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Improvement in the percutaneous absorption of beclomethasone dipropionate by γ-cyclodextrin complexation

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Inclusion complex formation of beclomethasone dipropionate (BMDP) with γ-cyclodextrin (γ-CyD) in water and in solid state was assessed by solubility analysis and X-ray diffractometry. The solid complex of BMDP with γ-CyD in a molar ratio of 1:2 was prepared and its in-vitro release behaviour was investigated using an ointment release simulator. The release of BMDP from hydrophilic ointment was significantly improved by the γ-CyD complexation. Permeation and uptake studies indicated that the enhanced release of BMDP from the ointment may be due to the faster dissolution and the lower binding affinity of the complex in the ointment base. The vasoconstrictor activity of BMDP in man was found to be increased by γ-CyD complexation, suggesting an improvement in the percutaneous absorption of BMDP.

Many in-vitro drug release studies have been made in attempts to relate with in-vivo percutaneous absorption from ointments (Idson 1975: Inagi et al 1981). Washitake et al (1980) reported that the vasoconstrictor activities of various ointments containing betamethasone 17-valerate depended upon the rate of drug release from the ointments. We have previously reported (Otagiri et al 1984) that the release of betamethasone from gel and hydrophilic ointments was significantly improved by inclusion complexation with β- and γ-cyclodextrins (β- and γ-CyD). However, it was recently found that β-CyD caused skin irritation at high concentrations, while no noticeable effect was observed for γ-CyD (Uekama et al 1982a). Thus, the present study was undertaken to survey the usefulness of γ-CyD in topical applications by examining the in-vitro/in-vivo relation of percutaneous drug absorption from ointments containing the γ-CyD complex. Beclomethasone dipropionate (BMDP), which has a powerful vasoconstrictor activity, was used as a test compound because its γ-CyD complex was readily obtainable in a pure form.

MATERIALS AND METHODS

Materials

Beclomethasone dipropionate (BMDP) was donated by Mitsubishi Yuka Pharmaceutical Co. (Ibaraki, Japan). α-, β- and γ-cyclodextrins (α-, β- and γ-CyD) were obtained from Nihon Shokuhin Kako Ltd (Tokyo, Japan) and recrystallized from water. All other materials and solvents were of analytical reagent grade. Deionized and double distilled water was used throughout.

The powder X-ray diffraction patterns were obtained with a Rigaku Denki Geiger Flex 2012 (Tokyo, Japan) using Ni-filtered Cu-K_α radiation. High performance liquid chromatography (HPLC) was with a Hitachi 635A (Tokyo, Japan) equipped with a variable-wavelength detector.

Solubility studies

Solubility measurements were carried out according to Higuchi & Lach (1954). Excess amounts of BMDP were added to aqueous solutions containing various concentrations of CyDs, and shaken at 34 ± 0·5 °C. After equilibration (approximately 10 days), an aliquot was centrifuged and pipetted through a cotton plug. A portion of the sample (1 ml) was then diluted with ethanol–water (1:1 v/v%) and analysed spectrophotometrically. An apparent stability constant, K′ was calculated from the initial linear portion of phase solubility diagrams according to Higuchi & Connors (1965). The solid complex was derived by mixing appropriate amounts of BMDP and γ-CyD in water. The amounts were calculated from the descending curvature of the phase solubility diagram (see Fig. 1). That is, 0·7 g of BMDP and 31·1 g of γ-CyD were added to 300 ml water, then the mixture was sealed in a flask and stirred at 34 °C for 10 days. The complex, which precipitated as a microcrystalline powder, was filtered and dried under a vacuum at 25 °C for 48 h. This powder

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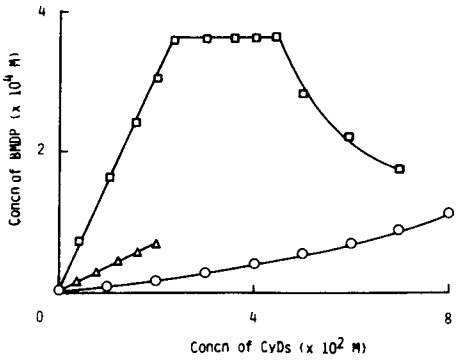

FIG. 1. Phase solubility diagrams of BMDP-CyD systems in water at 34 °C. ○, α-CyD system; △, β-CyD system; □, γ-CyD system.

corresponded to a 1:2 BMDP-γ-CyD complex and had a molecular weight of 3115.

Ointment release studies

Hydrophilic ointment base was prepared according to the Japanese Pharmacopoeia X. BMDP and its γ-CyD complex were mixed and homogenized with the base, and the content of BMDP adjusted to 1·0 w/w% in the base. The release of BMDP from ointment bases containing BMDP or its complex, was determined using an ointment release apparatus (Sartorius Co. Ltd, Göttingen, FRG) with a cellophane membrane as reported by Otagiri et al (1984). At appropriate intervals, 10 ml samples were removed from the release phase, and were then extracted with chloroform. The concentration of BMDP was determined by HPLC in which Lichrosorb RP-18 column (4 mm × 25 cm containing 15 μm particles, Merk, Darmstadt, FRG), with acetonitrile–methanol–water (35:30:35) as the mobile phase was used. Detection was at 241 nm and peak heights were compared with those obtained from the internal standard, butyl *p*-hydroxybenzoate.

Uptake by ointment base

The general procedure is essentially the same as the method of Nakano & Patel (1970). The ointment base was packed in one compartment cell and a 100 ml portion of a 2 μM BMDP solution in the absence and presence of 1 mM γ-CyD was placed in the release compartment. The decrease in BMDP content of the release solution was determined spectrophotometrically.

Membrane permeation studies

Permeation behaviour of BMDP through a cellophane membrane was examined using a permeation cell apparatus described previously (Uekama et al 1980). The sample powder (20 mg) of BMDP or its equivalent amount of γ-CyD complex was placed in 50 ml of water in a donor cell. The solutions in the permeation cell were stirred with a magnetic bar at 91 rev min^{-1} at 34 °C. Corrections were applied for the cumulative dilution. At appropriate intervals, 1 ml samples were pipetted from the receptor solution and were then extracted with 6 ml of chloroform. After centrifugation (2000 rev min^{-1}, 10 min), the organic phase (5 ml) was transferred to a new tube, and the solvent was evaporated to dryness on a water-bath at 40 °C under reduced pressure. The residue was dissolved in 100 μl methanol and assayed for BMDP by HPLC.

Vasoconstrictor activity

The vasoconstrictor activity was measured according to the modified method of McKenzie & Stoughton (1962). The test samples were prepared as dilutions in four-fold dilutions of BMDP and its γ-CyD complex with hydrophilic ointment base ranging from 1:10 to 1:10204. About 50 mg of each ointment was applied in random order to two sites on the backs of 10 healthy male volunteers concurrently at intervals of 5 min. Occlusion was achieved by using an adhesive patch test plaster (diameter: 16 mm, Torri Pharm. Co. Ltd, Tokyo, Japan). At 4 h after application of the ointments, the degree of skin blanching was visually assessed by 3 independent observers at 8 h after application. The skin blanching at each application site was noted as 'present' or 'absent', and the vasoconstrictor activity was expressed as the incidence of response.

RESULTS AND DISCUSSION

Inclusion complexation of BMDP with γ-CyD

Fig. 1 shows the phase solubility diagrams obtained for BMDP and three CyDs in water. The differences in the solubility curves are obvious. The solubility of BMDP increased with increasing concentrations of α- and β-CyDs within the limit of their aqueous solubilities, showing a feature of A-type phase solubility diagram (Higuchi & Connors 1965). On the other hand, the γ-CyD system showed a typical B_S-type solubility curve (Higuchi & Connors 1965) with precipitation of the microcrystalline complex occurring at high γ-CyD concentrations. The 1:2 stoichiometry observed for BMDP with γ-CyD on the basis of the data in the plateau region was in excellent agreement with that obtained by isolation and analysis of the crystalline complexes. Since the molecular size of BMDP is too large to be included,

even in a cavity of γ-CyD, it is reasonable to assume that at least one complex with a stoichiometric host-to-guest molecules ratio of greater than unity may be formed, in particular for higher concentrations of CyDs. Thus, the apparent stability constant, K′, assuming that a 1 : 1 complex is initially formed, increased in the order of γ- > β- > α-CyD. The K′ values obtained at 34 °C were small compared with those at 25 °C reported previously (Uekama et al 1982b). The decrease in K′ values with increasing temperature shows the exothermic nature of inclusion complexation, which is generally observed for CyD-organic drug molecule interactions (Ikeda et al 1975; Hardee et al 1978).

Fig. 2 shows the powder X-ray diffraction patterns of the BMDP-γ-CyD system. The diffraction pattern of the physical mixture was simply the sum of those of the components, while that of the complex was apparently different from the patterns of each constituent, as shown in Fig. 2. It was also found that the complex gave a somewhat diffuse diffraction pattern with decreased intensity, suggesting that it is less crystalline than the physical mixture. The ir spectra of the BMDP-γ-CyD complex was also compared with those of the physical mixture. The band near 1750 cm^{-1} assigned to the carbonyl-stretching of C_3 in BMDP shifted by about 10 cm^{-1} to a lower wave number.

In addition, an endothermic peak due to the melting of BMDP observed around 210 °C disappeared completely in the γ-CyD complex formation. These data apparently indicate that BMDP forms an inclusion complex with γ-CyD in aqueous solution and solid phase.

Drug release from ointment base

The release behaviour of the γ-CyD complex from the hydrophilic ointment base was compared with that of BMDP alone. Fig. 3 shows the amount of BMDP released from hydrophilic ointments containing BMDP and its complex as a function of the square root of time. It is evident that the release rate of BMDP was significantly improved by complexation. The linearities of plots, except the initial delay

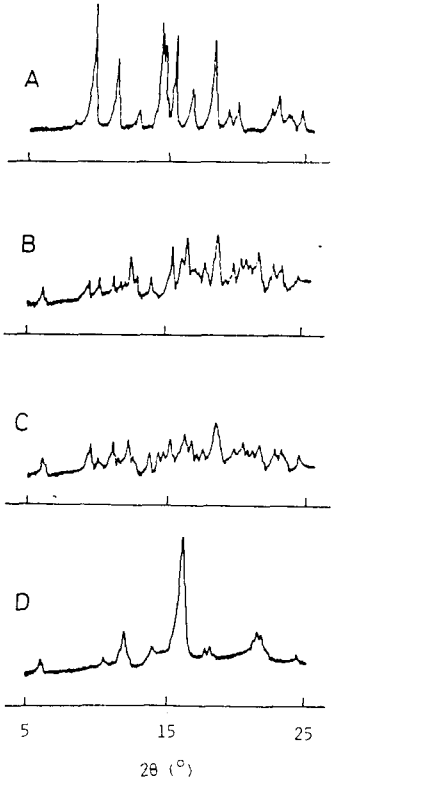

FIG. 2. Powder X-ray diffraction patterns of BMDP-γ-CyD systems. A, BMDP; B, γ-CyD; C, physical mixture of BMDP and γ-CyD; D, complex of BMDP with γ-CyD.

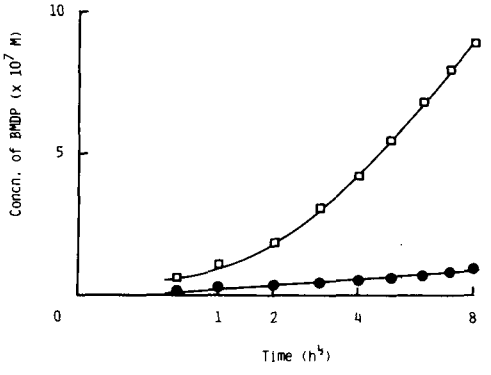

FIG. 3. Release profiles of BMDP from hydrophilic ointment containing BMDP and its γ-CyD complex in water at 34 °C. ●, BMDP; □, γ-CyD complex.

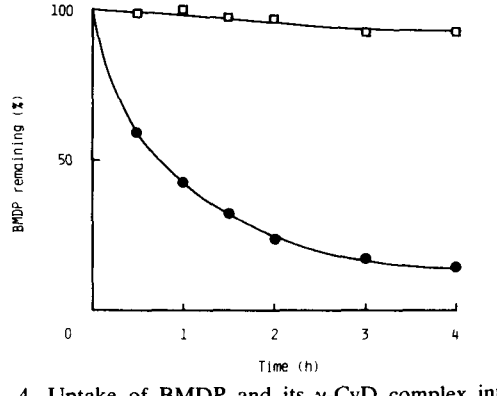

FIG. 4. Uptake of BMDP and its γ-CyD complex into hydrophilic ointment base at 34 °C. ●, BMDP; □, γ-CyD complex.

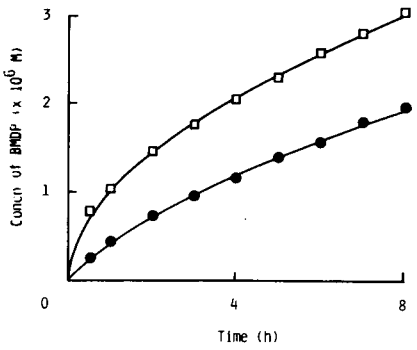

FIG. 5. Permeation profiles of BMDP from the suspension of BMDP powder and its γ-CyD complex in a donor cell through a cellophane membrane in water at 34 °C. ●, BMDP; □, γ-CyD complex.

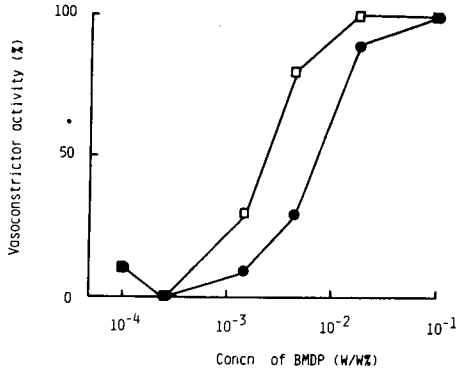

FIG. 6. Vasoconstrictor activities of the hydrophilic ointments containing BMDP and its γ-CyD complex. ●, BMDP; □, γ-CyD complex.

for the complex, may indicate that release of BMDP follows the diffusion control (Higuchi 1960).

To gain insight into the mechanisms of enhanced release due to γ-CyD complexation, the uptake and membrane permeation studies were further examined. The drug uptake from aqueous solution through a cellophane membrane into the hydrophilic ointment was measured to evaluate the relative affinity of the drug and its complex for the base. As shown in Fig. 4, the uptake of BMDP from the complex into the base was fairly slow compared with that of the drug itself by the base. Fig. 5 shows the permeation profiles of BMDP through a cellophane membrane following the dissolution from BMDP or its complex in a donor cell. The rapid dissolution of the γ-CyD complex resulted in an increase in the net amount of BMDP permeating into the receptor cell. The bulky γ-CyD complex may have poorer permeability because the permeation mechanism is mainly pore size-controlled (Iwaoku et al 1982). In fact, the increase in permeation rate of the complex was small compared with that expected from the dissolution profiles. Therefore, the rapid dissolution of the complex more than cancels out the negative effect due to the poor permeability and produces a net increase in drug permeation. These results indicate that the enhanced release by γ-CyD is mainly due to lower affinity and faster dissolution of the complex in the ointment base.

Vasoconstrictor activities of BMDP and its complex

The vasoconstrictor activities of hydrophilic ointments containing BMDP and its γ-CyD complex were examined. Fig. 6, shows the vasoconstrictor activity of BMDP was increased on complexation. In addition, the ED50 value, estimated by the method of Litchfield & Wilcoxon (1949) was lowered by complexation (ED50 = $9{\cdot}0 \times 10^{-3}$ w/w% for BMDP alone; $2{\cdot}7 \times 10^{-3}$ w/w% for γ-CyD complex). This may also be due to the faster release of drug from the ointment containing the γ-CyD complex. The present data clearly suggest that an improvement of topical bioavailability of BMDP can be obtained by means of γ-CyD complexation.

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