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Improvement of oral bioavailability of carbamazepine by inclusion in 2-hydroxypropyl-β-cyclodextrin

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Summary

The purpose of this study was to enhance the solubility and bioavailability of carbamazepine (CBZ) through complexation with 2-hydroxypropyl β-cyclodextrin (HPβCD). CBZ is a poorly water soluble antiepileptic drug. Reportedly, it has slow, erratic, and complete absorption after oral administration. This present report describes the study of the phase solubility diagram, preparation of the inclusion complex, characterization of the physico-chemical properties of the complex, and determination of the bioavailability of the complex after oral administration in rats. An A_L-type phase solubility diagram indicated a 1:1 complex of CBZ-HPβCD with the constant of complex formation of 665 M^{-1} at 37°C. The complex formation was confirmed by DSC, IR, and X-ray diffraction. The extent of absorption of the complex was determined in rats and was compared with that of pure drug. The peak plasma concentration of 21.4 ± 4.9 μg/ml of CBZ (C_{max}) appeared at 1.55 ± 0.19 h, whereas with pure drug the value was 10.7 ± 0.21 μg/ml at 3.98 ± 0.29 h. The AUC_0^{12} of the complex was 2.09 times as much as that of the pure drug. There was no change in the elimination rate constant (0.44 ± 0.049 h^{-1}). Thus, the extent of oral absorption is improved significantly from the inclusion complex.

Introduction

Cyclodextrins have extensively been used to increase the solubility (Hamada et al., 1975; Glomot et al., 1988; Hassan et al., 1990), dissolution rate (Uekema et al., 1983; Chow and Karara, 1986), and bioavailability of poorly water soluble drugs (Nambu et al., 1978; Seo et al., 1983; Kikuchi et al., 1987). The ability of cyclodextrins to modify these characteristics has been attributed to the formation of inclusion complex between cyclodextrins and 'guest' drug molecules. 2-Hydroxypropyl-β-cyclodextrin [HPβCD] is an amorphous mixture of modified β-cyclodextrins (βCD). It has the ability to form inclusion complexes but does not have the limitations associated with crystalline cyclodextrins such as renal toxicity (Pitha et al., 1988; Brewster et al., 1990). Further, the aqueous solubility of HPβCD is far greater than that of the parent βCD which results in vastly improved solubility of numerous compounds.

Carbamazepine (CBZ) is a major antiepileptic drug for the treatment of different form of

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seizures (Blom, 1962; Jongmans, 1964). The drug is practically insoluble in water and has poor wettability properties. It is absorbed slowly and erratically after oral administration. Peak concentrations in plasma are usually attained 4–8 h after oral ingestion but may be delayed by as much as 24 h. Reportedly, it has an oral bioavailability of less than 50% (Morselli et al., 1975; Rall and Schleifer, 1985). These properties of CBZ led to the belief that it can be a good candidate for complexation with HPβCD to increase solubility and bioavailability. The primary objective of the present study is to investigate the possibility of improving the aqueous solubility and oral bioavailability of CBZ via complexation with HPβCD. The formation of such complex was confirmed by a variety of techniques such as solubility determination, infrared spectrophotometry (IR), differential scanning calorimetry (DSC) and X-ray diffraction studies. The work also included determination of the in vitro dissolution profiles and bioavailabilities in rats of pure drug, physical mixture and CBZ:HPβCD complex system.

Materials and Methods

Materials

Carbamazepine was a gift from Ciba-Geigy (U.S.A.). βCD and HPβCD were kindly supplied by American Maize-Products Co. (U.S.A.). Acetonitrile, ethanol, methanol, and water were obtained from Fisher Scientific (U.S.A.) and all were of HPLC grade. All other chemicals were of analytical reagent grade.

Methods

Analysis of carbamazepine The CBZ concentrations for phase solubility study and in vitro dissolution study were determined spectrophotometrically (Perkin-Elmer) at 285 nm in a methanol/water mixture (80:20). The HPLC method of Hartley et al. (1986) was utilized to determine the plasma concentrations of CBZ for bioavailability studies. Briefly, a solid-phase extraction technique, using Bond-Elut C_{18} (Waters Associates, U.S.A.) as the stationary phase and acetonitrile as the mobile phase, was employed to extract the drug from plasma. Freshly prepared degassed mobile phase (acetonitrile/ethanol/water 25:25:50) was used at a flow rate 1.5 ml/min through a C_{18} μ-Bondapak (Waters Associates, U.S.A.) analytical column and the eluent was detected at 285 nm. 50 μl samples were injected into a Shimadzu LC-600 instrument equipped with a Beckman 164 variable-wavelength UV detector.

Phase-solubility studies Phase-solubility studies were performed according to the method reported by Higuchi and Connors (1965). Exactly 50 mg of CBZ were weighed into each 20 ml scintillation vial, to which were added 10 ml of water containing various concentrations of HPβCD (5–100 mM). The sealed vials were shaken for 2 days at a controlled temperature of 37 $\pm$ 0.5°C. After 2 days, an aliquot was withdrawn and filtered through a 0.45 μm Millipore filter. The concentration of CBZ in each aliquot was determined spectrophotometrically at 285 nm with reference to a suitably constructed standard curve.

Preparation of solid complex The CBZ:HPβCD complex in a 1:1 molar ratio was prepared by mixing 236 mg of CBZ and 1450 mg of HPβCD in a flask with 100 ml of water. The mixture was heated under reflux for 2 h and then stirred for 1 day at room temperature. Subsequently, this was stored at −70°C overnight and then lyophilized. The powder was then dried in a 40°C vacuum oven overnight. Chemical analysis was performed to confirm the stoichiometry of the complex system.

Physicochemical properties IR spectroscopy of the KBr pellet was performed using a Perkin-Elmer Model 257 FTIR spectrophotometer. X-ray diffraction spectroscopy was carried out using a Norelco X-ray diffractometer with a Norelco's Ni filter CuK(α) radiation detector. The scanning speed used was 2° 2θ/min. The DSC scans were recorded by a Perkin-Elmer Model 1B apparatus equipped with a low-temperature cell and nitrogen as the purging gas. The above scans were performed on CBZ, HPβCD, the physical mixture, and the complex.

Dissolution rate Dissolution studies were performed using the U.S.P. dissolution apparatus

(paddle method) in 500 ml phosphate buffer of pH 7.4 as the dissolution medium. The stirring rate was 100 ± 2 rpm and temperature was maintained at 37 ± 0.5°C. Dissolution samples were passed through 30 mesh and retained on 80 mesh USP standard and contained the equivalent of 100 mg carbamazepine.

Bioavailability studies in rats The bioavailability of the prepared complex and the drug was tested by administering the drug orally to male Sprague-Dawley rats. Six rats (200–250 g) were fasted overnight and dosed in the morning with 25 mg CBZ equivalent/kg suspended in 0.1% NaCMC solution (5 ml/kg). Blood samples were withdrawn at appropriate intervals up to 24 h through an indwelling catheter implanted in the right jugular vein. Plasma samples were frozen (−4°C) until analyzed. The plasma concentration-time data were analyzed using the PCNONLIN (Statistical Consultant Inc.) computer program. The area under the curve (AUC), peak plasma concentration and time to reach the peak plasma concentration were determined from the estimated pharmacokinetic parameters to compare the bioavailability of the inclusion complex with that of the pure drug.

Results and Discussion

The phase solubility diagrams shown in Fig. 1 can be classified as type A_L according to Higuchi and Connors (1965). Fig. 1 was plotted according to the following equation:

$$S/S_0 - 1 + \frac{K_F[\text{HP}\beta\text{CD}]}{1 + K_F S_0} \quad (1)$$

where S_0 is the solubility of CBZ in the absence of HPβCD, and S represents the solubility in the presence of different concentrations of HPβCD. Because the straight line had a slope less than unity, it was assumed that the increase in solubility observed was due to the formation of a 1:1 complex. The observed rate constants for the formation of the complex (K_F) were calculated for HPβCD and βCD according to Eqn 2 and were found to be 655 and 332 M^{-1}, respectively.

$$K_F = \frac{\text{slope}}{(1 - \text{slope}) \cdot \text{intercept}} \quad (2)$$

As observed from Fig. 1 a considerable increase in solubility of CBZ was obtained with HPβCD. In contrast, no appreciable increase in solubility was achieved with βCD. This might be due to the greater aqueous solubility of HPβCD as compared with βCD.

The IR spectra of the physical mixture (Fig. 2) did not show any significant differences from the respective spectra of the pure compounds. However, the IR spectrum of the inclusion complex exhibits some significant differences. The absorption peaks characteristic of the carbonyl group of CBZ in the range 1600–1800 cm^{-1} and the amino groups in the range 3200–3600 cm^{-1} have disappeared from the spectrum of the inclusion complex. These spectral changes may have resulted from the inclusion of CBZ within the cavity of HPβCD.

Supporting evidence for the complex formation was also obtained from thermal analysis studies (Fig. 3). DSC analysis of the complex did not show any endothermic peak between 204 and 206°C which corresponds to the melting point of the pure CBZ. The disappearance of the endothermic peak of the CBZ from the complex provided a further indication of the formation of the inclusion complex.

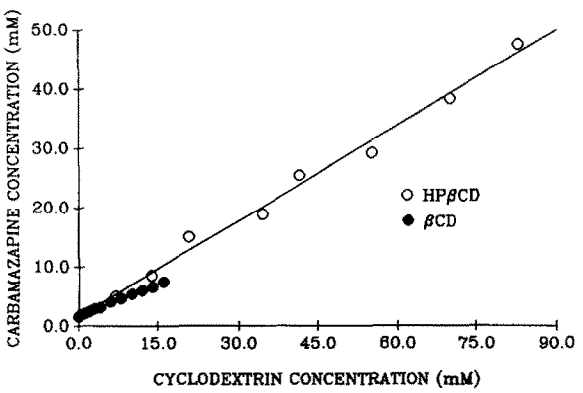

Fig. 1. Phase solubility diagram of carbamazepine-cyclodextrin systems in distilled water at 37°C.

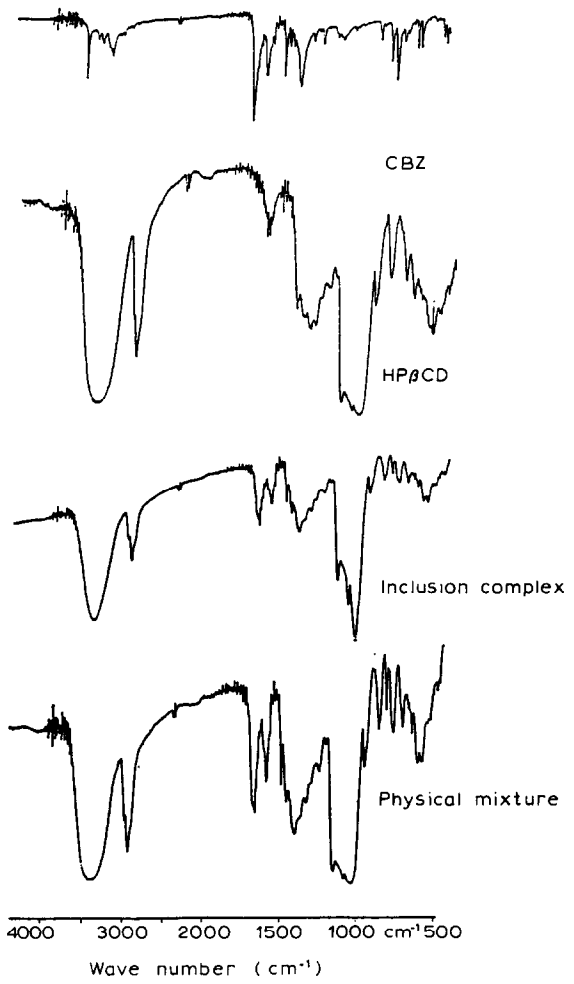

Fig. 2. IR spectra of carbamazepine alone, HPβCD alone, complex system of CBZ with HPβCD, and physical mixture of CBZ with HPβCD.

Further evidence of complex formation was obtained from X-ray powder diffraction patterns (Fig. 4). The diffraction pattern of the physical mixture was simply the superimposition of each component with the peaks having lower intensity. This may be attributed to a reduction in particle size during the preparation of the physical mixture. On the other hand, the diffraction pattern of the complex showed only a very few peaks with very low intensity. This indicated that the inclusion complex is markedly less crystalline than the physical mixture or the pure component.

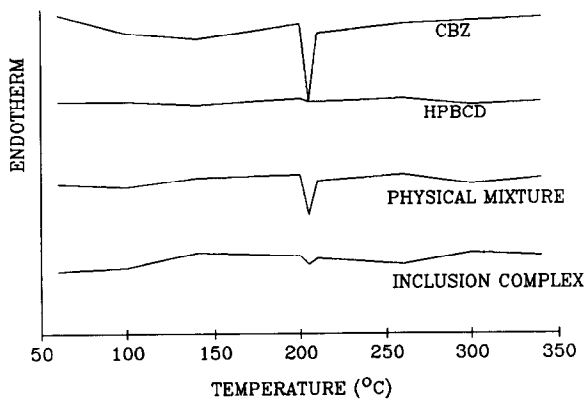

Fig. 3. DSC curves of pure drug, HPβCD, physical mixture, and inclusion complex.

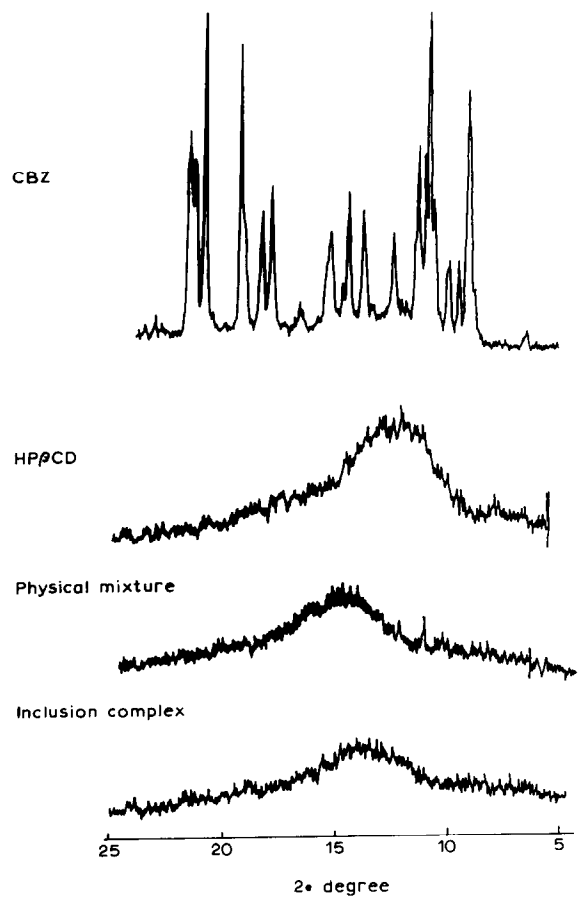

Fig. 4. Powder X-ray diffraction pattern of carbamazepine alone, HPβCD alone, complex system of CBZ with HPβCD, and physical mixture of CBZ with HPβCD.

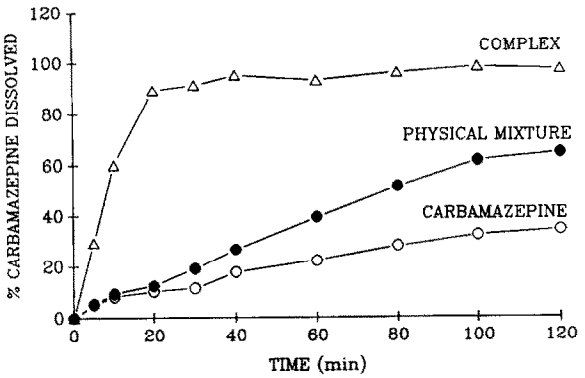

Fig. 5. Dissolution profiles of carbamazepine, its complex with HPβCD, and physical mixture in phosphate buffer pH 7.4 at 37°C by USP dissolution apparatus (paddle method).

Fig. 5 shows the dissolution profiles of CBZ from complex, physical mixture, and powders. It is evident that the release of CBZ was significantly enhanced by complexation. After 20 min, the percentage of drug released was approx. 20 and 90% for the powdered drug and the complex samples, respectively. Additionally, in this study, improved wettability of CBZ in the samples of complex was observed. The enhanced dissolution rate is probably due to increased solubility, decreased crystallinity and improved wettability (Goldberg et al., 1966).

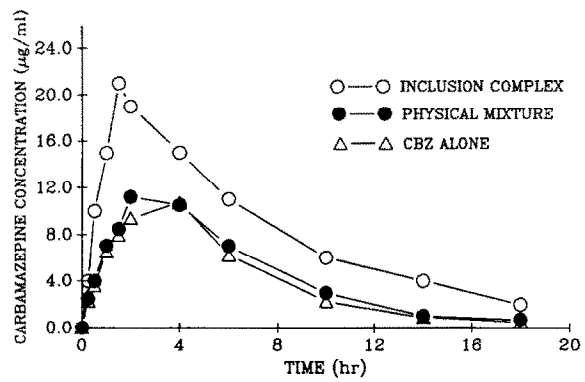

Fig. 6. Plasma concentration-time profiles of carbamazepine after oral administration of the pure drug, the physical mixture, and the inclusion complex (equivalent to 25 mg CBZ/kg) in rats.

TABLE 1

Mean bioavailability parameters obtained after single oral doses of carbamazepine, physical mixture, or inclusion complex (equivalent to 25 mg CBZ / kg) in aqueous suspension in rats

Parameters	Mean ± S.E. ($n = 6$)		
	CBZ	Physical mixture	Inclusion complex
Peak plasma concentration (μg/ml)	10.7 ±2.1	12.2 ±2.5	21.4 ±4.9
Time to peak concentration (h)	3.98 ± 0.29	3.42 ±0.21	1.55 ±0.19
AUC_0^{12}	21.29 ± 2.94	25.71 ±3.22	46.74 ±5.42
Elimination rate constant (h^{-1})	0.44 ± 0.049	0.432 ± 0.052	0.394 ± 0.031

Fig. 6 illustrates the mean plasma concentration-time profile of CBZ after oral administration of pure drug, the physical mixture, and the inclusion complex in the rat. The plasma concentration-time curve can be appropriately described by the one-compartment open model and is in agreement with results reported in the literature (Bundgaard et al., 1982; Mendez-Alvarez et al., 1990). The derived pharmacokinetic parameters are presented in Table 1. In the case of CBZ alone, the maximum plasma level (C_{max}) of 10.7 ± 2.1 μg/ml was observed at 3.98 ± 0.29 h. On the other hand, the physical mixture and the HPβCD complex showed C_{max} values of 12.2 ± 2.5 μg/ml at 3.42 ± 0.21 h and 21.4 ± 4.9 μg/ml at 1.55 ± 0.19 h, respectively. The area under the curve of the complex up to 12 h post-administration was 2.09 times as much as that of the CBZ alone.

The above data clearly indicate that the inclusion complex is much more bioavailable in rats than the pure drug itself. This enhancement in drug absorption is probably due to increased solubility of the drug and fast dissolution rate of the complex. Thus, HPβCD could be a useful additive to solid CBZ formulations as it may result in a more rapid absorption and improved bioavailability of the drug.

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