Improvement in Percutaneous Absorption of Prednisolone by β- and γ-Cyclodextrin Complexations

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In vitro release characteristics of prednisolone (PD) and its β- and γ-cyclodextrin (β- and γ-CyD) complexes were investigated by using an ointment release simulator with artificial double-layer membranes. The release of PD from hydrophilic ointment was significantly improved by β- and γ-CyD complexations. Permeation and uptake studies indicated that the enhanced release of PD from the ointment may be mainly due to the faster dissolution of PD in the base and the lower binding affinity of PD to the ointment base as a result of the CyD complexations. The percutaneous absorption of PD from hydrophilic ointment after application to the rabbit skin was also increased by CyD complexations. The in vitro and in vivo data suggest that CyDs can improve the topical bioavailability of PD.

Keywords—prednisolone; β-cyclodextrin; γ-cyclodextrin; inclusion complex; membrane permeation; ointment release; percutaneous absorption; rabbit; hydrophilic ointment

Introduction

Since the introduction of topical corticosteroid formulations, their use has become widespread because of the potent antiinflammatory activity of these drugs. The activities of topical corticosteroids are expected to depend upon the physicochemical properties of the drugs, such as solubility and partition coefficient. Moreover, it was found that the release of drugs, including steroid hormones, from topical dosage forms is affected by the composition of the vehicle and the thermodynamic activity of the drugs in the vehicle. We have previously reported that the release rate of betamethasone from gel and hydrophilic ointments was significantly improved by inclusion complexation with β- and γ-cyclodextrins (β- and γ-CyDs). It was also shown that the apparent rates of dissolution and membrane permeation of prednisolone (PD) were greatly improved by β- and γ-CyD complexations, resulting in increased serum levels of the drug following oral and rectal administrations. Thus, the present paper deals with the effects of β- and γ-CyDs on the release rate of PD from hydrophilic ointment in vitro, in comparison with in vivo data.

Experimental

Materials—PD was donated by Nakarai Chemicals Ltd. (Kyoto, Japan), and recrystallized from ethanol-water. β- and γ-CyDs were purchased from Nihon Shokuhin Kako Co., 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 complexes of PD with β- and γ-CyDs in the molar ratio of 1:2 and 2:3 were prepared in the same manner as previously described.

Ointment Release Studies——Hydrophilic ointment base was prepared according to JPX. After being passed through a 100 mesh sieve, CyD complexes were kneaded thoroughly with the base, and the content of PD was adjusted to 1.0% in the base. By means of polarized microscopic observations (×1000), fine solid particles of PD or
its CyD complexes were found to be distributed in both the inner and outer phases of o/w type ointment. The release of PD from ointment bases containing PD or its complexes was determined by using an ointment release apparatus (Sartorius Co., Ltd., Göttingen, FRG) with artificial double-layer membranes, as previously reported. The temperature of the release phase (100 ml of saline) was held at 34°C. At appropriate intervals, 1 ml samples were removed from the release phase, and mixed with 5 ml of methylene chloride. After centrifugation (2000 rpm, 10 min), the organic phase (3 ml) was transferred to a 15-ml glass-stoppered centrifuge tube and evaporated to dryness at 40°C. The extract was reconstituted in 100 μl of methanol and then 10 μl of the solution was injected into a high-performance liquid chromatography (HPLC) equipped with a LiChrosorb RP-18 column (10 μm in 4.6 i.d. × 250 mm, Merck) operating at a flow rate of 1.0 ml/min, with methanol-acetonitrile-water mixture (3 : 3 : 4) as a mobile phase. The eluate was monitored spectrophotometrically at 248 nm by measuring the peak height in comparison with those of known amounts of internal standard (cortisone acetate).

Membrane Permeation Studies——The permeation behavior of PD through the double-layer membranes was examined by using the permeation cell apparatus described previously. The artificial double-layer membranes used were the same as those in the ointment release experiments. The sample powder (100 mg) of PD or an equivalent amount of β- or γ-CyD complex was placed in 50 ml of saline solution in a donor cell. The solution in the permeation cell was stirred with a magnetic bar at 91 rpm at 34°C. At appropriate intervals, 1 ml samples were pipetted from the receptor solution and extracted with 4 ml of chloroform. After centrifugation (2000 rpm, 10 min), the organic phase (3 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 μl of methanol and assayed for PD by HPLC, as described above. Corrections were made for the cumulative dilution caused by replacement of samples with equal volumes of the original medium.

Uptake by Ointment Base——The general procedure is essentially the same as that of Nakano and Patel. The ointment base was packed in one compartment cell and a 100 ml portion of 4.7 × 10⁻⁴ M PD solution in the absence or presence of 9.4 × 10⁻⁴ M β-CyD or 7.1 × 10⁻⁴ M γ-CyD was placed in the release compartment. The decrease in PD content of the release solution was determined by HPLC, as described above.

In Vivo Studies——Five rabbits weighing 2.7–3.0 kg were used at intervals between applications of more than two weeks. The hair was removed with electric hair clippers from the intended dosing region of the back, 24 h prior to application of the ointment. The ointment base was packet uniformly over the surface of six sheets of thin plastic films (2 × 2 cm²), and the sheets were applied to the shaved surface of the dorsal skin of rabbits. To ensure close contact between the ointment and the skin, the films were covered with adhesive tape. The ointment samples were recovered periodically from the dorsal skin by wiping with absorbent cotton. The ointment samples and the absorbent cotton were then transferred to a new tube and dissolved with 10 ml of methanol with sonication for 30 min. After centrifugation (2000 rpm, 10 min), the supernatant was discarded and a 10 μl aliquot of the aqueous layer was assayed by HPLC as described above. In the pretreatment experiment, the ointment containing β-CyD alone was applied on the rabbit skin. After the removal of that ointment, the ointment containing PD was then applied, as described above.

Results and Discussion

Drug Release from Ointment Base

The release behavior of the β- and γ-CyD complexes from the hydrophilic ointment base was compared with that of PD alone. Figure 1 shows the amount of PD released from hydrophilic ointments containing PD or its complexes as a function of the square root of time. It is evident that the release rate of PD was significantly increased by complexation, particularly with β-CyD. The linearity of the plots, except for the initial delay in the case of the β-CyD complex, may indicate that release of PD is diffusion-controlled. However, there was no detectable amount of CyDs in the release phase under these experimental conditions. This suggests that only the free form of the drug can penetrate into the release phase from the ointment base through the artificial membranes.

To gain insight into the mechanism of enhanced drug release due to β- and γ-CyD complexations, uptake and membrane permeation studies were carried out. The drug uptake from saline solution through a cellophane membrane into the hydrophilic ointment was measured to evaluate conveniently the relative affinities of the drug and its complexes for the base in a manner similar to that reported recently. As shown in Fig. 2, the uptake of PD from the complexes into the base was fairly slow compared with that of the drug itself. Figure 3 shows the permeation profiles of PD through the artificial double-layer membranes.
following the dissolution from PD or its complexes in the donor cell. The rapid dissolution of β- and γ-CyD complexes resulted in an increase in the net amount of PD permeating into the receptor cell. The bulky and hydrophilic complexes seem to have poorer permeability because the permeation through the double-layer membrane is mainly pore size- and partition-controlled. Therefore, the increase in the permeation rate of the complexes was small compared with that expected from the dissolution profiles. Therefore, the rapid dissolution of the complexes may overcome the negative effect of the poor permeability, resulting in a net increase in drug permeation.

These results indicate that the enhancement of drug release may be mainly ascribable to the decrease in binding affinity of PD to the inner phase of the o/w type ointment together with the increase in the dissolution rate of PD in the outer phase of the ointment, owing to the hydrophilic CyD complex formation.

In this regard, it is anticipated that PD in CyD complexes may be displaced by some components of the ointment base. Microscopic observation revealed that small amounts of PD crystallites were present in the ointment containing CyD complexes. However, it seems likely that the extent of displacement is not significant because of the relatively large stability constant of PD–CyD complexes (3600 M⁻¹ for β-CyD complex and 3240 M⁻¹ for γ-CyD complex), as reported previously. In fact, it is apparent from Fig. 1 that β-CyD complex (having a larger stability constant) is much more effective in enhancing the drug release than γ-CyD complex.

**In Vivo Studies**

From the in vitro observations, it was suggested that β- and γ-CyDs may be useful to improve the topical bioavailability of PD from ointment preparations. Therefore, as a preliminary study on percutaneous absorption, the decrease in PD concentration in the ointment base was measured after application of the ointment to the rabbit skin. Figure 4 shows the time course of the residual amount of PD in the ointment base after the application of hydrophilic ointment containing PD or its complexes in the dorsal region of rabbits. As shown in Fig. 4, application of both CyD complexes resulted in extensive elimination of PD from the ointment base as compared with the drug alone. The average amounts of PD eliminated from the ointment base at 24 h after application of β- and γ-CyD complexes to
rabbits were 31.5% and 33.4% of the dose, respectively, while that in the case of the drug alone was 23.2%. Although little difference was found between β- and γ-CyD complexes, the degree of elimination of the drug in vivo was well correlated with that in the in vitro release studies. Interestingly, it was observed that pretreatment of rabbit skin with CyDs did not significantly alter the elimination rate of PD from the ointment base. Therefore, the superior percutaneous absorption expected for CyD complexes may be mainly owing to the faster dissolution and the lower binding affinity of the complex to the ointment bases rather than to direct interaction of CyD with the skin. However, recent studies have demonstrated that hydrophobic derivatives of CyDs such as dimethyl-β-CyD significantly enhanced the percutaneous absorption of drugs owing to the extraction of membrane components, which may result in modification of the skin barrier. In this regard, detailed data on the PD–dimethyl-β-CyD system will be reported elsewhere.

References