[Chem. Pharm. Bull. 36(12)4908—4913(1988)]

Dissolution and Bioavailability of Phenytoin in Solid Dispersion with Phosphatidylcholine[1)]

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(Received April 13, 1988)

An attempt was made to improve the dissolution behavior of phenytoin(PHT), a poorly water-soluble drug, with phosphatidylcholine (PC) solid dispersion (SD). The powder X-ray diffraction patterns indicated that PHT was present in an amorphous state in SD when the molar fraction of PHT was under 0.33. Infrared spectra suggested a weak interaction, probably hydrogen bonding, between PC and PHT in SD. The solubility of PHT was 30 μg/ml in both pH 1.2 and 6.8 test solutions. The PHT concentration increased temporarily to 36 μg/ml in both pH 1.2 and 6.8 test solutions with SD in which the molar fraction of PHT was 0.25 (SD (0.25)). This is only 1.2 times the PHT solubility, but the dissolution rate in pH 1.2 test solution was significantly improved: the PHT concentration reached 24 μg/ml in the first 5 min, whereas it was 6 μg/ml in the case of PHT crystals. The dissolution rate was slightly higher even with physical mixture (PM) because of improved wettability.

After oral administration of SD (0.25) to rabbits, plasma concentrations up to 8 h were significantly higher than those in the cases of PM (0.25) and PHT crystals, but there was no significant difference in the area under the plasma concentration curve. PM (0.25) did not show improved bioavailability. These results were consistent with the results of the dissolution test at pH 1.2. PM gave an increased plasma concentration of PHT if PC was used in a large excess over PHT (PM (0.10)), it was considered that PC functioned as an oil in this case.

Keywords——phenytoin; phosphatidylcholine; solid dispersion; dissolution rate; amorphous; bioavailability; rabbit oral administration

Phenytoin (PHT) is used extensively in the treatment of epilepsy, but its bioavailability is variable because of its low solubility in water.[2)] Many investigators have attempted to improve the dissolution rate and solubility of PHT by the use of inclusion compounds with cyclodextrin[3)]; solid dispersion (SD) with polyethylene glycol (PEG)[4)] or polyvinylpyrrolidone (PVP)[4b)]; coprecipitate with PVP and deoxycholate[5)]; roll-mixing with PVP[6)]; or a spherical crystallization technique with PEG.[7)] Hydrophilic and water-soluble agents were used in all cases.

We previously reported that the SD with phosphatidylcholine (PC), an amphoteric but water-insoluble lipid, improve the solubilities of antiinflammatory drugs, *i,e.*, indomethacin (IM), ketoprofen and flurbiprofen.[8)] The improvement of solubilities was considered to have occurred because drugs were present in the amorphous state in PC. This time we prepared a solid dispersion of PHT and PC, and its physicochemical properties were examined. Its the dissolution and bioavailability following oral administration to rabbits were also investigated.

Experimental

Materials——PHT was purchased from Dainippon Pharmaceutical Co., Ltd. (Aleviatin®, lot 4661). PC was purified from hydrogenated soybean phospholipids (Nikko Chemicals, Lecinol S-10), containing 5—7% phosphati-

dylethanolamine as an impurity; its acyl chains composition was stearic acid : palmitic acid = 85 : 15.[9] Other chemicals were of reagent grade.

Preparation of SD——The required amounts of PHT and PC were weighed out and dissolved in ethanol or xylene, then the solvent was evaporated off *in vacuo*. Glassy powder or film thus obtained was collected, crushed in an electric coffee mill and sieved with an 80 mesh screen to obtain a powder. The powder was stored in a desiccator over silica gel at room temperature. Such a powder is described as SD (0.25), for example, where the figure in parentheses is the molar fraction of PHT.

For physical mixture (PM), required amounts of PHT and PC were weighed out and mixed with a spatula.

Physicochemical Properties of SD——Powder X-ray diffraction patterns were obtained with an X-ray diffractometer (Rigaku Denki, Geigerflex 2027). The X-rays were Ni-filtered CuK_α radiation (30 kV and 40 mA; scanning speed 4 degrees/min).

Infrared (IR) spectra were obtained on an IR spectrophotometer (Nippon Bunko, A-102) by the KBr semimicro disk technique.

Solubility Studies——A sample equivalent to 50 mg as PHT was put into a flat-bottomed flask and 50 ml of the 1st fluid (pH 1.2) or the 2nd fluid (pH 6.8) of the JP XI disintegration test was added. The flask was set in a water bath incubator (Yamato, BT-47) and shaken at 100 cycles/min at 37 °C. Aliquots of test solution were taken at appropriate intervals and filtered through a 0.20 μm membrane filter. The concentration of PHT was determined by high-pressure liquid chromatography (HPLC).

Dissolution Studies——The dissolution patterns of PHT were tested in a JP XI dissolution test apparatus with the paddle method. As test solution, 500 ml of the 1st fluid (pH 1.2) or the 2nd fluid (pH 6.8) of the JP XI disintegration test was kept at 37 °C. A sample equivalent to 10 or 25 mg as PHT was dispersed in 5 ml of test solution and dropped into the test solution with stirring at 100 rpm by a paddle. An aliquot of the test solution was withdrawn periodically and immediately filtered through a 0.20 μm membrane filter (Toyo Roshi, Dismic-25). The same volume of fresh test solution was added to the test solution. PHT concentration was determined by HPLC. All studies were done in triplicate.

Animal Experiment——Six white male rabbits (body weight 2.4—3.2 kg) were fasted for 24 h with a collar to prevent coprophagy, but allowed to take water freely; this procedure reduced the gastric contents.[10] A sample equivalent to 30 mg/kg as PHT suspended in 30 ml of water was orally administered to a rabbit. Blood samples were taken at an appropriate interval with heparinized syringes and centrifuged for 15 min at 3000 rpm to obtain plasma fractions. About 100 g of feed was given after 12 h of blood sampling. Doses were administered by the cross-over arrangement after a one-week interval.

Determination of PHT in Plasma——Plasma concentration of PHT was determined by modification of the method of Slonek *et al.*[11] A 100 μl aliquot of plasma was added to 250 μl of acetonitrile and the mixture was mixed with vortex mixer for about 5 s. After centrifugation for 10 min at 2500 rpm, 25 μl of the clear supernatant was subjected to HPLC.

Condition of HPLC——HPLC was performed with a Waters model 510 pump, and a variable-wavelength ultraviolet detector (Waters, model 490). The stationary phase, μBondapak C_{18} (3.9 × 150 mm), was kept at 30 °C. The mobile phase was a mixture of acetonitrile–water (30 : 70, v/v) acidified with phosphoric acid to pH 2.5. The flow rate was 1.5 ml/min for plasma samples and 2.0 ml/min for solubility and dissolution tests. PHT was detected at 230 nm. The standard solutions were chromatographed and calibration curves were constructed on the basis of peak area measurement.

Statistical Analysis——Statistical analysis was performed using one-way analysis of variance. Follow-up analysis was performed using Dunnett's test. A p value of <0.05 was accepted as demonstrating statistical significance.

Results and Discussion

Preparation and Physicochemical Properties

In the case of IM, xylene was suitable for the preparation of a solid dispersion,[8] so the preparation was attempted with xylene as a solvent. PHT dissolved slightly in xylene (1 mg/1.5 ml), but dissolved freely in the presence of a 2-fold molar excess of PC. The preparation of SD was tried in the range of molar fraction of PHT from 0.25 to 0.40 with xylene. SD was also prepared with ethanol which dissolves well both PHT and PC.

Figure 1 shows powder X-ray diffraction patterns. With either solvent, SD (0.25) and (0.33) showed only the PC short spacing peak, $2\theta = 21$ °. They indicated that PHT was present in an amorphous state in SD (0.25) and (0.33). But some crystals occurred in SD (0.40) with either solvent. IM existed in an amorphous state in PC solid dispersion prepared with ethanol

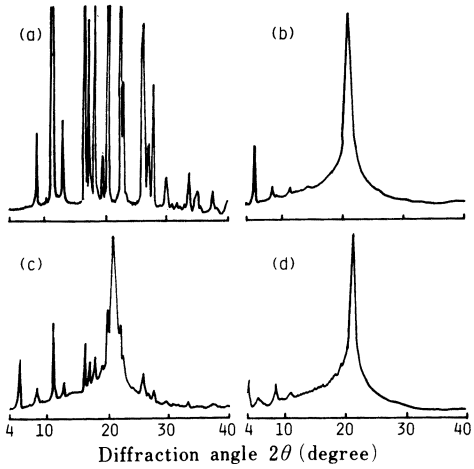

Fig. 1. X-Ray Diffraction Patterns of PHT (a), PC (b), PM (0.33) (c) and SD (0.33) (d)

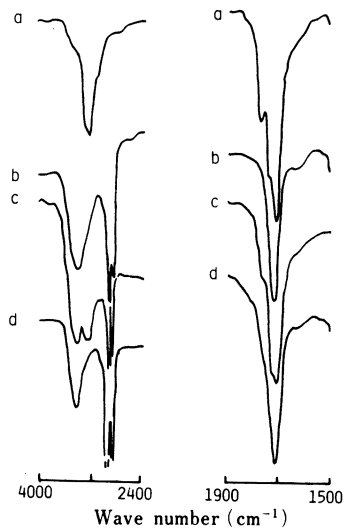

Fig. 2. IR Spectra of PHT (a), PC (b), PM (0.33) (c) and SD (0.33) (d)

if the molar fraction of IM was under 0.50, and in SD prepared with xylene if the molar fraction was under 0.75.[8] The limit of molar fraction of PHT which existed in the amorphous state was lower than that of IM, though the molecular weight of PHT is smaller than that of IM. It was considered that the molecular shape of PHT is bulky, whereas that of IM may be plate-like, so it was more difficult for PHT to be accommodated.

Figure 2 shows IR spectra. The spectrum of PM (0.33) appeared to be the sum of those of PHT and PC. Some difference was observed near 1700 and 3200 cm^{-1} between SD (0.33) and PM (0.33). Near 1700 cm^{-1}, PHT showed peaks at 1717, 1740 and 1768 cm^{-1}, and PC, at 1730 cm^{-1}. Three peaks were observed at 1717, 1730 and 1760 cm^{-1} with PM (0.33), though only 1 peak was observed at 1720 cm^{-1} with SD (0.33). On the other hand, PHT also showed a broad peak at 3200—3275 cm^{-1} (NH band) and PC, at 3400 cm^{-1} (OH band). PM (0.33) showed both peaks but SD (0.33) showed only the PC peak of 3400 cm^{-1}. These differences were also observed between SD (0.25) and PM (0.25). It suggests that some interaction, probably hydrogen bonding, occurs between PHT and PC in solid dispersion.

Further investigation was undertaken with SD (0.25) because the amorphous state of PHT was considered to be more stable than in SD (0.33), and SD (0.25) contained about 10% of PHT.

Dissolution and Solubility Studies

The solubility of PHT in pH 1.2 and 6.8 test solutions was 30 μg/ml, in agreement with reported data.[2,3] The pK_a of PHT was reported to be 8.3,[2a] so the solubility would not be expected to change between pH 1.2 and 6.8. With PM (0.25), no change of solubility was observed. In both pH 1.2 and 6.8 test solutions, the PHT concentration initially increased to 36 μg/ml with SD (0.25), but it decreased with time, being 32 μg/ml after 30 h. In the case of IM, IM, existed in an amorphous state in PC solid dispersion, dissolved to form a supersaturated solution (4 times its solubility) and crystallized with time.[8] It was considered that the same phenomenon occurred in the case of PHT solid dispersion.

The maximum PHT concentration with SD (0.25) was only 1.2 times the PHT solubility, but the dissolution rate was increased. Figure 3 shows the dissolution patterns in pH 1.2 (a) and pH 6.8 (b) test solution with a dose of 10 mg (PHT equivalent). At this dose, PHT should

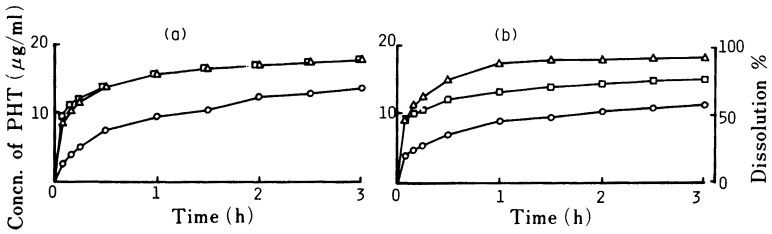

Fig. 3. Dissolution of PHT from PHT Crystals (○), PM (0.25) (△) and SD (0.25) (□) in 500 ml of pH 1.2 (a) and pH 6.8 (b) Test Solutions with a Dose of 10 mg PHT Equivalent

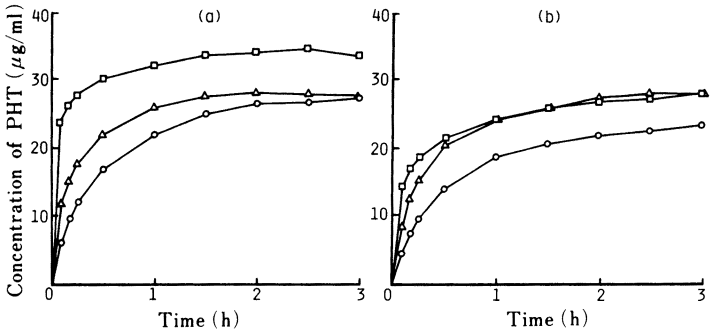

Fig. 4. Dissolution of PHT from PHT Crystals (○), PM (0.25) (△) and SD (0.25) (□) in 500 ml of pH 1.2 (a) and pH 6.8 (b) Test Solutions with a Dose of 25 mg PHT Equivalent

be 100% dissolved. In the pH 1.2 test solution, SD (0.25) and PM (0.25) showed the same dissolution pattern; 50% of PHT (10 μg/ml) was dissolved within 5 min and 90% (18 μg/ml) within 3 h. The dissolution from PHT crystals was slow and only 70% of PHT was dissolved within 3 h. In pH 6.8 test solution, the dissolution pattern from PM (0.25) was the same as that at pH 1.2, and that from PHT crystals was slightly slower. From SD (0.25), the dissolution pattern within 5 min was the same as that at pH 1.2, but only 75% of PHT was dissolved in 3 h.

Figure 4 shows the dissolution patterns of PHT with a dose of 25 mg (PHT equivalent). At this dose, PHT would not be 100% dissolved. This is likely to reflect the situation in the digestive system. In pH 1.2 test solution the PHT concentration with SD (0.25) became 24 μg/ml at 5 min, which was 4 times that with PHT crystals and twice that with PM (0.25). After 3 h, the PHT concentration became 28 μg/ml with powder and PM (0.25), and 33 μg/ml with SD (0.25). In pH 6.8 test solution, similarly to the test with 10 mg of PHT, PM (0.25) showed a similar dissolution pattern to that pH 1.2. PHT crystals showed slightly slower dissolution and SD (0.25) obviously slower dissolution than at pH 1.2. The dissolution test performed with SD (0.25) (50 mg PHT equivalent) showed a similar dissolution pattern to that with 25 mg PHT equivalent in the pH 1.2 test solution.

The initial dissolution from SD (0.25) was faster than that from PHT crystals because of the amorphous state of PHT. But at pH 6.8, dissolution from SD (0.25) was depressed to 75%. The solubility of PHT and maximum PHT concentration from SD (0.25) at pH 1.2 and 6.8 were the same, so the difference must arise from PC. It was considered that PHT inside SD (0.25) did not wet easily because PC is a water-insoluble lipid, so PHT was not 100% dissolved. But PC is zwitterionic and is charged positively at pH 1.2 (apparent pK value: 2—

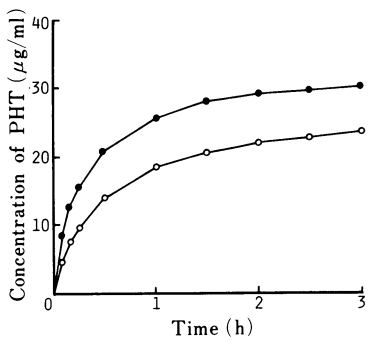

Fig. 5. Dissolution of PHT from PHT Crystals with (●) or without (○) Polysorbate 80 in 500 ml of pH 6.8 Test Solution

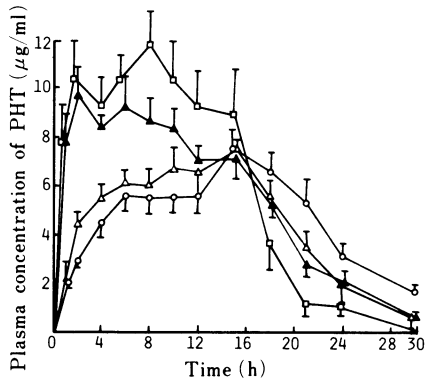

Fig. 6. Plasma Concentration of PHT after Oral Administration to Rabbits at a Dose of 30 mg/kg PHT Equivalent

—○—, PHT crystals; —□—, SD (0.25); —△—, PM (0.25); —▲—, PM (0.10). Each point represents the mean ± S.E. of six rabbits.

TABLE I. Bioavailability Parameters after Oral Administration of PHT at a Dose of 30 mg/kg to Rabbits

	C_{max} (μg/ml)	T_{max} (h)	AUC_{0-30h} (μg·h·ml^{-1})
PHT crystals	8.3 ± 0.6	15.8 ± 2.0	139.5 ± 11.1
SD (0.25)	14.7 ± 0.8[a]	5.5 ± 1.5[a]	174.9 ± 16.4
PM (0.25)	8.5 ± 0.7	10.5 ± 2.3	132.8 ± 12.3
PM (0.10)	10.1 ± 1.1	3.7 ± 1.3[a]	161.6 ± 12.2

Each value represents the mean ± S.E. of six rabbits. *a*) Significantly different ($p < 0.05$) from PHT crystals.

3[12]), so its dispersibility would be improved at pH 1.2 and the dissolution ratio at pH 1.2 was higher than that at pH 6.8. Consequently, the dissolution test performed with SD (0.25) (50 mg PHT equivalent) showed a similar dissolution pattern to that with 25 mg PHT equivalent in the pH 1.2 test solution.

PM (0.25) also improved the dissolution of PHT even when PHT was present as crystals. PHT crystals were not dispersed well in test solution and some of them floated on the surface of the test solution, especially at pH 6.8. The dissolution from PHT crystals in pH 6.8 test solution with 0.01% Polysorbate 80 was similar to that from PM (0.25) without Polysorbate 80 (Fig. 5). PC is a kind of surface active agent, and presumably improved the wettability of PHT.

Bioavailability Studies

Figure 6 shows the plasma concentration of PHT following oral administration at a dose of 30 mg/kg PHT equivalent. The bioavailability parameters (maximum concentration (C_{max}), time to maximum concentration (T_{max}) and area under plasma concentration curve (AUC)) are shown in Table I. Following administration of PHT as SD (0.25), the plasma concentration from 1 to 8 h and C_{max} became significantly higher and T_{max} was faster than those with PM (0.25) or PHT crystals. The plasma concentrations following administration of PM (0.25) and PHT crystals showed no significant difference. AUC_{0-30h} after administration of SD (0.25) tended to be higher, but not significantly so. These results are consistent with the dissolution test with a dose of 25 mg PHT equivalent in pH 1.2 test solution, because drugs

administered orally are first dispersed in the stomach. PM (0.25) improved the wettability but it seemed to have no marked effect on the absorption.

The plasma concentration following PM (0.10) administration was significantly higher than that in the case of PHT crystals up to 6 h, whereas the dissolution rate from PM (0.10) was similar to that from PHT crystals. PC is a kind of lipid, and it has been demonstrated that PHT bioavailability is improved if it is administered as an oil suspension.[13] Thus, the effect of a large amount of PC in increasing the initial plasma concentration was considered to arise because PC functioned like an oil in this case.

Conclusion

PHT is present in an amorphous state in PC solid dispersion if the molar fraction of PHT is below 0.33. IR spectra suggested a weak interaction between PC and PHT, probably hydrogen bonding. With SD (0.25), the maximum PHT concentration is only 1.2 times the PHT solubility, but the dissolution rate is high especially in pH 1.2 test solution. The plasma concentration following oral administration of SD (0.25) to rabbits was significantly higher than that of PHT crystals up to 10 h, and $AUC_{0-30\,h}$ tended to be higher than those of PM (0.25) and PHT crystals, though the difference was not significant.

Acknowledgments We thank Taisho Pharm. Co., Ltd. for measurement of X-ray diffraction patterns. We also thank Nikko Chemicals Co., Ltd. for providing samples.

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