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Effects of β - and γ -Cyclodextrins on Release of Betamethasone from Ointment Bases¹⁾

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In vitro release of betamethasone (BeM) from ointment bases containing BeM or its β - and γ -cyclodextrin (β - and γ -CyD) complexes was investigated by using an ointment release simulator with artificial double-layer membranes. The release of BeM from gel and hydrophilic ointments was significantly improved by CyD complexation, owing to increases in the apparent rates of dissolution and membrane permeation of the drug. The enhanced release of BeM from the two ointments may be attributed to the faster dissolution of the complex and the lower binding affinity of the complex to the ointment bases. The present data suggest that an improvement of topical bioavailability of BeM can be obtained by means of inclusion complexation.

Keywords—betamethasone; β -cyclodextrin; γ -cyclodextrin; inclusion complex; release from ointment base; gel ointment; hydrophilic ointment; artificial double-layer membrane; dissolution rate; permeation rate

Various methods have been employed to evaluate *in vitro* release from topical preparations, because the drug release from ointment bases can be considered as a reliable indication of the bioavailability.²⁻⁴⁾ Recently, Washitake *et al.*⁵⁾ reported that the vasoconstrictor activities of various ointments containing betamethasone 17-valerate correlated well with the drug release from the ointments. It was also found that topical corticosteroid activities depend upon the physicochemical properties of the drugs, such as solubility and partition coefficient.⁶⁾

Cyclodextrin (CyD) complexations have been extensively applied in the pharmaceutical fields to improve the physical and chemical properties of drug molecules through inclusion complexation.⁷⁻⁹⁾ We have previously reported that some pharmaceutical properties of steroid hormones, such as dissolution, membrane permeation and absorption, were improved by CyD complexation.^{10,11)} Thus, the present work was undertaken to survey the possible utility of CyD in ointments. Betamethasone (BeM) was used as a test compound, because its complexes with β - and γ -CyDs were readily obtainable in pure form. The release of BeM and its CyD complexes from gel and hydrophilic ointments were examined by using an ointment release simulator with artificial double-layer membranes.¹²⁾

Experimental

Materials—BeM was kindly supplied by Showa Yakuhin Kako Co., Ltd. (Kawasaki, Japan) and recrystallized from ethanol-water. β - and γ -CyDs were purchased 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 study. The solid CyD complexes were prepared in the same manner as described previously.¹⁰⁾ That is, 0.6 g of BeM and 4.54 g of β -CyD were added to 200 ml of water, and sealed in a flask, then the mixture was stirred with a magnetic stirrer at 25°C for 7 d. The complex, which precipitated as a microcrystalline powder, was filtered and dried under a vacuum at 40°C for 48 h. This powder corresponded to a 1:2 BeM- β -CyD complex which had a molecular weight (M.W.) of $2660.5 \pm 5\%$. Similarly, 2:3 BeM- γ -CyD complex

TABLE I. Apparent Stability Constants (K') and Stoichiometries for BeM-CyD Systems at 25 °C

System	K' (M^{-1})	Stoichiometry (BeM : CyD)	
		Solubility analysis	Chemical analysis
α -CyD	220		
β -CyD	5420	1.00 : 1.98	1.00 : 2.08
γ -CyD	21600	2.00 : 2.98	2.00 : 3.00

(M.W. = $4672.9 \pm 5\%$) was prepared on the basis of the phase solubility diagrams.¹⁰ The apparent 1:1 stability constants (K') and the stoichiometries of the complexes are listed in Table I. These two complexes and the BeM powders were passed through a screen (100 mesh) and used for the following studies.

Dissolution and Membrane Permeation Studies—An amount equivalent to 40 mg of BeM was weighed and put in 25 ml of water in a 60-ml beaker. The jacket of the beaker was maintained at 34 °C throughout the measurement, corresponding to the dermal temperature in humans. The suspension was stirred with a magnetic bar at 91 rpm. At appropriate intervals, 0.5 ml samples were pipetted through a cotton filter, diluted with water, and assayed spectrophotometrically. Corrections were applied for the cumulative dilution caused by replacing the samples with equal volumes of the original medium.

Permeation behavior of BeM through the double-layer membranes was examined by using the permeation cell apparatus described previously.¹³ The artificial double-layer membranes used were the same as those in the ointment release experiments. In the permeation cell, 50 ml of BeM solution (1.6×10^{-4} M) in the absence or presence of β - and γ -CyDs (3.2×10^{-4} and 2.4×10^{-4} M) was put into the donor compartment, while the same volume of water was put into the acceptor compartment. In the case of the suspension sample, the sample powder (60 mg) of BeM or an equivalent amount complex was put in 50 ml of water in the donor cell. The solutions in the permeation cell were stirred with a magnetic bar at 91 rpm at 34 °C. Corrections were again applied for the cumulative dilution. At appropriate intervals, 1 ml samples were pipetted from the receptor solution and the samples were then extracted with 5 ml of chloroform. After centrifugation (2000 rpm, 10 min), the organic phase (4 ml) was transferred to a new tube, and the solvent was evaporated off on a water-bath at 40 °C under reduced pressure. The residue was dissolved in 100 μ l of methanol, and assayed for BeM by high performance liquid chromatography (HPLC). HPLC was performed using an ATTO model HSLC-013 instrument (Tokyo, Japan). The eluent was monitored spectrophotometrically at 242 nm. The separation utilized a reverse-phase column (LiChrosorb RP-18, 5 μ m, 4 ϕ \times 250 mm; Merck, Darmstadt, FRG) operating at a flow rate of 0.7 ml/min with ethanol-water (7:3) as a mobile phase. Butyl *p*-hydroxybenzoate was used as an internal standard.

Ointment Release Studies—Hydrophilic ointment base was prepared according to JPX, and gel ointment base was prepared by the method of Shima *et al.*⁴ BeM and its CyD complexes were dissolved or suspended in these ointment bases, and the concentration of BeM was adjusted to 0.2 w/w% in all bases. The release of BeM from BeM or its complexes in the ointment bases was determined by using an ointment release apparatus (Sartorius Co., Ltd., Göttingen, FRG)¹² with artificial double-layer membranes, as illustrated in Fig. 1. The artificial membranes, which consist of a cellulose nitric acid ester membrane soaked in a Component N[®] and a cellophane membrane, were combined and then inserted directly into the release chamber with the hydrophilic side on the ointment, as shown in Fig. 1. The temperature of the release phase (100 ml of water) was held at 34 °C. At appropriate intervals, 10 ml samples were removed from the release phase, and were extracted with chloroform. The concentration of BeM was determined by HPLC.

All the experiments were done in triplicate, and results were reproducible within $\pm 5\%$.

Results and Discussion

Dissolution and Membrane Permeation

Figure 2 shows the dissolution profiles of BeM from β - and γ -CyD complexes and BeM powder in water at 34 °C. It is evident that the CyD complexes dissolved much more rapidly than BeM alone. The enhanced dissolution rate of BeM may be due to the increase in solubility and/or the decrease in crystallinity resulting from inclusion complexation. In fact, it

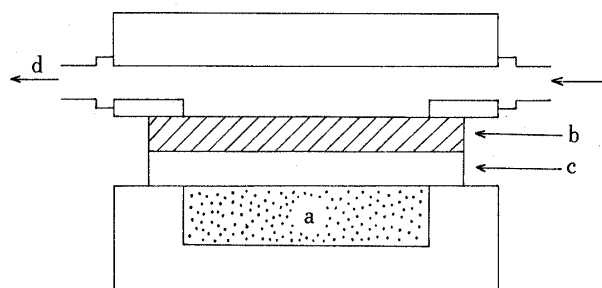


Fig. 1. Schematic Diagram of the Ointment Release Chamber

a, ointment; b, lipophilic membrane; c, hydrophilic membrane; d, circulating release phase.

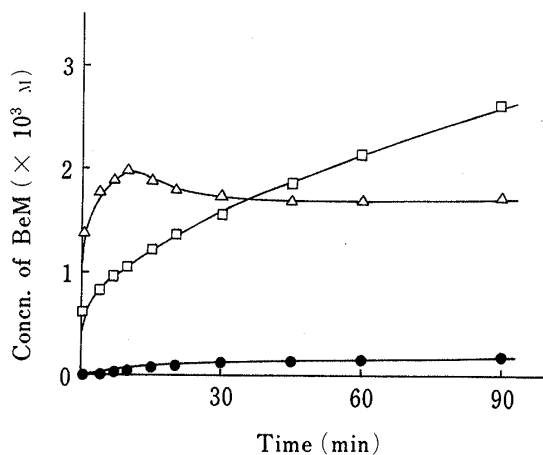


Fig. 2. Dissolution Profiles of BeM from BeM Powder and BeM-CyD Complexes in Water at 34°C, Measured by the Dispersed Amount Method

●, BeM alone; △, β-CyD complex; □, γ-CyD complex.

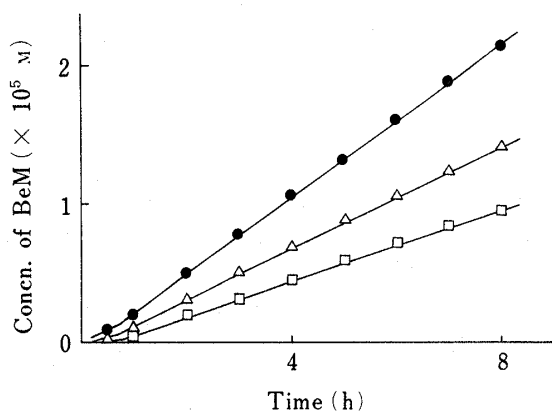


Fig. 3. Effects of CyDs on Permeation of BeM through Artificial Double-Layer Membranes in Water at 34°C

●, without CyDs; △, with β-CyD; □, with γ-CyD.

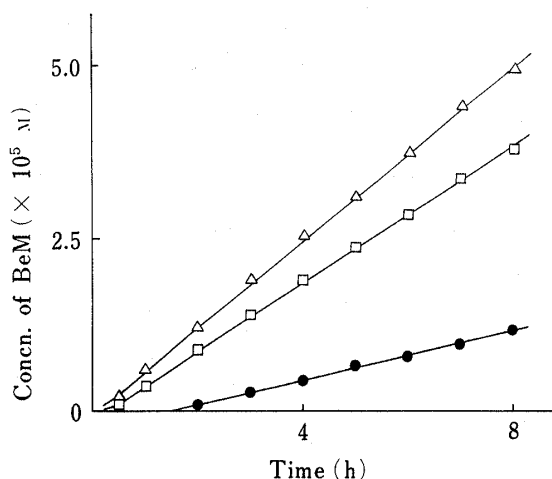


Fig. 4. Permeation Profiles of BeM from Suspensions of BeM Powder and BeM-CyD Complexes through Artificial Double-Layer Membranes

●, BeM alone; △, β-CyD complex; □, γ-CyD complex.

was found that the apparent solubilities ($S_{\beta} = 1.7 \times 10^{-3} \text{ M}$, $S_{\gamma} = 3.2 \times 10^{-3} \text{ M}$)¹⁴⁾ of the β- and γ-CyD complexes were about 7 and 13 times higher, respectively, than that of BeM alone, and also that the CyD complexes gave somewhat diffuse X-ray diffraction patterns compared with the drug itself.¹⁰⁾

Figure 3 shows the permeation of BeM from the donor solution through the artificial double-layer membranes in the absence and presence of β- and γ-CyDs. In the presence of CyDs, it appeared that the greater the free drug concentration in the donor cell (β-CyD system > γ-CyD system, as expected from their K' values), the greater the permeation of the drug. This may be due to the poor permeability of the bulky and hydrophilic complex (γ-CyD complex > β-CyD complex) because the permeation through the double-layer membranes

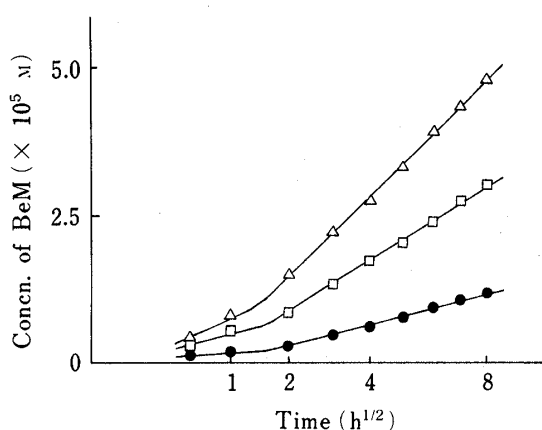


Fig. 5. Release Profiles of BeM from Gel Ointments Containing BeM and BeM-CyD Complexes in Water at 34°C

●, BeM alone; △, β -CyD complex; □, γ -CyD complex.

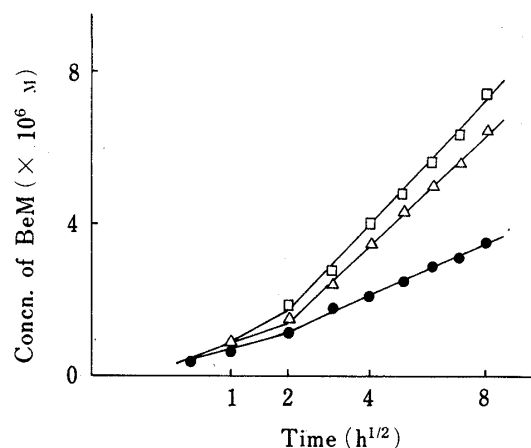


Fig. 6. Release Profiles of BeM from Hydrophilic Ointments Containing BeM and BeM-CyD Complexes in Water at 34°C

●, BeM alone; △, β -CyD complex; □, γ -CyD complex.

may be mainly pore size- and partition-controlled.^{15,16)} On the other hand, when the test powders were suspended in the donor cell, an increase in permeation of BeM as a result of CyD complexation was observed, as shown in Fig. 4. In this case, the enhanced permeation of BeM from the complex can be explained on the basis of the dissolution characteristics of the test samples. That is, the rapid dissolution of the complex more than cancels out the negative effect of the poor permeability of the complex and produces a net increase in drug permeation.

Drug Release from Ointments

The release behavior of CyD complexes from various ointments was examined and compared with that of BeM alone. Figure 5 shows the amount of BeM released from gel ointments containing BeM and its CyD complexes as a function of the square root of time. It is evident that the release rate of BeM was significantly improved by inclusion complexation, particularly with β -CyD. The enhanced release of BeM from the β - and γ -CyD complexes was also obtained from the hydrophilic ointment, as shown in Fig. 6. The linearities of the plots except for the initial delay in Figs. 5 and 6 may indicate that the release of BeM is diffusion-controlled.¹⁷⁾ The enhanced release of BeM from CyD complexes may be due to the faster dissolution of the complexes and lower binding affinity of the complexes in the ointment bases. The differences in the results shown in Fig. 5 and Fig. 6 for β - and γ -CyD complexes may be ascribed to the different binding affinities of the complexes to the two ointment bases, depending upon their hydrophilicities. However, further investigation is necessary to elucidate the release mechanism of CyD complexes across the double-layer membranes, since some effect of interaction of the drug with membrane components after dissociation of the complex cannot be excluded.

In any case, the present results suggest that CyD complexation may provide good topical bioavailability of BeM. A study is in progress on the correlation between the *in vitro* results and *in vivo* percutaneous absorption by using these CyD complexes.

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References and Notes

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