IMPROVEMENT OF ORAL BIOAVAILABILITY OF PREDNISOLONE BY β-CYCLODEXTRIN COMPLEXATION IN HUMANS

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Inclusion complex of prednisolone with β-cyclodextrin (β-CyD) in 1:2 molar ratio was prepared and its dissolution, membrane permeation and oral absorption behaviors were examined. The rates of dissolution and permeation through a cellophane membrane in water were significantly increased by β-CyD complexation. A crossover bioavailability study was performed using human subjects with lower doses of prednisolone tablets, where the plasma levels of the drug were determined by radioimmunoassay. The enhanced bioavailability of prednisolone by β-CyD complexation suggested the possibility of smaller doses and fewer side effects in prednisolone therapy.

Keywords—prednisolone; β-cyclodextrin; inclusion complex; dissolution; membrane permeation; oral absorption in human; enhanced bioavailability; radioimmunoassay

INTRODUCTION

Prednisolone is a potent, therapeutically important synthetic corticosteroid mainly used for its anti-inflammatory activity in a wide spectrum of diseases. Because of its low aqueous solubility, prednisolone has been included in a list of drugs susceptible to bioavailability problems.1) Until recently, effective bioavailability studies of prednisolone have been hampered by the lack of a sensitive assay method for determining the drug in biological fluids.

Cyclodextrin (CyD) complexations have been extensively utilized to increase the dissolution and absorption rates of poorly soluble drugs.2,3) We have recently reported that prednisolone forms a soluble complex with β-CyD in water and in solid phase.4) The present study was conducted to investigate the dissolution in water, permeation through a cellophane membrane with prednisolone and its β-CyD complex. Furthermore, a crossover bioavailability study was performed using adult male volunteers with lower doses of prednisolone tablets, measuring the plasma levels by means of radioimmunoassay.

MATERIALS AND METHODS

Materials—Prednisolone was donated by Mitsubishi Yuka Pharmaceuticals (Ibaraki, Japan), and recrystallized from ethanol–water. β-CyD was purchased from Nippon Shokuhin Kako (Tokyo, Japan), and recrystallized twice from water. All other materials and solvents were of analytical reagent grade. Deionized double-distilled water was used throughout.

A prednisolone–β-CyD complex was prepared according to the procedure reported previously.4) That is, 0.8 g of prednisolone and 6.8 g of β-CyD were added in 200 ml water, sealed in a flask, and the mixture was stirred with magnetic stirrer at 25°C for 7 d. The complex, which precipitated as a micro-crystalline powder, was filtered and dried under vacuum at 60°C for 48 h. This powder corresponded to a 1:2 prednisolone–β-CyD complex which had a molecular weight of 2628±5%.

Dissolution Studies—The pure powder (100 mesh) of prednisolone (40 mg) or its β-CyD complex (40 mg; equivalent to prednisolone) was put into 25 ml of water in a flask kept at 37°C. The suspension was stirred by a magnetic bar at a rate...
of about 300 rpm. A 0.5 ml of sample solution was pipetted through a cotton plug and its absorption at 246 nm was measured after appropriate dilution with ethanol-water (1:1).

The dissolution tests for 5 mg-prednisolone tablets (Fig. 2) were performed by the paddle method according to the specification of Japanese Pharmacopoeia X (JP X). The formulation of 5 mg-prednisolone tablet consisted of lactose (40 mg) as a diluent, magnesium stearate (0.5 mg) as a lubricant, and hydroxy propyl cellulose (5 mg) as a binder. The tablet was made by the direct compression method using a single-punch tablet machine (Okada Seiko KT-2, Tokyo, Japan). The β-CyD complex tablet (equivalent to 5 mg prednisolone) was also made similarly.

Membrane Permeation Studies — Permeation of prednisolone through a cellophane membrane (type 36/32, Visking Co.) was examined by using a permeation cell. The sample powder (400 mg, 100 mesh) of prednisolone or its β-CyD complex was put into 250 ml of water in a donor compartment while the same volume of water was placed in a receptor compartment. The solutions in permeation kept at 37°C by means of a thermostated water bath were stirred by a magnetic bar at a rate of 300 rpm. At predetermined intervals, a sample was pipetted from the receptor solution and the concentration of prednisolone which had permeated from donor cell was measured spectrophotometrically.

In Vivo Absorption Studies — A crossover bioavailability study was performed using six healthy adult male volunteers between ages of 22 and 30 years and weighing between 55 and 65 kg. According to the treatment schedule, each subjects ingested two compressed tablets of prednisolone or its β-CyD complex, which formulations were described in the section of dissolution studies. The evening before each treatment period, 1.0 mg of dexamethasone was administrated orally to each subject to suppress endogeneous secretion of cortisol. An interval of more than 10 d was allowed prior to the next treatment. Whole blood samples were taken from a fore-arm vein at predetermined intervals after prednisolone administration. The blood was centrifuged at 3000 rpm for 10 min and the plasma obtained was stored in a freezer until assay. According to the procedure reported, all plasma samples were assayed for prednisolone using a radioimmunoassay kit (Gamma Coat® Nippon Travenol, Japan). The amount of drug in the plasma samples was determined by interpolation from the calibration curve.

RESULTS AND DISCUSSION
Dissolution Behavior

Figure 1 shows the dissolution profiles of prednisolone from the complex and from prednisolone powder in water at 37°C. It is evident that the complexed form of prednisolone dissolved much more rapidly than prednisolone itself. The rapid dissolution of prednisolone-β-CyD complex from the compressed tablet was also obtained in the JP X dissolution test (Fig. 2). The enhanced dissolution rate may be due to the increase in solubility and/or the decrease in crystallinity of the drug in inclusion complexation as described previously.

Permeation Behavior

Figure 3 shows the permeation profile of prednisolone through a cellophane membrane, following the dissolution from prednisolone or its β-CyD complex powder in a donor cell. The faster the dissolution rate, the greater the net amounts of prednisolone permeated into the receptor cell was observed particularly for β-CyD complex. It appeared that the permeation rate of the complex was rather small compared with that expected from the dissolution profiles (Fig. 1). This may be due to the poor permeability of the bulky complex molecule, since the permeation mechanism through a cellophane membrane is mainly the pore-size control. In the case of the fast dissolution form of β-CyD complex, however, the negative effect due to poor permeability could be more than cancelled out by the net increase in the fraction of free drug available for permeation.

In Vivo Absorption Behavior

The bioavailability study was performed using adult male volunteers with lower dose (equiva-
lent to 10 mg prednisolone) of the compressed tablets, and the results are shown in Fig. 4. The plasma levels of drug following the oral administration of the complex were much higher during the initial 1 h period than those of drug itself. After administration of prednisolone, the maximum plasma level (C₀) of 0.36 μg/ml was

**FIG. 1.** Dissolution Profiles of Prednisolone and Its β-CyD Complex in Water at 37°C

○ : prednisolone; ● : β-CyD complex.

All the points are the average of three determinations.

**FIG. 2.** Dissolution Profiles of Prednisolone and Its β-CyD Complex Tablets in Water at 37°C measured by JPX Paddle Method

○ : prednisolone; ● : β-CyD complex.

All the points are the average of three determinations.

**FIG. 3.** Permeation Profiles of Prednisolone and Its β-CyD Complex through a Cellophane Membrane in Water at 37°C

○ : prednisolone; ● : β-CyD complex.

All the points are the average of three determinations.

**FIG. 4.** Plasma Concentrations of Prednisolone following the Oral Administration of 10 mg Prednisolone or Its β-CyD Complex Tablet to Human Subjects

○ : prednisolone; ● : β-CyD complex.

Each point represents the mean ± S.E. of 6 human subjects.

a) p < 0.001 in (●) versus (○).

b) p < 0.002 in (●) versus (○).
observed at 2.2 h \( (T_{\max}) \), then the concentration decreased gradually. In contrast the complex resulted in a rapid appearance of prednisolone in the plasma, and the concentration reached 0.54 \( \mu g/ml \) at 0.8 h after administration, followed by a rapid concentration decrease. The area under plasma concentration-time curve (AUC) of the complex up to 8 h was found to be about 1.2 times as much as that of prednisolone itself. It is interesting to note that the increase in AUC was rather small, regardless of the rapid dissolving form of \( \beta \)-CyD complex. This may be due to the relatively large stability constant of prednisolone-\( \beta \)-CyD complex (3600 \( M^{-1} \)) as reported previously, which may result in lesser release of free drug available for gastrointestinal absorption. However, the present approach of using rapid dissolving \( \beta \)-CyD complex seems to be promising for improving the oral bioavailability of prednisolone.

CONCLUSION

Although \( \beta \)-CyD complexation may retard the absorption of prednisolone due to the poor permeability and/or poor lipophilicity of the complex, a greatly enhanced dissolution rate of the drug more than cancels out the negative effects and produces a net increase in absorption rate. The rapid plasma appearance and subsequent clearance of the drug observed for the complex would be highly advantageous for the use of this form in oral prednisolone therapy. Furthermore, the 1:2 complexation of prednisolone with \( \beta \)-CyD results in 7.3-fold increase in molecular weight of the drug, which should facilitate the pharmaceutical preparation of the tablets, particularly from the viewpoint of content uniformity.

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