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Improvement of dissolution and absorption characteristics of phenytoin by a water-soluble β-cyclodextrin-epichlorohydrin polymer

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Summary

Complexes of phenytoin with 3 water-soluble cyclodextrin-epichlorohydrin polymers (α-CyD · EP, β-CyD · EP and γ-CyD · EP) in aqueous solution and in the solid phase were studied by a solubility method, spectroscopic analyses and X-ray diffractometry. Binding to CyD polymers increased the solubility and dissolution rate of phenytoin in the order of β-CyD · EP > α-CyD · EP ≧ γ-CyD · EP. The rapidly dissolving form of phenytoin–β-CyD · EP complex was found to significantly increase the plasma levels of the drug after oral administration to dogs. Data are presented suggesting that β-CyD · EP is particularly useful for improving the oral bioavailability of phenytoin.

Introduction

Although the natural cyclodextrins (CyDs) have been used in many fields (Szejtli, 1982), they contain some undesirable inclusion characteristics due to definite cavity sizes (5.2 Å, 6.4 Å and 8.3 Å for the larger entrance sides of α-, β- and γ-CyDs, respectively). The limited applications of CyDs in the pharmaceutical field seems to

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be related to the relatively low aqueous solubility (Uekama, 1981). This is particularly the case for β-CyD (1.8 w/v% at 25°C). Recently, the chemically modified CyDs have received considerable attention because their physicochemical properties and inclusion behaviors may be different from those of the natural CyDs (Saenger, 1980). For example, a condensed polymer of β-CyD with epichlorohydrin (β-CyD · EP) is extremely soluble in water (more than 50 w/v%) and interacts with a variety of guest molecules (Harada et al., 1981). Thus, the present study was undertaken to survey the possible utility of CyD · EP, in anticipation of improving dissolution and absorption characteristics of poorly soluble drugs. Phenytoin, an anticonvulsant drug, was employed as a model compound because it has a potential for poor oral bioavailability when formulated into solid dosage forms (Glazko and Chang, 1972).

Materials and Methods

Materials

Phenytoin was donated by Dainippon Pharmaceuticals (Osaka, Japan). CyDs were purchased from Nihon Shokuhin Kako (Tokyo, Japan). Three CyD polymers were synthesized according to the method of Hoffman (1973) at our laboratories, and their average molecular weights were estimated to be 3000–20,000 by gel permeation chromatography using a Shodex B-803 (Tokyo, Japan). However, the exact molar ratio of the product was not clarified. All other materials and solvents were of analytical reagent grade. Deionized double-distilled water was used throughout the study.

Apparatus

The circular dichroism (CD) spectra were taken by a Jasco J-50A recording spectropolarimeter (Tokyo, Japan) at 25 ± 0.5°C. The infrared (IR) spectra were measured as a KBr disc, using a Jasco DS-701G diffraction grating spectrophotometer (Tokyo, Japan). The powder X-ray diffraction patterns were taken by a Rigaku Denki Geiger Flex-2012 diffractometer (Tokyo, Japan). The gas chromatography (GC) was performed on a Shimadzu GC-6AM equipped with a flame ionization detector (Tokyo, Japan).

Preparation of solid complexes

The solid complexes of phenytoin with CyD polymers were prepared according to the method reported previously (Tsuruoka et al., 1980). Appropriate amounts of phenytoin and CyD polymers were dissolved in an aqueous ammonium solution and the solutions were then freeze-dried. After freeze-drying, no ammonia was detected in the complex powders, using Nessler's reagent.

Solubility studies

Solubility measurements were carried out according to Higuchi and Connors (1965). Excess amounts of phenytoin were added to aqueous solutions containing

various concentrations of CyD polymers and shaken at 25 ± 0.5°C. After equilibrium was attained (approximately 7 days), an aliquot was centrifuged and pipetted through a cotton plug. A portion of the sample was adequately diluted with 0.1 M phosphate buffer (pH 11.0) and analyzed spectrophotometrically at 260 nm.

Dissolution studies

Dissolution rates of phenytoin from the preparations were measured by the method of Nogami et al. (1969). In brief, the sample powder (100 mesh) of drug (100 mg) or its equivalent amount of the complex was put into 25 ml of water in a dissolution cell which was kept at 25°C and the dissolution medium was stirred at 91 rpm by a stainless propeller. At appropriate intervals, 0.5 ml samples were pipetted through a cotton plug, diluted with 0.1 M phosphate buffer (pH 11.0) and assayed spectrophotometrically. A correction was applied for cumulative dilution caused by replacing the sample by equal volumes of the original medium.

In vivo absorption studies

Four female beagle dogs, weighing 12–14 kg, were fasted for 18 h prior to drug administration. Intervals of at least one week were taken in a cross-over matrix to minimize the residual or cumulative effect of the preceding dose. A test powder (300 mg as equivalent of phenytoin, 100 mesh) packed in a gelatin capsule was administered orally with 20 ml of water. At predetermined intervals, a 3 ml blood sample was taken from the cephalic vein. The blood samples, collected in heparinized test tubes, were centrifuged to obtain 1 ml of plasma for analysis. To 1 ml of plasma was added 1 ml of 6 N HCl and then the mixture was diluted with 8 ml of $CHCl_3$–iso-BuOH (4 : 1) containing 1 ml of 5-phenyl-5-(*p*-tolyl)-hydantoin sodium hydroxide solution as an internal standard. After centrifugation, the organic phase (5 ml) was transferred to a new tube, and the solvent was evaporated to dryness on a water-bath under reduced pressure. The residue was dissolved in 40 μl of 0.1 M phenyltrimethylammonium hydroxide solution, and 2.5 μl of the solution was injected into a gas chromatograph. The chromatograph was operated at a flow rate of 40 ml/min using pure N_2 gas as a carrier. The column was of coiled glass (2 diameter × 100 mm) packed with 3% Silicone OV-17 on 80–100 mesh Chromosorb W AW (Tokyo, Japan). The temperature of the injection port and the detector were 200 and 250°C, respectively.

Results and Discussion

Complex formation in aqueous solution and in the solid state

Fig. 1 shows the effects of 3 CyD polymers on the solubility of phenytoin. Linear plots were obtained in all cases, indicating a first-order dependency of the interactions on the polymer concentrations. Solubility of phenytoin increased in the order of β-CyD · EP > α-CyD · EP ≧ γ-CyD · EP. Since this order is almost the same as that obtained for the inclusion complexes of phenytoin with natural CyDs (Tsuruoka et al., 1981), ring availability of phenytoin may be the primary factor for interaction

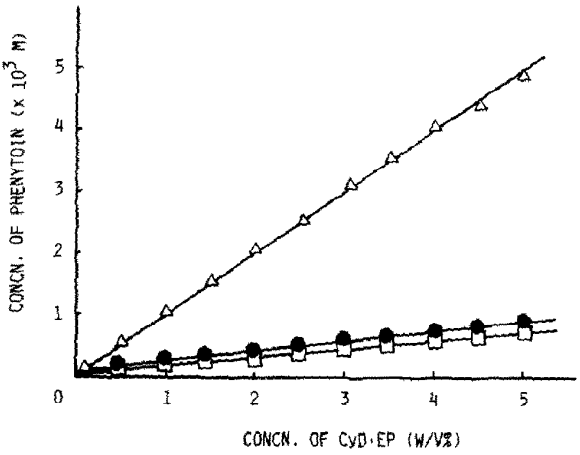

Fig. 1. Effects of CyD·EPs on the solubility of phenytoin in water at 25°C. The ordinate represents the total concentration of phenytoin including the complexed phenytoin. ●, α-CyD·EP; △, β-CyD·EP; □, γ-CyD·EP.

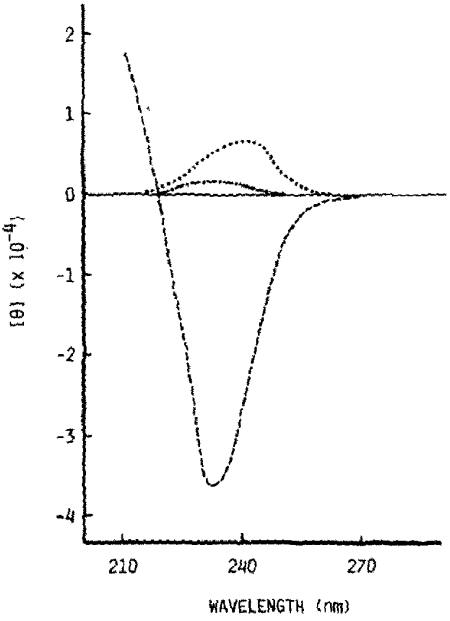

Fig. 2. Circular dichroism spectra of phenytoin complexes with CyD·EPs in 0.1 M phosphate buffer (pH 11.0) at 25°C. [phenytoin] = 5×10^{-4} M; [CyD·EPs] = 5×10^{-3} M. ·····, with α-CyD·EP; - - - -, β-CyD·EP; — · — · —, γ-CyD·EP.

with CyD polymers. That is, the cavity size of β-CyD in the polymer seems to be optimum to entrap the phenytoin molecule, and consequently provides the greatest solubilization effect. The interactions of phenytoin with CyD polymers in aqueous solution were further examined by CD spectroscopy. Fig. 2 shows the CD spectra of phenytoin in the presence of 3 CyD polymers. Since the CyD polymers themselves show no CD band at wavelengths longer than 200 nm, the observed optical activities can be ascribed to the induced Cotton effects of phenytoin by the binding to CyD polymers. The β-CyD · EP system showed a strong negative peak in the UV absorption region of phenytoin. In contrast, α- and γ-CyD polymer systems showed very weak positive peaks, suggesting that the interaction mode of β-CyD · EP complex might be somewhat different from those of α- and γ-CyD · EP complexes. The magnitude of these CD spectral changes correlated well with the solubility changes.

Since no precipitation was observed in the solubility studies (Fig. 1), the solid complexes of phenytoin with 3 CyD polymers were prepared by a freeze-drying method, and used for further studies. The interaction in the solid state was examined by X-ray diffractometry and IR spectroscopy in comparison with the corresponding physical mixtures. Fig. 3 shows the powder X-ray diffraction patterns of the phenytoin–CyD polymer systems. The diffraction patterns of the physical mixtures were simply a superposition of each component, that is, the sharp peaks due to drug and the broad peaks due to CyD polymers were observed. In the case of the complexes, these peaks disappeared to give an amorphous form, indicating a new solid phase. The IR spectra of the complexes were also compared with the physical mixtures in the carbonyl-stretching region of phenytoin. In the case of the complexes, the 1715 cm^{-1} band shifted to 1725 cm^{-1}, suggesting the formation of hydrogen bonds between phenytoin and CyD polymers. In contrast, no appreciable spectral changes were observed for the physical mixtures. The above data clearly indicate that phenytoin–CyD polymer complexes exist in solution and in the solid phase.

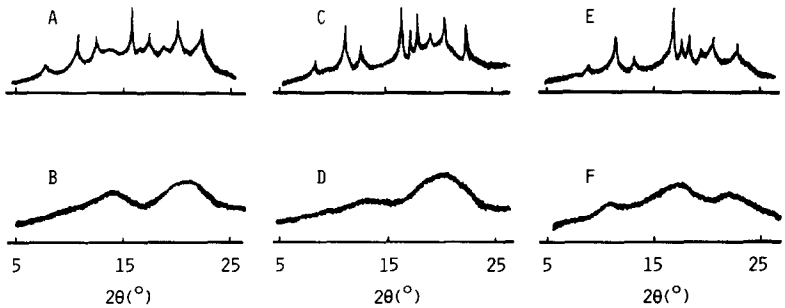

Fig. 3. Powder X-ray diffraction patterns of phenytoin–CyD · EP systems (1 : 5 weight ratios). A: physical mixture of phenytoin with α-CyD · EP. B: complex of phenytoin with α-CyD · EP. C: physical mixture of phenytoin with β-CyD · EP. D: complex of phenytoin with β-CyD · EP. E: physical mixture of phenytoin with γ-CyD · EP. F: complex of phenytoin with γ-CyD · EP.

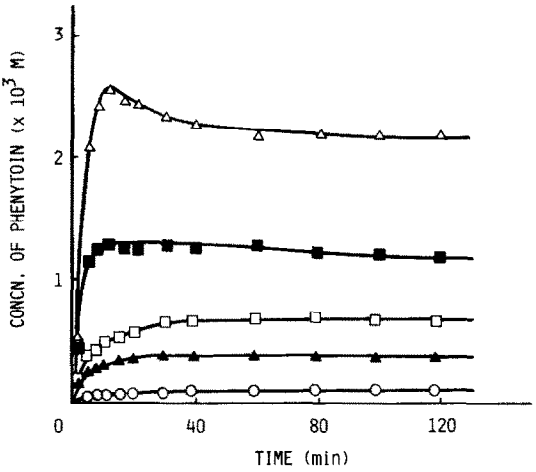

Fig. 4. Dissolution profiles of phenytoin–β-CyD·EP complexes at various phenytoin to β-CyD·EP weight ratios in water at 25°C. The ordinate represents the total concentration of phenytoin including the complexed phenytoin. ○, phenytoin; ▲, phenytoin–β-CyD·EP complex (1:0.5); □, phenytoin–β-CyD·EP complex (1:1); ■, phenytoin–β-CyD·EP complex (1:3); △, phenytoin–β-CyD·EP complex (1:5).

Dissolution behavior

The dissolution profiles of phenytoin–β-CyD polymer complexes at various drug-to-polymer weight ratios and that of phenytoin alone in water appear in Fig. 4. The complexes exhibited a faster drug dissolution rate than phenytoin alone. The dissolution rate of phenytoin in the complexes was greater when the ratio of drug : β-CyD polymer was smaller. The enhanced dissolution rate may be due to the

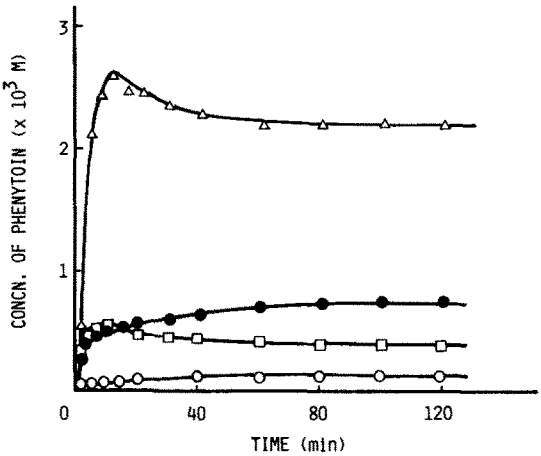

Fig. 5. Dissolution profiles of phenytoin from phenytoin–CyD polymer complexes (1:5 weight ratios) in water at 25°C. The ordinate represents the total concentration of phenytoin including the complexed phenytoin. ○, phenytoin; ●, phenytoin–α-CyD·EP; △, phenytoin–β-CyD·EP; □, phenytoin–γ-CyD·EP.

increase in solubility and the decrease in crystallinity of the drug by complexation with CyD polymers as expected from Fig. 1 and 3, respectively. The dissolution profiles of phenytoin in the complexes with various CyD polymers at a 1 : 5 weight ratio of phenytoin–CyD polymers are shown in Fig. 5. The initial dissolution rate of the drug from the complexes increased in the order of β-CyD · EP > α-CyD · EP $\geqq$ γ-CyD · EP. This tendency is in accord with the solubility changes shown in Fig. 1. In the case of β-CyD · EP complex, the drug concentration following dissolution exceeded (about 25-fold) its normal solubility in water, indicating supersaturation. This supersaturation was stable and no recrystallization of the drug occurred under the experimental conditions used. Thus, the remarkable increase in dissolution characteristics suggested that the complexed form of phenytoin, particularly for β-CyD · EP, might improve oral bioavailability.

Bioavailability of phenytoin-β-CyD · EP complex

The mean plasma levels of phenytoin following the oral administration of phenytoin or its β-CyD · EP complex appear in Fig. 6. When the equivalent dose of phenytoin (300 mg) was administered to dogs, a significant difference in the peak concentrations was observed between the complex and drug itself. The area under plasma concentration–time curve (AUC) of the complex for up to 24 h post-administration was about 2 times as much as that from phenytoin alone. Unfortunately, a reliable value for the absorption rate of the complex was not obtainable because no sensitive assay is currently available for such a low CyD · EP concentration. However, it is reasonable to assume that the absorption rate of the complex is negligibly small compared with phenytoin alone, owing to the poor permeability and/or the low lipophilicity of the complex. Therefore, a greatly enhanced dissolution rate of

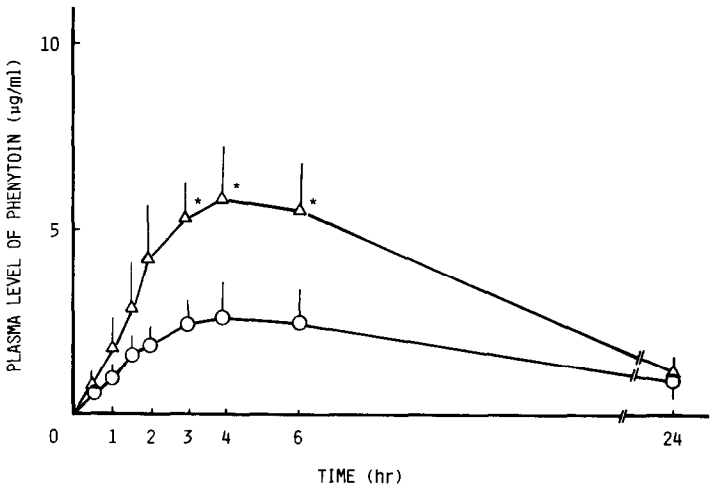

Fig. 6. Plasma levels of phenytoin following the oral administration of phenytoin and its β-CyD·EP complex (equivalent to 300 mg phenytoin) to dogs. ○, phenytoin; △, phenytoin–β-CyD·EP (1 : 5 weight ratio). Values represent the mean ± S.D. of 4 dogs. * $P < 0.01$ in △ versus ○.

phenytoin may cancel out these negative effects and produce a net increase in the concentration of phenytoin available for gastrointestinal absorption.

The present approach of using a rapidly dissolving form of β-CyD · EP complex is promising for improving the oral bioavailability of phenytoin. The present data also suggest the possible utility of the water-soluble CyD polymers in various pharmaceutical dosage forms such as oral solutions and injections, although the practical application will have to await the toxicological studies of the CyD polymers.

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