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Enhanced Bioavailability of Poorly Water-Soluble Clotrimazole by Inclusion with β-Cyclodextrin

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Clotrimazole, a poorly water-soluble antimycotic agent, is a promising agent for various diseases including cancer and sickle cell anemia. To improve the oral bioavailability of clotrimazole, the inclusion compound of clotrimazole with β-cyclodextrin was prepared by spray-drying method and characterized by phase solubility, differential scanning calorimetry and dissolution. Furthermore, the pharmacokinetics after oral administration in rats was then performed compared with clotrimazole powder. The solubility of clotrimazole increased linearly as a function of β-cyclodextrin concentration, resulting in A_L type phase solubility diagram which revealed a formation of inclusion compound in a molar ratio of 1:2, with the apparent association constant of 230.2 M^{-1}. The dissolution rate of clotrimazole in the inclusion compound increased greatly compared to clotrimazole powder in pH 7.4 phosphate buffer solution. The inclusion compound gave significantly higher initial plasma concentrations, Cmax and AUC of clotrimazole than did clotrimazole powder when they were administered as suspension form, indicating that the drug from inclusion compound could be more orally absorbed in rats. Thus, the oral bioavailability of clotrimazole could be improved markedly by inclusion complexation, possibly due to an increased dissolution rate.

Key words: Clotrimazole, Inclusion compound, Bioavailability, β-Cyclodextrin

INTRODUCTION

Clotrimazole [bis-phenyl-2-(chloro-phenyl)-1-imidazolyl-methane], a lipophilic, imidazole derivative, is an antimycotic agent with a broad spectrum (Pedersen *et al.*, 1998). Furthermore, it is a promising agent for various diseases including cancer and sickle cell anemia, neuroprotective effect (Wojtulewski *et al.*, 1980) and anti-inflammatory effect in patients with rheumatoid arthritis (Ning *et al.*, 2005). However, clotrimazole when administered orally, exhibits large differences in bioavailability due to its low aqueous solubility (solubility=0.49 mg/L) (Pedersen *et al.*, 1998; Pedersen, 1993) and slow dissolution in water (Pedersen, 1993). It has been attempted to improve the solubility of clotrimazole by microcapsule (Abdel-Moety *et al.*, 2002), thermosensitive gels (Chang *et al.*, 2002), liposome (Ning *et al.*, 2005), suspension with hydroxypropylmethyl cellulose and nanosphere (Memisoglu *et al.*, 2003).

Cyclodextrin complexation has been extensively applied to enhance the solubility, dissolution rate and bioavailability of slightly water-soluble drugs (Bekers *et al.*, 1991; Jarvinen *et al.*, 1995; Nakai *et al.*, 1984). The cyclodextrin complexes with slightly water-soluble drugs have been prepared using co-precipitation, kneading, neutralization, spray drying and freeze drying method (Abosehmah-Albidy *et al.*, 1997: Ammar *et al.*, 1996; Bekers *et al.*, 1991; Jarvinen *et al.*, 1995). Among them, spray drying has been extensively applied to prepare the inclusion complexes, since it has many advantages of a good yield in a short operating and suitability for extension to manufacturing scale (Nakai *et al.*, 1984; Pedersen, 1997).

Thus, in this study, to enhance the oral bioavailability of clotrimazole, the inclusion complex of clotrimazole with β-cyclodextrin was prepared by spray drying method. The phase solubility and dissolution of inclusion complex were carried out, and its pharmacokinetics after oral administration in rats was then performed compared with clotrimazole powder.

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MATERIALS AND METHOD

Materials

Clotrimazole was provided by Boryung Pharmaceutical Co. (Anyang, Korea). β-Cyclodextrin was purchased from Aldrich Chemical Co. (Milwaukee, WIS, U.S.A.). Semipermeable membrane tube (Spectra membrane tubing No. 1) was purchased from Spectrum Medical Industries Inc. (Los Angeles, California, U.S.A.). Ethanol was obtained from Duksan Pharmaceutical Co. (Seoul, Korea). All other chemicals were of reagent grade and used without further purification.

Preparation and physicochemical properties of inclusion complex

Preparation

A Büchi 190 nozzle type mini spray dryer (Flawil, Switzerland) was used for the preparation of clotirmazole-β-cyclodextrin inclusion complex. In brief, 0.2 g of clotrimazole and 1.6 g of β-cyclodextrin (molar ratio, 1:2) were dissolved in 100 mL ethanol and 100 mL water, respectively, and then mixed. The resulting clear solution was delivered to the nozzle at a flow rate of 5 mL/min using a peristaltic pump and thereafter spray-dried at 120°C inlet temperatures with a flow rate of 10 mL/min. The residue, clotrimazole-β-cyclodextrin inclusion complex was collected (Choi *et al.*, 2001; Lee *et al.*, 1999).

On the other hand, the physical mixture of clotrimazole and β-cyclodextrin (molar ratio 1:2) was prepared by gentle mixing 0.2 g of clotrimazole and 1.6 g of β-cyclodextrin in a mortar.

DSC curve

The formation of inclusion complex was conformed by DSC (Perkin-Elmer DSC7, U.S.A.) at a heating rate of 20/min over a 25-200 temperature range. A nitrogen purge was maintained throughout runs and base line optimization was performed before each run (Choi *et al.*, 2003).

Phase-solubility

Phase solubility studies were performed according to the method of Higuchi and Conners (1965). Briefly, excess of clotrimazole powder (50 mg) and various amounts of β-cyclodextrin were added in 5 mL water, respectively. They were shaken at 25°C for 5 days, filtered through a 0.45 μm filter (Millipore, Bedford, MA), and analyzed by UV/visible spectrophotometer (Shimadzu, UV-1201, Tokyo, Japan) at 262 nm.

Dissolution

Clotrimazole powder (50 mg) and inclusion complex (0.45 g) (equivalent to 50 mg clotrimazole) were packed in semipermiable membrane and placed in a dissolution tester (DST-600, Fine Chemical, Korea), respectively. Dissolution test was performed at 36.5°C using the paddle method at 50 rpm with 500 mL of phosphate buffer (pH 7.4) as a dissolution medium. At appropriate time intervals, 5 mL aliquots of solution were withdrawn, filtered immediately through a membrane filter and analyzed directly by UV/visible spectrophotometer at 262 nm (Beckers *et al.*, 1991; Becket *et al.*, 1999; Gandhi and Karara, 1988; Hassan *et al.*, 1990).

Pharmacokinetic study

In vivo experiments

Male Sprague-Dawley rats weighing 250±20 g were fasted for 12 h prior to the experiments but allowed free access to water. Tweleve rats were divided into two groups. All animals care and procedures were conducted according to the Guiding Priciples in the Use of Animals in Toxicology, as adopted by the Society of Toxicology (USP) in 1989.

Administration and blood-collecting

Each rat, anesthetized in an ether-saturated chamber, was secured on a surgical board in the supine position with a thread. A polyethylene tube was inserted into the right femoral artery of the rat, all of the incision was covered with wet cotton and the cannula was flushed with 0.2 mL of heparinized normal saline (80 U/mL) to prevent blood clotting. Clotrimazole powder and inclusion compound (0.36 g/kg equivalent to clotrimazole 80 mg/kg) were suspended in 1% povidone solution, and orally administered to rats in each group, respectively. Then, 0.25 mL of blood was collected from the right femoral artery at predetermined time and centrifuged at 3000 g for 20 min using a centrifuge 5415C (Eppendorf, U.S.A.) (Chang *et al.*, 2002; Ficarra *et al.*, 2000).

Blood sample analysis

Plasma (100 μL) was mixed with 10 μL of ethanol solution containing ibuprofen (100 μg/mL), as internal standard, 50 μL of 85% phosphoric acid, and extracted with 500 μL of dicloromethane. After vortexing vigorously for 1 min, it was then centrifuged at 3000 g for 2 min to separate organic phase. After evaporation of the organic phase in centrifugal vacuum concentrator, the residue was reconstituted with 50 μL of the mobile phase. Then, a 20 μL aliquot was analyzed by HPLC (Jasco UV-975, Japan) equipped with an Inertsil ODS-2 C_{18} column (GL science, 0.5 μm, 15 cm × 0.46 cm i.d.) and UV detector (Model L-7450). The mobile phase consisted of a mixture of methanol and 25 mM dibasic potassium phosphate buffer pH (6.3) (70: 30, v/v) adjusted pH to 4.8 with 1M phosphoric acid. The mobile phase was filtered through a 0.45 μm filter (Millipore, Bedford, MA) and ultrasonically deaerated

prior to the use. The eluent was monitored at 230 nm with a flow rate of 1.2 mL/min (Beckers *et al.*, 1991).

Pharmacokinetic data analysis

The non-compartmental pharmacokinetic parameters including area under the drug concentration-time curve (AUC) were calculated using RSTRIP II program (Salt Lake City, UT, U.S.A.). The maximal plasma concentration of drug (Cmax) and time to reach maximum plasma concentration (Tmax) were also obtained from plasma data. The data from different formulations were compared for statistical significance by the student t-test. All results were expressed as mean ± standard deviation (S.D.).

RESULTS AND DISCUSSION

The clotrimazole-b-cyclodextrin (1:2) inclusion complex was prepared easily by spray-drying 0.2 g clotrimazole and 1.6 g β-cyclodextrin. Fig. 1 illustrates DSC thermal curves of clotrimazole, b-cyclodextrin and inclusion complex (1:2, molar ratio). The DSC curve of clotrimazole shows one characteristic sharp endothermic peak at around 150 indicating the melting point of the drug. DSC curve showed that the sharp endothermic peak at around 150°C, which was observed for clotrimazole, decreased in the inclusion complex (1:2). Furthermore, the wide peak at 90°C, which was observed for β-cyclodextrin, shifted to 60°C in the inclusion complex, indicating that the inclusion complex did not contain a much residue of clotrimazole or β-cyclodextrin and thus suggesting that the drug is well dispersed in the β-cyclodextrin cavity (Ahmed *et al.*, 1998; Davis *et al.*, 1997; Linares *et al.*, 1997).

The apparent association constant (K_c) often plays an important role in explaining the various results obtained. The K_c for the complex formed was calculated from the slope of the phase-solubility diagram and the clotrimazole solubility at 25 in water for S_0, according to the following equation (Grant and Higuchi, 1990):

$$K_c = \text{Slope} / S_0(1 - \text{Slope}) \quad (1)$$

The aqueous phase solubility profile for the complex formation between clotrimazole and β-cyclodextrin at 25°C is shown in Fig. 2. This diagram illustrates that the apparent solubility of clotrimazole is maximum at 1:2 ratio and increased linearly as a function of the β-cyclodextrin concentration (correlation coefficient, 0.955) resulting in A_L type phase solubility curve. Moreover, Fig. 3 showed that the solubility of clotrimazole from inclusion complex (1:2) is much higher than other ratios. These findings suggested that the formation of the soluble clotrimazole/â-cyclodextrin inclusion complex with 1:2 stoichiometry according to Highuchi and Connors (1965). The apparent association constant of clotrimazole/β-cyclodextrin complex was found to be 80.2 M^{-1}. The aqueous solubility of clotrimazole determined in this study was 0.055 mg/mL which was in good agreement with that reported by Pedersen (1993).

To evaluate whether inclusion compound affected the dissolution rate of clotrimazole, the dissolution studies were performed for clotrimazole powder, physical mixture (1:2) and inclusion compound (1:2). The dissolution profiles of clotrimazole from the clotrimazole-loaded preparations are illustrated in Fig. 4. The dissolution rate of clotrimazole in the inclusion compound increased greatly compared to clotrimazole powder in pH 7.4 phosphate buffer solution. The amounts of clotrimazole dissolved from inclusion

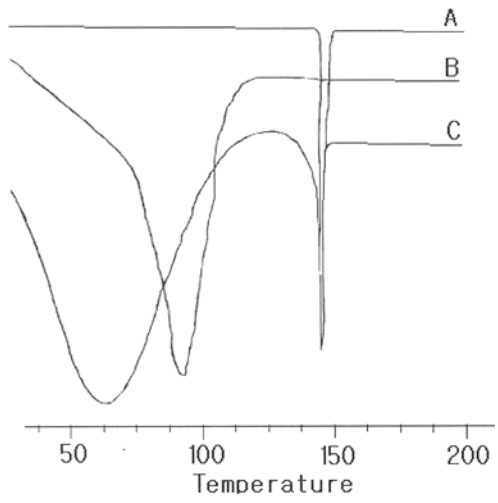

Fig. 1. DSC curves: (A) clotrimazole alone; (B) β-cyclodextrin; (C) inclusion complex.

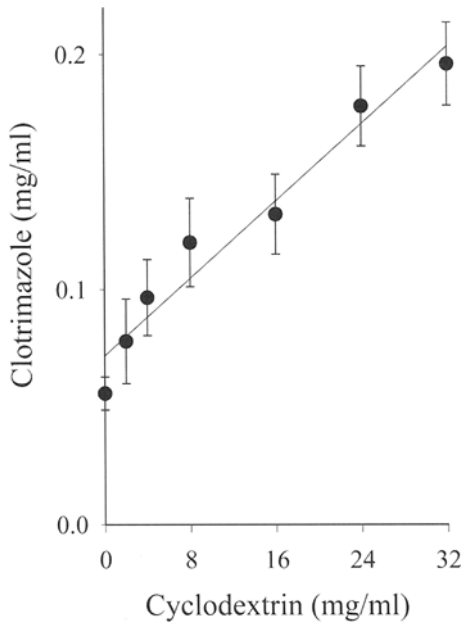

Fig. 2. Phase solubility diagram of clotrimazole-β-cyclodextrin system in distilled water at 25°C. Each value represents the mean ± S.D. (n =3).

complex in pH 7.4 phosphate buffer solution for 4 h increased about 2-fold compared to clotrimazole powder (13.2±1.6 vs. 29.0±5.7%). Corresponding physical mixture also demonstrated higher dissolution profile compared to pure drug. The significant enhancement in the dissolution rate of clotrimazole from the inclusion complex could be explained from the increased solubility wettability of the drug by the inclusion complexation (Gandhi and Karara, 1988; Hassan *et al.*, 1990). Furthermore, the increased dissolution rate of clotrimazole in physical mixture might be possibly attributable to the wetting effect of the β-cyclodextrin at the initial stage of the dissolution process (Özkan *et al.*, 2000; Stella and Rajewski, 1997). Thus, the inclusion complexation was useful for improving the dissolution rate of poorly water-soluble clotrimazole.

The plasma concentration profile of clotrimazole shown in Fig. 5, obtained after oral administration of clotrimazole powder and inclusion compound, showed the changes in mean plasma concentration of clotrimazole in rats. The total plasma concentrations of clotrimazole obtained from inclusion compound were higher compared with those in clotrimazole alone when they were administered as suspension form. In particular, the initial plasma concentrations of clotrimazole from inclusion compound, until 2 h, were significantly higher compared with those of clotrimazole powder ($P<0.05$). Our results suggested that the higher initial plasma concentrations of clotrimazole in inculsion compound were due to the increased dissolution rate of clotrimazole from the inclusion compound in rats (Kimura *et al.*, 1997; Stella and Rajewski, 1997).

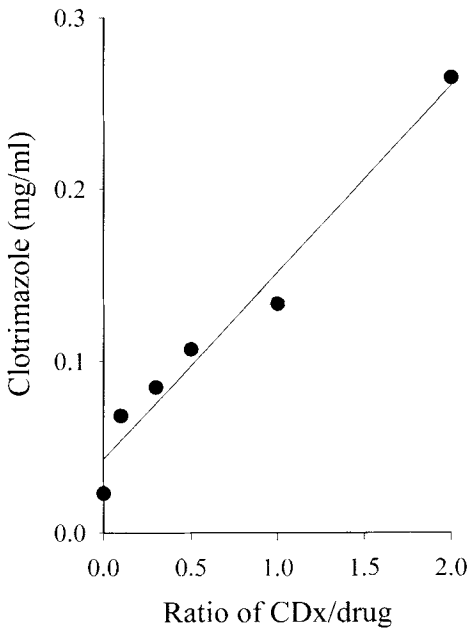

Fig. 3. Solubility diagram of different ratios clotrimazole-β-cyclodextrin inclusion complexes in distilled water at 25°C. Each value represents the mean ± S.D. (n =3).

The pharmacokinetic parameters are shown in Table I. The inclusion compound gave significantly shorter Tmax and higher AUC and Cmax of clotrimazole than did clotrimazole powder ($P<0.05$). In particular, the AUC of clotrimazole from inclusion compound was about 3-fold

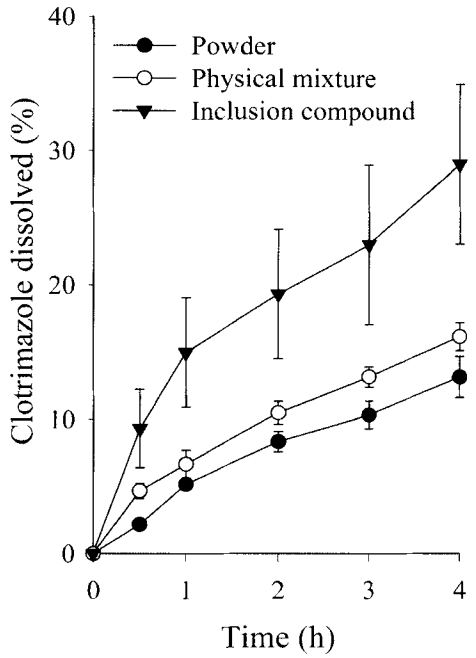

Fig. 4. Dissolution profiles of clotrimazole from powder, physical mixture and inclusion compound in pH 7.4 buffer solution. Each value represents the mean ± S.D. (n =6).

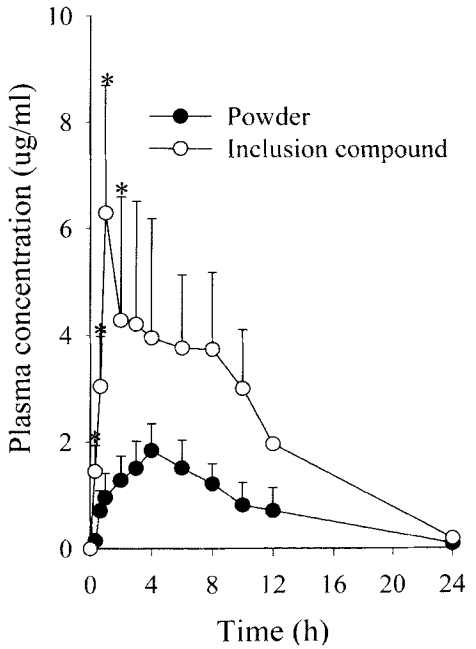

Fig. 5. Plasma concentration-time profiles of ibuprofen after oral administration of suspension form of clotrimazole powder and inclusion compound to rats. Each value represents the mean ± S.D. (n=6). *$P<0.05$ compared with clotrimazole powder.

Table I. Pharmacokinetic parameters of clotrimazole after oral administration of suspension form of clotrimazole powder and inclusion complex at a dose of 80 mg/kg to rats

Parameters	Clotrimazole powder	Inclusion compound
AUC (h·μg/mL)	18.90±6.24	56.15±35.70*
Tmax (h)	4.16±0.98	1.00±0.00*
Cmax ($_m$g/mL)	1.93± 0.46	6.29±3.39*
K_{el} (h^{-1})	0.19±0.10	0.20±0.08
$T_{1/2}$ (h)	4.50±2.47	3.90±1.32

* P<0.05 compared with clotrimazole powder.
** Each value represents the mean±S.D. (n=6).

higher than that from clotrimazole powder when they were administered as suspension form, indicating that the enhanced oral relative bioavailability of clotrimazole in the inclusion compound was contributed by the marked increase in the absorption rate of clotrimazole due to the increased dissolution rate of clotrimazole from the inclusion compound in rats (Becket *et al.*, 1999; Gandhi and Karara, 1988; Wong *et al.*, 2001). However, the K_{el} and $t_{1/2}$ values of clotrimazole from inclusion compound were not significantly different from those of clotrimazole powder. Our results suggested that the inclusion compound would be useful to deliver clotrimazole in a pattern that allows fast absorption in the initial phase, leading to better absorption.

CONCLUSION

It is concluded that the inclusion compound gave significantly higher initial plasma concentrations, Cmax and AUC of clotrimazole than did clotrimazole powder when they were administered as suspension form, indicating that the drug from inculsion compound could be more orally absorbed in rats. Thus, the inculsion compound was a more effective oral dosage form for poorly water-soluble clotrimazole. The further study on the oral bioavailability in human subjects of inclusion compound will be performed.

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