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Particle Size Dependency of Dissolution Rate and Human Bioavailability of Phenytoin in Powders and Phenytoin–Polyethylene Glycol Solid Dispersions

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Commercially available phenytoin crystals were fractionated by passing them through 44—350 μm sieves, then blended with diluents to prepare phenytoin–diluent physical mixture or made into solid dispersions with polyethylene glycol 4000 (PEG 4000). Dissolution studies of phenytoin in artificial gastric juice suggested that less than 1 to 10 ratio of phenytoin to PEG 4000 was required to disperse amorphous phenytoin completely in the carrier. A comparative dissolution study of various sizes of phenytoin crystals revealed the existence of a critical particle size between 74—149 μm, but phenytoin in solid dispersions showed far faster dissolution and higher solubility than any of the particle fractions. A three-way cross-over study was carried out for phenytoin crystals of particle size 44—53 μm, phenytoin–diluent physical mixture and the phenytoin solid dispersion in five healthy human volunteers. The solid dispersion gave the highest bioavailability of phenytoin, followed by the physical mixture. Thus, phenytoin solid dispersion in PEG 4000 appears to have the clinical advantages of quick release and excellent bioavailability. It also appears that there is a critical particle size for dissolution of phenytoin from powders *in vitro*. The phenytoin solid dispersion gave a larger bioavailability than crystals below the critical size *in vivo*.

Keywords——phenytoin; phenytoin solid dispersion; dissolution; human bioavailability; critical particle size

It was reported that water-soluble materials of large molecular size and of high viscosity represent potential carriers of scarcely water-soluble drugs in the form of solid dispersions,[1] although the exact physicochemical characteristics of solid dispersions have not been fully studied. Chiou and Riegelman used polyethylene glycol polymers as a matrix for a poorly water-soluble drug to increase the rate of dissolution, and accordingly, the *in vivo* absorption.[2] Phenytoin is scarcely soluble in water and the rate of phenytoin absorption is controlled by the dissolution rate from its dosage form in the gastrointestinal tract. Moreover, it has been reported that phenytoin absorption is variable and incomplete after oral administration, and the drug also has a very narrow therapeutic range even after controlled administration.[3,4]

Rate of dissolution is influenced by particle size,[5] characteristics of excipients,[6] manufacturing procedures,[7] and dosage form.[8] The authors have previously reported that the manufacturing procedure of phenytoin affects the dissolution behavior and human bioavailability of phenytoin to a great extent.[7]

The purpose of the present investigation was to evaluate the dissolution characteristics and human bioavailability of phenytoin solid dispersion prepared by fusion with polyethylene glycol 4000 (PEG 4000) and to investigate the possible existence of a critical particle size of phenytoin.

Experimental

Materials——Phenytoin (PHT) (JP X) was obtained from Fujinaga Pharmaceuticals Co., Ltd. (Hydantol, lot ZJ 301). 5-(*p*-Methylphenyl)-5-phenylhydantoin was obtained from Aldrich Chemical Co., Milwaukee, Wis. A 20—25% methanol solution of phenyltrimethylammonium hydroxide was purchased from Tokyo Kasei Industrial Co. PEG 4000, lactose and potato starch used were of JP X grade and all other chemicals were of reagent grade.

Preparation of Dosage Forms——Flow charts for the preparation of dosage forms are shown in Fig. 1.

a) Simple Blend Method: Phenytoin crystals were passed through 42, 80, 100, 280 and 325 mesh sieves and the residue on each sieve was collected. The sieved phenytoin crystals were blended with granules (lactose : potato starch = 7 : 3, w/w) uniformly to make physical mixtures (10% powder) (phenytoin granules physical mixture) in a blender.

b) Solid Dispersion Method: Solid dispersions of polyethylene glycol 4000 and phenytoin were prepared from physical mixtures of specified proportions of both components by a fusion method. The mixture was heated at 250 °C on a thermostatic plate with constant stirring until a clear homogeneous melt was formed. The molten mixture was quickly poured onto an air-cooled stainless steel plate. The solid dispersions were stored in a desiccator for 3 d and subjected to pulverization. The white mass thus obtained was crushed in a cutter mill and particles in the size range of 105—177 μm were used for further study.

Differential Scanning Calorimetry (DSC)——DSC was performed with a Perkin Elmer differential calorimeter. Samples weighing 2.6—8.2 mg were used in a conventional aluminium pan and a scan speed of 10 °C/min was employed.

Powder X-Ray Diffraction Study——Powder X-ray diffractometry was carried out with a Rigaku Denki Ru-200 diffractometer by using monochromated Cu-K_α radiation.

Dissolution Study——Dissolution of phenytoin was tested in a USP dissolution test apparatus, Method II, in 500 ml of JP X disintegration test medium No. 1 (pH 1.2) at an agitation speed of 100 rpm at 37 °C. Two hundred mg of phenytoin crystals and solid dispersion (powder) equivalent to 200 mg of phenytoin were subjected to the test. The test was triplicated.

Human bioavailability Study——a) Formulation and Dose: Phenytoin crystals (size, 44—53 μm; dose, 300 mg), phenytoin granules physical mixture (10% powders: crystal size of PHT, 44—53 μm: dose, 200 mg) and the solid dispersion powders (dose: 200 mg) were administered orally to healthy male volunteers.

b) Subjects: A three-way cross over study was carried out in five healthy male volunteers aged between 23 and 36 (mean 29.2), and weighing between 56 and 88 kg (mean 73.4 kg).

The volunteers gave their written consent after the object and the procedures of the trial had been fully explained to them. No abnormalities were found on clinical examination, in the results of hematological and biochemical profiles, or in their electrocardiograms.

c) Trial Design: A preparation was administered to each subject with 200 ml of water following an overnight fast. The volunteers continued fasting for 3 h after the dose was given. Blood samples were taken by venipuncture into plastic centrifuge tubes containing heparin at 2, 4, 6, 8, 12, 24, 28, 32, 36 and 48 h after administration and stored at 4 °C. Urine was collected at the same intervals. Following a wash-out period of seven days, the volunteers received another preparation and a series of samples was taken following the same schedule as described above.

d) Assay of the Plasma Level: The plasma samples were assayed for phenytoin by enzyme immunoassay.[9]

e) Assay of the Urinary Level: The levels of the intact phenytoin in the urine were assayed by the gas-liquid chromatography (GLC) method described previously.[10]

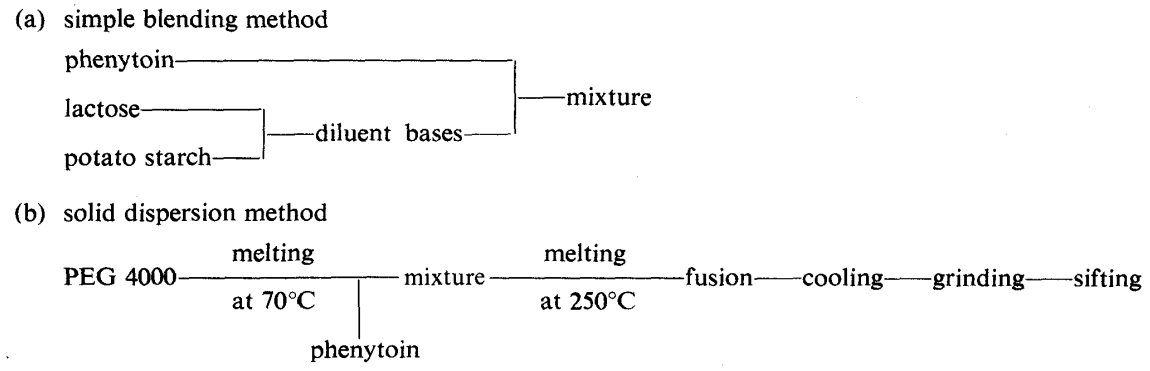

Fig. 1. Preparation of Phenytoin Powders and PEG 4000–Phenytoin Solid Dispersion

Results and Discussion

Figure 2 shows the result of DSC of phenytoin and phenytoin–PEG solid dispersion. Phenytoin has a sharp endothermic peak at 299 °C which corresponds to the melting point. The solid dispersion showed an endothermic peak at 60 °C which was identified as the melting point of PEG, and the sharp peak at 299 °C was not seen.

Figure 3 shows powder X-ray diffraction spectra of phenytoin and phenytoin–PEG 4000 solid dispersion. The characteristic peaks of phenytoin were not seen in the solid dispersion, and two major peaks at 18.5 and 23 °C were observed, which were identified as being due to PEG 4000. Based on these data, it was concluded that phenytoin was uniformly dispersed in an amorphous state in a solid matrix of PEG 4000.

Figure 4 shows the dissolution of phenytoin from phenytoin solid dispersion prepared at various ratios of PEG 4000 to phenytoin. At lower ratios of phenytoin to PEG than 1 to 10, the bulk concentration reached a plateau of 43 μg/ml in less than 5 min and a gradual decline occurred to 40 μg/ml after 120 min, while higher ratios gave rapid rises to 32 μg/ml in 5 min with subsequent small increases. These results suggest that a ratio of less than 1 to 10 is

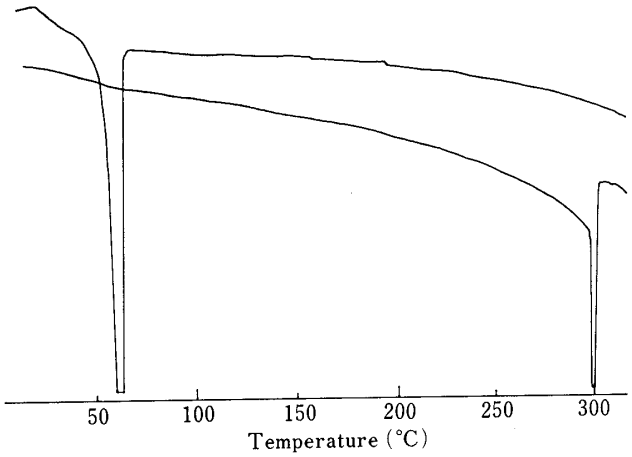

Fig. 2. DSC of Phenytoin (Bottom) and Phenytoin–PEG 4000 (1 : 10, w/w) Solid Dispersion (Top)

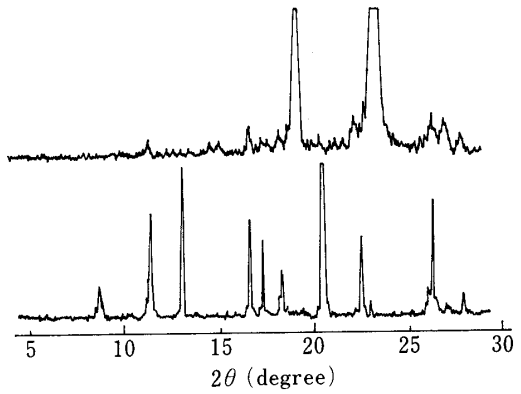

Fig. 3. X-Ray Diffraction Spectra of Phenytoin (Bottom) and Phenytoin–PEG 4000 (1 : 10, w/w) Solid Dispersion (Top)

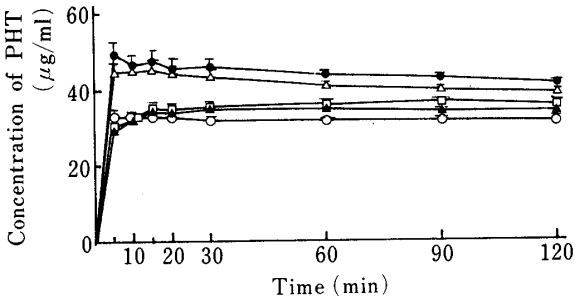

Fig. 4. Dissolution of Phenytoin from Phenytoin Solid Dispersion Prepared at Various Ratios of PEG 4000 to Phenytoin Crystals (w/w) in 500 ml of JP 1st Disintegration Test Fluid as Determined by the USP Paddle Method (Rotation Speed: 100 rpm) at 37 °C

Each point denotes mean of three determinations ± S.E. Phenytoin : PEG = 0.5 : 10 (●), 1 : 10 (△), 2 : 10 (○), 3 : 10 (▲), 4 : 10 (□).

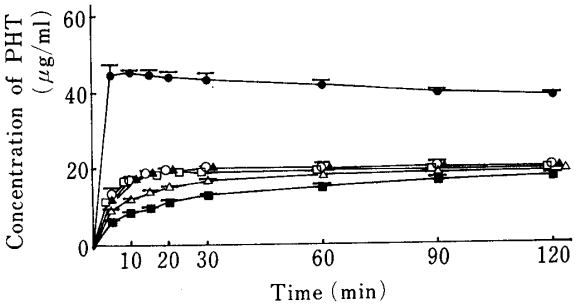

Fig. 5. Effect of Particle Size on the Dissolution of Phenytoin from Phenytoin Crystals and Phenytoin–PEG 4000 (1 : 10) Solid Dispersion

Dissolution test: 500 ml of JP 1st disintegration test fluid, according to the USP paddle method (rotation speed; 100 rpm) at 37 °C.

●, Phenytoin–PEG 4000 solid dispersion; ○, 44—53 μm; ▲, 53—74 μm; □, 74—149 μm; △, 149—177 μm; ■, 177—350 μm.

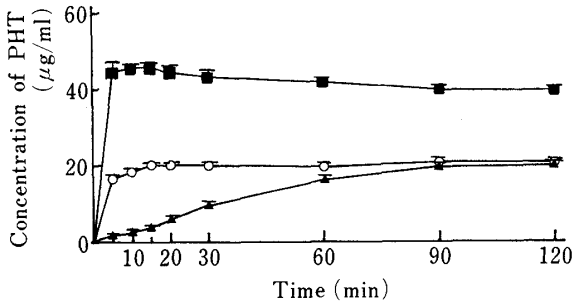

Fig. 6. Dissolution of Phenytoin Crystals (▲), Phenytoin–PEG 4000 (1 : 10) Solid Dispersion (■) and Phenytoin–PEG 4000 (1 : 10) Physical Mixture (○) in 500 ml of JP 1st Disintegration Test Fluid According to the USP Paddle Method (Rotation Speed: 100 rpm) at 37 °C

Each point denotes the mean of three determinations ± S.E.

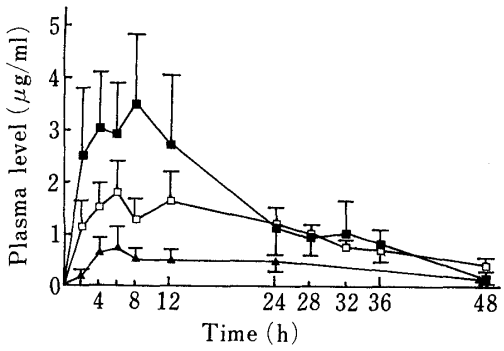

Fig. 7. Mean Plasma Level of Phenytoin in Human Volunteers Following Oral Administration of 300 mg of Phenytoin Crystals (▲), 2200 mg of Phenytoin–PEG 4000 (1 : 10) Solid Dispersion (■) and 2200 mg of Phenytoin Granules Physical Mixture (□)

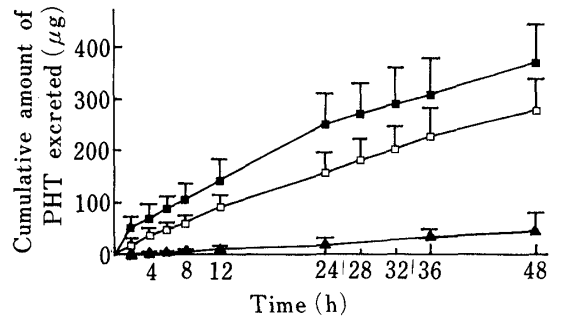

Fig. 8. Cumulative Urinary Excretion of Phenytoin Following Oral Administration of 300 mg of Phenytoin Crystals (▲), 2200 mg of Phenytoin–PEG 4000 (1 : 10) Solid Dispersion (■) and 2200 mg of Phenytoin Granules (1 : 10) Physical Mixture (□)

required to disperse amorphous phenytoin completely in PEG.

Figure 5 shows the dissolution of various sizes of phenytoin crystals and phenytoin–PEG 4000 (1 : 10) solid dispersion. The dissolution rate increased with decreasing of particle size of phenytoin crystals, and dissolution of smaller particle fractions than 74—149 μm converged to a definite dissolution profile. This result suggests the existence of a critical particle size between 74—149 μm for the dissolution of phenytoin crystals. Phenytoin solid dispersion showed far faster dissolution than any of the particle fractions. It gave plateau of 42 μg/ml within 5 min, while phenytoin crystals gave 20 μg/ml at 120 min after initiation of the dissolution test.

Figure 6 shows the dissolution profiles of unsieved phenytoin crystals, solid dispersed phenytoin (mixing ratio of phenytoin to PEG 4000: 1/10), and the physical mixture of phenytoin (crystal size: 44—53 μm, 1 part) and PEG 4000 (10 parts). The apparent dissolution rate of unsieved phenytoin crystals was approximately 1/10 and 1/26 of those of the physical mixture and the phenytoin solid dispersion, respectively. Simple blending of phenytoin with a hydrophilic diluent such as PEG 4000 improves the wetability of phenytoin crystals and facilitates dissolution, while phenytoin crystals themselves are extremely water-repellent. Phenytoin solid dispersion dramatically improved dissolution and should show improved bioavailability if *in vitro* dissolution reflects absorption *in vivo*. However, it is assumed that a particle size of around 50 μm is the critical particle size for the absorption of phenytoin *in vivo*, it is possible that the phenytoin solid dispersion and phenytoin crystals of smaller size fraction

than 53—74 μm might give equivalent bioavailability.

Figures 7 and 8 show the results of the human bioavailability study on phenytoin crystals (crystal size: 44—53 μm), phenytoin solid dispersion (phenytoin–PEG 4000 (1 : 10)), and the physical mixture of phenytoin and lactose–potato starch granules.

For phenytoin crystals, the dose was adjusted so as not to exceed the total daily dose specified in JP X. A dose equivalent to 200 mg of phenytoin was administered in the cases of phenytoin solid dispersion and the physical mixture of phenytoin lactose–potato starch granules because of the comparatively large dissolution rate and solubility. Phenytoin solid dispersion gave the highest bioavailability, followed by the physical mixture. Phenytoin crystals of particle size 44—53 μm gave the lowest plasma level even they were given at the highest dose.

It was reported that fine powder of PHT (mean particle size: 4.1 μm) showed significantly better bioavailability than commercial PHT powder.[11] However, the fine powder could only be prepared on a laboratory scale. The area under the plasma level–time curve (*AUC*) of the PHT–PEG solid dispersion described in this study is almost as high, although the subjects in the clinical studies were not the same, and the solid dispersion could be produced on a large scale.

The improved bioavailability of PHT achieved by the solid dispersion should make it possible to achieve a therapeutic blood concentration with a lower dose. It has been suggested[12] that inter-subject variation of PHT bioavailability in epileptic patients and healthy subjects could be explained by poor absorption due to the low solubility of PHT in the aqueous medium of the gastro-intestinal tract. In the present study, no significant difference of inter-subject variation was observed, but in an expanded clinical study, the PEG–PHT solid dispersion might have the advantage of lower variation in absorption.

Urinary excretion data (depicted in Fig. 8) support the above interpretation. Urinary excretion of phenytoin was monitored in trials where the subjects received the same dose level, *i.e.*, 2200 mg of phenytoin solid dispersion and 2200 mg of phenytoin granules physical mixture. In earlier studies, the urinary excretion of the metabolite of PHT was not directly proportional to the plasma level of the drug,[13] though a direct correlation between the plasma level of the metabolite and its urinary excretion rate was indicated.[14] The major metabolites of PHT are the *p*-hydroxyphenyl derivative and its conjugate with glucuronic acid.[15] In this study the metabolites were excluded and intact PHT in urine was assayed by GLC. Total excreted PHT from solid dispersed PHT exceeded that from the physical mixture or the crystals at any time.

The results reflected well the plasma levels of PHT. In Table I lists plasma levels observed. The plasma levels of phenytoin produced by phenytoin solid dispersion and the physical mixture were significantly different at 4, 6 and 8 h. Table II shows the mean values of the time-to-peak, peak plasma level and *AUC*. Phenytoin solid dispersion gave a four times larger *AUC* than phenytoin crystals, although the dose (as phenytoin) was 2/3 of that for phenytoin crystals. The physical mixture gave about 2.5 times larger *AUC* than the crystals but the *AUC* was still only about 70% of that of phenytoin solid dispersion. The time-to-peak values of the preparations were not significantly different. These results suggest an important role of such formulation factors as preparation method and physical characteristics of the bulk chemical in determining the human bioavailability of phenytoin preparations.

A study to seeking a direct correlation between particle size of phenytoin crystals and bioavailability was performed in dogs, and the *AUC* was reported to be inversely related to the particle size.[5] On the other hand, serum phenytoin levels produced by different brands and particle sizes of phenytoin were compared in a human bioavailability study, where amorphous phenytoin and 17.5—45 μm particles of phenytoin were used. It concluded that there were statistically significant differences among the mean serum phenytoin levels

TABLE I. Mean Plasma Levels of Phenytoin Compared by the Paired *t*-Test

Time (h)	Plasma level[a] (μg/ml)			Significance of difference[b] between PHT granules and PHT–PEG 4000	
	PHT crystals[c]	PHT granules[d] Physical mixture	PHT–PEG 4000[e] Solid dispersion		
2	0.22±0.05	1.14±0.49	2.50±1.29	$t=2.234$	N.S.
4	0.63±0.30	1.53±0.44	3.01±1.11	$t=3.557$	$0.01<p<0.05$
6	0.71±0.43	1.80±0.60	2.94±0.98	$t=4.463$	$0.01<p<0.05$
8	0.51±0.22	1.25±0.42	3.50±1.33	$t=4.506$	$0.01<p<0.05$
12	0.48±0.24	1.65±0.55	2.72±1.35	$t=2.062$	N.S.
24	0.46±0.19	1.16±0.34	1.11±0.55	$t=-0.365$	N.S.
28	—	1.01±0.17	0.96±0.37	$t=-0.449$	N.S.
32	—	0.75±0.15	1.04±0.60	$t=1.237$	N.S.
36	—	0.71±0.24	0.81±0.31	$t=0.909$	N.S.
48	0.14±0.01	0.41±0.16	0.22±0.07	$t=-2.629$	N.S.

a) Mean±S.D. *b*) $t(4, 0.01)=4.604$, $t(4, 0.05)=2.776$. *c*) Dose: 300 mg/body. *d*) Dose: 200 mg/body. *e*) Dose: 200 mg/body.

TABLE II. Mean Time-to-Peak, Peak Plasma Level and *AUC* Following Oral Administration of 300 mg of Phenytoin Crystals and Phenytoin Preparations Equivalent to 200 mg of Phenytoin

	Time-to-peak (h)	Peak plasma level[a] (μg/ml)	*AUC* (h·μg/ml)
PHT crystals	5.7	0.75±0.40	18.45± 7.72
PHT granules (1 : 10) physical mixture	6.8	1.92±0.53	50.26±10.85
PHT–PEG 4000 (1 : 10) solid dispersion	5.6	3.74±1.15	73.86±27.15
Significance level between PHT granules and PHT–PEG 4000		$t=5.514$[b] $p<0.01$	$t=3.012$[c] $0.01<p<0.05$

a) Mean±S.D. *b*) $t(4, 0.01)=4.604$. *c*) $t(4, 0.05)=2.776$.

produced by different brands, but no correlation was found between particle size and serum phenytoin level.[16] The present study suggests that this inconsistency might arise from the difference in the diluents and/or the particle size distribution of phenytoin used in both studies. However, there is still a possibility that a larger particle size than about 50 μm might only be critical for release and absorption of phenytoin *in vivo*. In the present study it was demonstrated that there is a critical particle size of 74—149 μm for dissolution of phenytoin from its crystal form, and the particle size might also be critical for the bioavailability of phenytoin crystals, because an *in vitro* and *in vivo* correlation was well established, as discussed above. It was also found that phenytoin solid dispersion showed superior dissolution and human bioavailability to phenytoin crystals having a crystal size below the critical particle size. These characteristics of the phenytoin solid dispersion should offer the clinical advantages of quick drug release and excellent bioavailability in phenytoin therapy.

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