragastrically intubated via stainless steel dosing needles. i.e. 1.2 ml of phenytoin, 25 mg/ml emulsion or suspension were administered to 300±15 g rats. The molecular weight difference of 8.7% (phenytoin 252.3, phenytoin Na 274.3) were not considered for the adjustment of the dose. About 0.3 ml of blood samples was withdrawn via the indwelling cannula into heparinized 0.5 ml polypropylene microcentrifuge tube, before drug administration and thereafter at 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 5, 6, 7, 8, 10, 12, 16 and 24hr. The blood samples were immediately centrifuged, and from the resulting plasma, 100 μl samples were stored at -20°C until HPLC assay.

Pharmacokinetic and statistical evaluation

The plasma concentration versus time data in the rat after oral administration were fitted to a one com-

partment open model using the computer program, Boomer, elimination rate constant (k_{el}), absorption rate constant (k_a) and volume of distribution (Vd/F) were calculated by Boomer analysis (Bourne *et al.*, 1989). The time of maximum concentration (T_{max}) and the maximum concentration (C_{max}) were calculated by standard equations (DiPiro *et al.*, 1988). The area under the curve (AUC) was calculated by the linear trapezoidal rule using Boomer to the last blood concentration (Cp^{last}) and extrapolated to infinity by addition of the term (Cp^{last}/k_{el}), where Cp^{last} and kel are the estimated by Boomer regression analysis. The terminal disposition half-life was calculated as $t_{1/2}=(\ln 2)/k_{el}$ (Gibaldi *et al.*, 1982; Shargel *et al.*, 1993).

The statistical difference between plasma concentrations at each observed time point was analyzed using an unpaired Students t-test at the 95% confidence level.

RESULTS

HPLC assay

The chromatopaphic procedure separated phenacetin (I.S.) and phenytoin with retention times of 4.4 and 7.6 min, respectively. The correlation coefficient of the calibration graph for the microdialysis study was greater than 0.999 in the range of 1 to 100 μg/ml and more than 0.995 for plasma samples in the range of 0.5 μg/ml to 10 μg/ml. The recovery of the drug from plasma samples was greater than 95% in most instances. Within-day correlation coefficients of variation at 5 μg/ml were less than 5%. The detection limit of the method was 100 ng/ml for plasma sample.

Distribution of emulsion-droplet size

The particle size distribution stayed essentially constant with all the emulsions of corn oil, sunflower oil and canola oil for a 12 week of period with and without refrigeration (Fig. 1). In the case of peanut oil, the emulsion showed separation of the oil phase and resulted in oil floating on the surface of emulsion after 12 weeks refrigeration at -4°C. The average particle size of the corn oil emulsion was 227.4±63.6 nm after 10 min microfludizer emulsification, and further reduced after 15 min (187.4±62.8 nm) and 30 min (184 ±57.8 nm) of emulsification. From these results, a corn oil emulsion after 30 min of emulsification was selected for the oral absorption study. This corn oil emulsion maintained particle size of 184±57.8 nm during the study period.

Equilibrium dialysis study

Both the phenytoin Na emulsion and the phenytoin suspension dialyzed slowly to produce external concentrations of 1.7 and 1.9 μg/ml, respectively in 24 hr

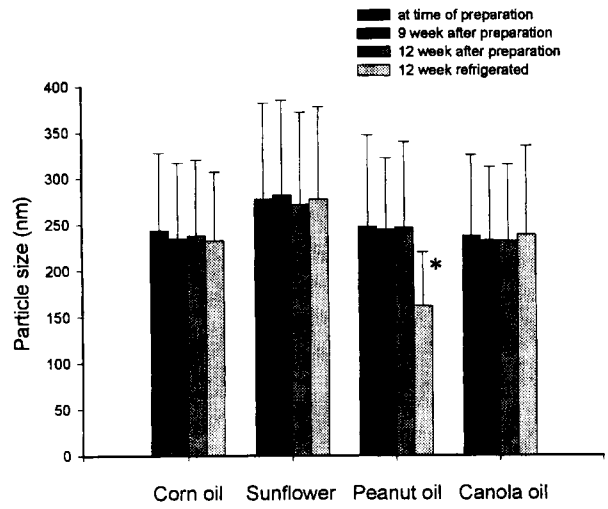

Fig. 1. Droplet size (mean±S.D. in diameter) of various oil-in-water emulsion in time course. The droplet size was determined at the time of preparation, 9 and 12 weeks at room temperature, and 12 weeks after refrigeration of the emulsion. *Peanut oil emulsion showed separation of oil phase afrer 12 weeks refrigeration.

time. Thus 1.9 and 1.7%, of the total phenytoin (100 mg/2 ml) diffused from the internal phase in 24 hr. The phenytoin Na emulsion prepared with and without 10 % ethanol showed higher diffusion rates with 41.2 and 33.2% of total phenytoin diffused, respectively, in 24 hr (Fig. 2). The diffusion rate constant from the internal phase to external phase (k_{IE}) was 0.00141 hr^{-1} for the phenytoin suspension and 0.0114 hr^{-1} for the phenytoin Na emulsion. The k_{IE} of the phenytoin Na emulsion appeared to be 0.0439 without ethanol and 0.0660 with 10% ethanol.

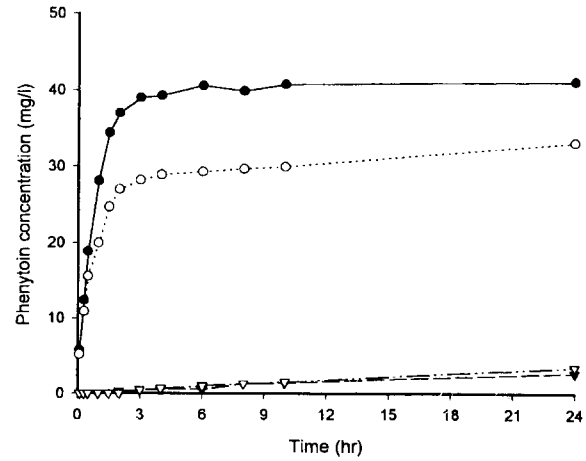

Fig. 2. Membrane equilibrium dialysis of phenytoin suspension (▽—▽), phenytoin emulsion (▼—▼), phenytoin Na emulsion (○—○), and phenytoin Na emulsion with ethanol 10% (●—●), respectively. Phenytoin concentrations in external phase were measured by HPLC. Mean of duplicated experiments.

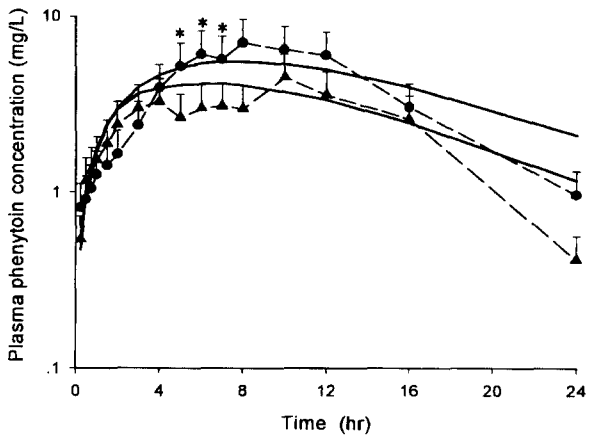

Fig. 3. Mean (±S.E.M.) plasma concentration versus time profile after administration 10 mg/kg of phenytoin suspention (▲—▲) or phenytoin Na emulsion (●—●) in the rat (n= 6-8). The lines express the predicted results after fitting the plasma concentration data to oral one compartment open model using computer program, Boomer. *: P<0.05.

Oral absorption study

Fig. 3 presents plasma phenytoin concentration versus time plots obtained following the oral administration of a single 100 mg/kg dose phenytoin Na emulsion or phenytoin suspention to each rat. The analysis of the plasma concentration versus time data was performed assuming linear kinetics by fitting the data with an oral one compartment open model with Boomer. The pharmacokinetic parameters estimated by Boomer are summerized in Table I. Examination of this figure reveals that no difference existed in the time to reach the peak plasma drug concentration following oral administration of phenytoin Na emulsion compared to phenytoin suspension (i.e. 7.70 hr versus 7.35 hr). The maximum plasma concentration (C_{max}) appeared to increase from 3.60 to 6.19 μg/ml followed by phenytoin suspension and phenytoin Na emulsion administration, respectively. And the magnitude of the plasma concentrations of phenytoin after the emulsion at each observed times were approximately 1~2 times higher than from the suspension. An interesting finding was that the absorption rate constant (k_a) and elimination rate constant (k_{el}) were not altered by emulsion, while Vd/F decreased from 3.01 L to 1.78 L. This suggests a 69% increase in the fraction absorbed (F) was observed after phenytoin Na emulsion administration, if we assume there was no change in volume of distribution. The AUC increased from 65.6 to 106.7 μg · hr/ml after phenytoin suspension and phenytoin Na administration, respectively. Students t-test indicated significant differences (P<0.05) between emulsion and suspension for the plasma concentrations at 5, 6 and 7 hr after oral administration.

Table I. Pharmacokinetic parameters of phenytoin following oral administratioin of 100 mg/kg of phenytoin suspension and phenytoin emulsion in the rat

Parameter	Suspension	Emulsion
Vd/F (L)	3.01	1.78
ka (hr^{-1})	0.134	0.129
kel (hr^{-1})	0.138	0.129
Terminal $t_{1/2}$ (hr)	5.01	5.37
T_{max} (hr)	7.35	7.70
C_{max} (μg/ml)	3.60	6.19
AUC (μg · hr/ml)	65.5	106.7

DISCUSSION

The aim of the present study was to investigate the increased bioavailability of phenytoin Na micro emulsion with mean droplet size of less than 200 nm. The prepared phenytoin Na emulsion was compared to a phenytoin suspension because of the absence of change in the extent of bioavailability in the case of phenytoin Na which suggested that solubility was not the only factor in phenytoin oral absorption (Macheras *et al.*, 1991).

Changes in the metabolic and distribution phase are often considered the principle features of phenytoin kinetics (Savio *et al.*, 1994; Perucca *et al.*, 1982). Savio *et al.*, (1994) presented no alteration of phenytoin absorption when the pharmacokinetics of phenytoin emulsion sign altered. Data from our single dose study indicate that phenytoin absorption rate and elimination rate were not much altered after phenytoin Na emulsion administration but the AUC of phenytoin Na emulsion increased 63% from the suspension. A significant increase (P<0.05) in plasma concentrations at time 5, 6 and 7 hr after administration were also observed. There were small differences in plasma concentrations until 3 hr after the phenytoin emulsion and suspension administration. These results may be explained in the two ways. One is the delayed processing of fine emulsion inside the intestine (Macheras *et al.*, 1991; Borel *et al.*, 1994; Carrigan *et al.*, 1973). Because of the limited absorption rate of phenytoin, delayed processing of fine emulsion in the small intestine can extend the extent of phenytoin absorption without altering the absorption rate. The other reason could be the effect of fat on phenytoin metabolism. Fat in the emulsion might reduce the metabolism of phenytoin and reduce overall elimination of phenytoin (Carrigan *et al.*, 1973; Splinter *et al.*, 1990; Bates *et al.*, 1977; Chakrabarti *et al.*, 1978). Although Michaelis-Menten enzyme kinetics are usually applicable for phenytoin pharmacokinetics (Mosby 1996; Wieland *et al.*, 1995), it is debatable for a single dose administration, especially when the plasma concentrations are low (Macheras *et al.*, 1991). By fitting an open one compartment model to the data, good correlation coeff-

cients were obtained for both of emulsion and suspension administration. Therefore, data fitting was performed assuming linear kinetics (Kirsh *et al.*, 1988; Neuvonen *et al.*, 1979).

The equilibrium dialysis showed remarkable differences between phenytoin and Phenytoin Na preparations. The diffusion rate also increased with the addition of 2.5% ethanol to the phenytoin Na emulsion. These results reveal that the solubility of drug into an aqueous phase mainly affected diffusion rate. Phenytoin Na dissolved well in ethanol and some in water but not in oil phase. No difference in diffusion rate was observed between phenytoin emulsion and phenytoin suspension. As the solubility of phenytoin is not the major factor for phenytoin bioavailability (Macheras *et al.*, 1991), the equilibrium dialysis may not be a good experimental model to predict the phenytoin absorption *in vivo*.

In conclusion, the results of this study indicate that the phenytoin Na micro emulsion was formulated in the form of a corn oil-in-water emulsion by microfludizer to less than 200 nm in mean diameter. The diffusion rate of phenytoin Na emulsion increased markably compared to phenytoin suspension. The bioavailability of phenytoin Na emulsion was enhanced 63% upon its administration to the rat.

ACKNOWLEDGEMENTS

OCAST (Oklahoma Center for the Advancement of Science and Technology) for finantial support. Mr. Jae Heung Lee and Bo Sin Kim for their help in experiments.

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