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Improvement in Biopharmaceutics of Prednisolone by β- and γ-Cyclodextrins

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Abstract. Inclusion complexes of prednisolone with β- and γ-cyclodextrins in the molar ratio of 1 : 2 and 2 : 3, respectively, were prepared, and their dissolutions, permeations through a cellophane membrane, releases from a suppository base, and *in vivo* absorption behaviors were examined. The apparent rates of dissolution and permeation of prednisolone were significantly increased by the formations of inclusion complexes with β- and γ-cyclodextrins. The release of the drug from Witepsol H_{15} suppositories was also increased by complexation. The serum levels of prednisolone following oral and rectal administrations of the cyclodextrin complexes to rabbits were higher than those of the drug alone. The enhanced initial absorption of prednisolone by cyclodextrin complexation suggested the possibility of smaller doses in prednisolone therapy.

Key words: Prednisolone; β- and γ-cyclodextrins; inclusion complex; dissolution; membrane permeation; suppository release; oral and rectal absorption; rabbit; serum level.

1. Introduction

Prednisolone is a potent therapeutically important synthetic corticosteroid used primarily for its anti-inflammatory activity. Because of its low solubility in water, the bioavailability of prednisolone preparations is known to vary significantly among brands and batches [1]. Cyclodextrin has been extensively applied to increase the solubility, dissolution rate, and absorption characteristics of poorly soluble drugs [2–6]. We have preliminarily reported the improvement of oral bioavailability of prednisolone by β-cyclodextrin complexation in humans [7]. In these continuing investigations, we now describe in detail the enhanced absorption of prednisolone in the form of its β- and γ-cyclodextrin complexes following the oral and rectal administrations in rabbits.

2. Materials and Methods

2.1. MATERIALS

Prednisolone was donated by Mitsubishi Yuka Pharmaceutical Co. (Ibaraki, Japan), and recrystallised from ethanol–water. β- and γ-cyclodextrins 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 doubly-distilled water was used throughout. The solid complexes of prednisolone with β- and γ-cyclodextrins in the molar ratio of 1 : 2 and 2 : 3, respectively, were prepared in the same manner as previously described [8].

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2.2. DISSOLUTION STUDIES

Dissolution rates of prednisolone and its cyclodextrin complexes were measured by the method of Nogami *et al.* [9]. For example, the equivalent amount of 50 mg of prednisolone as a 100-mesh powder was weighed and put into a dissolution cell. The dissolution medium (25 cm^3 of saline) was maintained at 37 °C and stirred at 91 rpm. At appropriate intervals, 1 cm^3 samples were removed from the flask. After the samples were centrifuged at 10000 rpm for 2 min, 0.5 cm^3 of the supernatant was assayed spectrophotometrically at 248 nm. Corrections were applied for cumulative dilution caused by replacing the sample by equal volumes of the original medium.

2.3. MEMBRANE PERMEATION STUDIES

The membrane permeation apparatus described previously [10] was used for the measurement of permeation behavior of the drug through a cellophane membrane. The cellophane membrane was distilled and washed with distilled water before use. In the permeation cell, 50 cm^3 of prednisolone (7.21×10^{-4} mol dm^{-3}) in the absence and presence of β- or γ-cyclodextrin (1.44×10^{-3} mol dm^{-3}, 1.08×10^{-3} mol dm^{-3}, respectively) was put into a donor compartment while the same volume of saline was put into a receptor cell. In the case of the suspension sample, the test powder (100-mesh) of prednisolone (100 mg) or its cyclodextrin complexes (equivalent to 100 mg prednisolone) was put directly into 50 cm^3 of saline in the donor cell and was stirred at 91 rpm in the thermostated water bath (37 °C). At predetermined intervals, 1 cm^3 samples were removed from the receptor cell and the concentration of prednisolone which had permeated from the donor cell was measured spectrophotometrically at 248 nm.

2.4. SUPPOSITORY RELEASE STUDIES

The suppositories were prepared by the fusion method using a typical hydrophobic base, Witepsol H_{15} (Dynamit Nobel Chemicals, West Germany). The drug or the complex was suspended in Witepsol H_{15} after the suppository base had been melted at 50 °C. The molten mass was poured into a suppository mold (Erweka GmbH, Frankfurt, West Germany). A sufficient quantity of the drug was suspended in each batch to give 15 mg of prednisolone in each 1 g suppository. The release of prednisolone from the suppositories was measured using a suppository release apparatus (Toyama Sangyo Co., Osaka, Japan) according to the procedure reported by Muranishi *et al.* [11]. In the cylindrical suppository chamber, the release phase was separated by a membrane filter (Milipore Filter SSWP 04700, 3.0 μm pore). The release phase used was a normal saline solution, stirred with a magnetic stirrer at 100 rpm at 37 °C. The rotation rate of the steel rod in the suppository chamber was 25 rpm. At appropriate intervals, 3 cm^3 samples were removed from the release phase, diluted with normal saline solution and assayed spectrophotometrically at 248 nm.

2.5. IN VIVO ABSORPTION STUDIES

Four rabbits weighing 2.3–2.7 kg were used at intervals of more than two weeks. They were fasted for 24 h prior to drug administration. Prednisolone or its cyclodextrin complex was administered orally (15 mg as equivalent of prednisolone) as a suspension in 30 cm^3 water, using a stomach catheter. The suppository (15 mg as equivalent of prednisolone) was inserted

into the rectum, and leakage from the rectum was prevented by a clip. Blood samples (3 cm^3) were taken from the ear vein at 0.5, 1, 2, 3, 5, and 7 h after the oral and rectal administrations. The blood samples were centrifuged (3000 rpm, 10 min), and the serum was stored in a refrigerator until assay.

2.6. ASSAY OF PREDNISOLONE IN SERUM

To 1 cm^3 of serum was added 1 cm^3 of 0.02 N NaOH and 6 cm^3 of dichloromethane. After centrifugation (2000 rpm, 10 min), the organic phase (5 cm^3) was transferred to a new tube. To the organic phase was added 1 cm^3 of 0.01 N H_2SO_4 and extraction with dichloromethane again performed. To 4 cm^3 of the organic phase was added ethylparabene as an internal standard, and the solvent was evaporated to dryness on a water bath at 40 °C under reduced pressure. The residue was dissolved in 100 µl of methanol, and assayed by high performance liquid chromatography (HPLC). The HPLC conditions were as follows: the chromatograph (FLC 700, Jasco, Tokyo, Japan) was equipped with a variable wavelength UV detector. The separation utilized a column of LiChrosorb RP-18 (10 µm in 4 mmϕ × 250 mm, Merck). The mobile phase consisted of methanol-distilled water (1 : 1), and the flow rate was of 0.9 $cm^3\ min^{-1}$. The mobile phase was filtered using a 0.20 µm filter and deaerated under vacuum. The eluent was monitored spectrophotometrically at 248 nm by measuring peak heights and comparing the height with that of known amounts of internal standard. The calibration curve in serum (0.2–2.5 µg ml^{-1}) had a slope of 0.537 and an intercept of 0.061 with a correlation coefficient of 0.998. The coefficient of variation was 2.5% (n = 6) for 1.5 µg ml^{-1} of prednisolone in serum.

3. Results and Discussion

3.1. IN VITRO STUDIES

Figure 1 shows the dissolution profiles of prednisolone from β- and γ-cyclodextrin complexes and prednisolone powders in solution saline at 37 °C. The dissolution patterns of the β- and γ-cyclodextrin complexes showed similar behaviors, however, the complexes dissolved much more rapidly than prednisolone alone. The enhanced dissolution rate of prednisolone may be due to the increase in solubility and/or the increase in surface area of the drug caused by inclusion complexation [8]. In fact, the apparent solubilities of the complexes (S_β = 5.2 × 10^{-3} mol dm^{-3}, S_γ = 3.0 × 10^{-3} mol dm^{-3}) in water at 25 °C were estimated to be about 4–7 times as much as that of prednisolone, on the basis of the B_s type phase solubility diagrams obtained [8]. In addition, the cyclodextrin complexes gave somewhat diffuse diffraction patterns, compared with that of prednisolone alone.

Figure 2 shows the permeation profiles of prednisolone from the donor solution through a Cellophane membrane in the absence and in the presence of β- and γ-cyclodextrins. It was shown that β- and γ-cyclodextrin complexes permeated poorly compared with prednisolone itself. This may be due to the poorer permeability of the bulky complex (relative to prednisolone) because the permeation mechanism through a cellophane membrane is mainly based on pore-size control [12]. On the other hand, when the test powders were suspended in the donor cell, an increase in permeation of prednisolone by cyclodextrin complexation was observed, as shown in Figure 3. In this case, the enhanced permeation of prednisolone from the complex can be explained on the basis of the permeation and dissolution characteristics of the test samples. That is, the rapid dissolution of the complex more than cancels out the

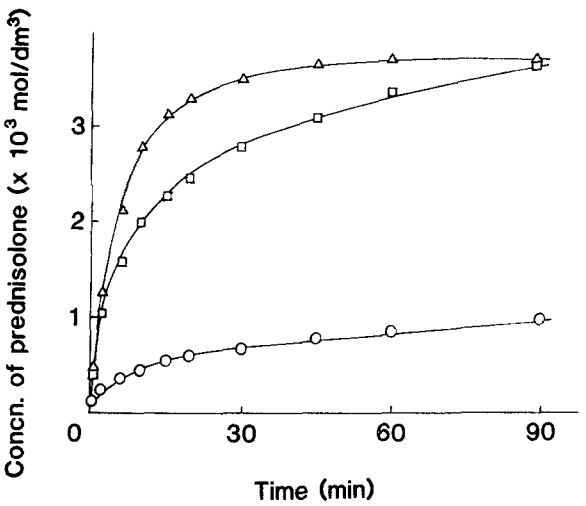

Fig. 1. Dissolution profiles of prednisolone and its cyclodextrin complexes in normal saline solution at 37 °C, measured by the dispersed amount method. All the points are the average of three determinations. ○: prednisolone alone, △: β-cyclodextrin complex, □: γ-cyclodextrin complex.

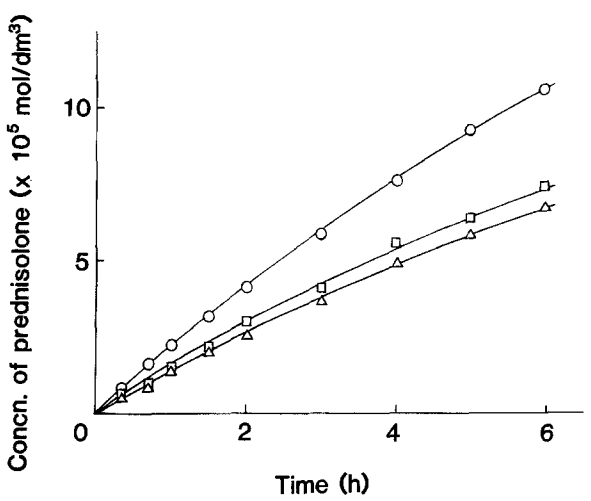

Fig. 2. Effects of cyclodextrins on the permeation of prednisolone through a cellophane membrane in normal saline solution at 37 °C. All the points are the average of three determinations. ○: without cyclodextrin, △: with β-cyclodextrin, □: with γ-cyclodextrin.

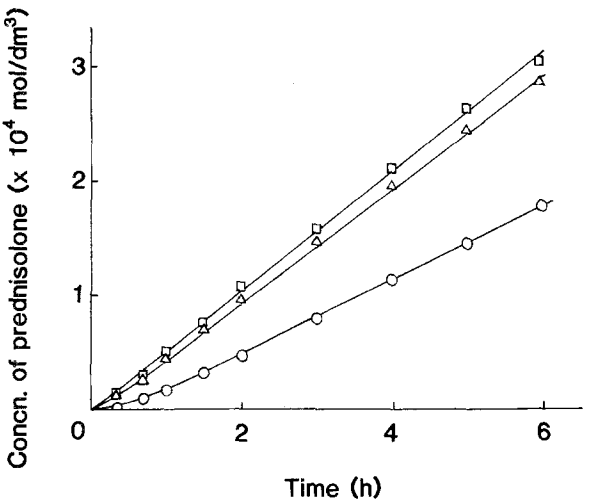

Fig. 3. Permeation profiles of prednisolone and its cyclodextrin complexes through a cellophane membrane in normal saline solution at 37 °C. All the points are the average of three determinations. ○: prednisolone alone, △: β-cyclodextrin complex, □: γ-cyclodextrin complex.

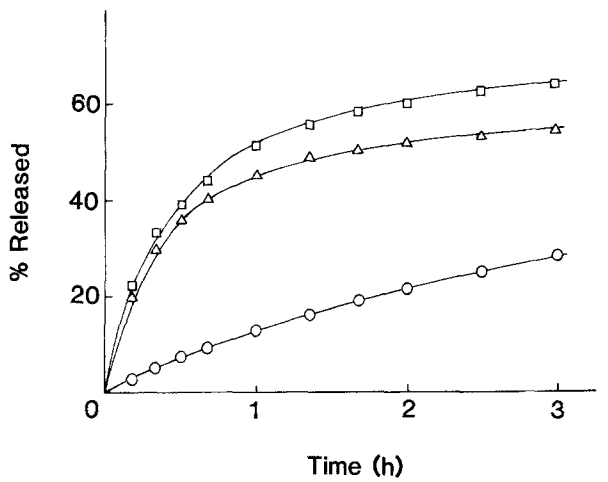

Fig. 4. Release profiles of prednisolone and its cyclodextrin complexes from Witepsol H_{15} suppositories in normal saline solution at 37 °C. All the points are the average of three determinations. ○: prednisolone alone, △: β-cyclodextrin complex, □: γ-cyclodextrin complex.

negative effect due to the poor permeability of the complex and produces a net increase in drug permeation.

Figure 4 shows the release profiles of prednisolone from the Witepsol H_{15} suppositories at 37 °C. It is evident that the release rate of prednisolone was significantly improved by inclusion complexation with β- and γ-cyclodextrins. In the case of the complexes, only 10 min

was required to release 20% of the drug from the suppositories, while 120 min was required to release the same amount (20%) of the drug from suppositories containing the drug alone. This may be attributed to the faster dissolution of complexes and the lower binding affinities of the hydrophilic complexes to the suppository base.

3.2. IN VIVO ABSORPTION STUDIES

β- and γ-cyclodextrin complexes of prednisolone were expected to have good bioavailability after oral and rectal administrations because the dissolution and release rate of the complexes were significantly superior to those of the drug alone. Thus, prednisolone and its complexes were administered orally and rectally to rabbits to evaluate their absorption characteristics. Figure 5 shows the mean serum levels of prednisolone followng the oral administration of 15 mg prednisolone or its cyclodextrin complexes to four rabbits. There was a significant difference in the peak concentrations between prednisolone and its cyclodextrin complexes. After administration of prednisolone, the maximum serum level (C_{max}) of 0.93 mg dm^{-3} was attained 2 h. On the other hand, the β-and γ-cyclodextrin complexes resulted in the rapid appearance of prednisolone in the serum, showing C_{max} values of 1.87 and 2.25 mg dm^{-3} after 1 h, respectively. The areas under the serum concentration time curves (AUC) of β- and γ-cyclodextrin complexes up to 7 h post administration, were about 1.5 times as much as that from prednisolone alone.

Figure 6 shows the mean serum levels of prednisolone following the rectal administration of Witepsol H_{15} suppositories containing 15 mg prednisolone or the complex. After administration of the drug alone, C_{max} of 0.252 mg dm^{-3} was attained after 2 h. On the other hand,

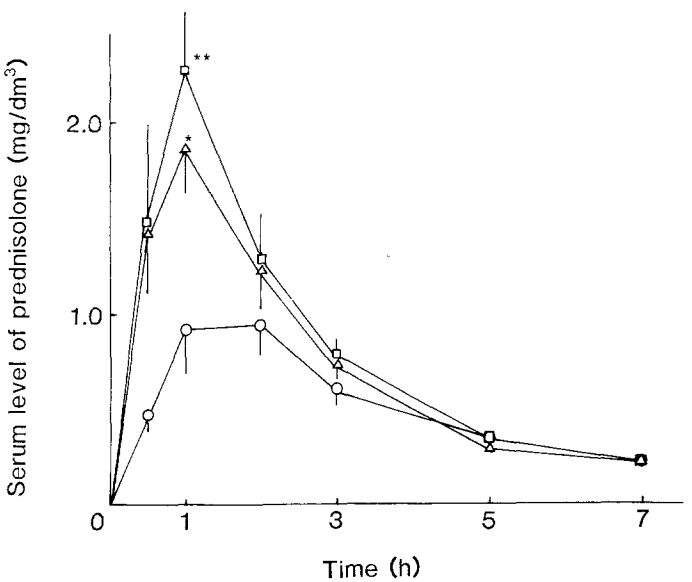

Fig. 5. Serum levels of prednisolone following the oral administration of 15 mg prednisolone and its cyclodextrin complexes to rabbits. ○: prednisolone alone, △: β-cyclodextrin complex, □: γ-cyclodextrin complex. Values represent the mean ± S.E. of 4 rabbits. *: $p < 0.05$ in (△) versus (○), **: $p < 0.02$ in (□) versus (○).

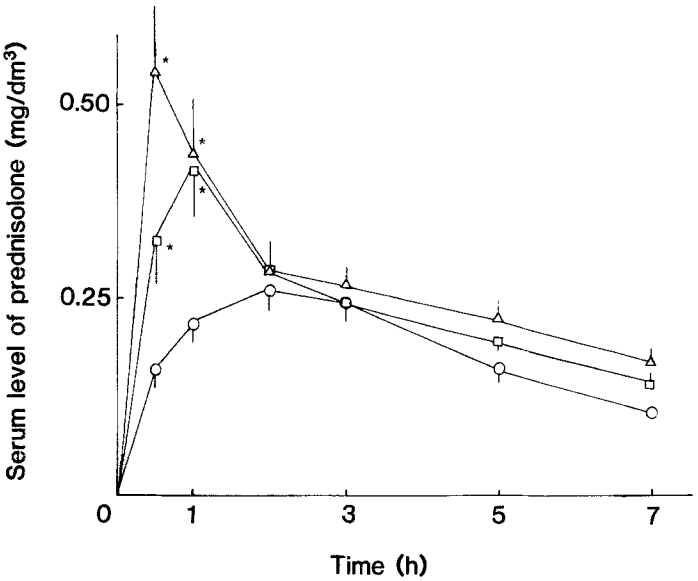

Fig. 6. Serum levels of prednisolone following the rectal administration of 15 mg prednisolone and its cyclodextrin complexes to rabbits. ○: prednisolone alone, △: β-cyclodextrin complex, □: γ-cyclodextrin complex. Values represent the mean ± S.E. of 4 rabbits. *: $p < 0.05$ in (△, □) versus (○).

the β- and γ-cyclodextrin complexes resulted in the rapid appearance of prednisolone in the serum as in the case of oral administration, showing C_{max} values of 0.592 mg dm^{-3} at 30 min and 0.402 mg dm^{-3} at 1 h, respectively. The AUC values of β- and γ-cyclodextrin complexes up to 7 h post administration were about 1.3–1.6 times as much as that from prednisolone alone. This may be ascribed to the rapid release of the drug from the suppository base containing the hydrophilic complexes. It is interesting to note that the extent of bioavailability of prednisolone after the rectal administration was almost one third as large as that of oral administration. The lower serum levels produced by the rectal administration route may be explained by the lesser amount of secretory fluid for dissolution of the drug compared with that in gastrointestinal tract. However, no significant difference between the β- and γ-cyclodextrin complexes was obtained following oral and rectal administrations, as would be expected from Figures 1 and 4.

The present investigation showed that the fast dissolving forms of prednisolone by β- and γ-cyclodextrin complexations apparently resulted in an increase in oral and rectal bioavailabilities of the drug, suggesting the possibility of smaller doses in prednisolone therapy. From a practical point of view, β-cyclodextrin may be a more suitable host molecule for prednisolone because it is easier to prepare the inclusion complex, compared with the more costly γ-cyclodextrin.

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