AAPS PharmSciTech, Vol. 14, No. 2, June 2013 (© 2013)
DOI: 10.1208/s12249-013-9973-x

Research Article

Formulation and *In Vivo* Evaluation of Orally Disintegrating Tablets of Clozapine/Hydroxypropyl-β-cyclodextrin Inclusion Complexes

Fan Zeng,[1] Ling Wang,[1] Wenjing Zhang,[1] Kejing Shi,[1] and Li Zong[1,2]

Received 29 January 2013; accepted 16 April 2013; published online 7 May 2013

Abstract. The aim of this study was to improve the solubility and oral bioavailability of clozapine (CLZ), a poorly water-soluble drug subjected to substantial first-pass metabolism, employing cyclodextrin complexation technique. The inclusion complexes were prepared by an evaporation method. Phase solubility studies, differential scanning calorimetry, X-ray powder diffraction, and Fourier transform infrared spectroscopy were used to evaluate the complexation of CLZ with hydroxypropyl-β-cyclodextrin (HP-β-CD) and the formation of true inclusion complexes. Characterization and dissolution studies were carried out to evaluate the orally disintegrating tablets (ODTs) containing CLZ/HP-β-CD complexes prepared by direct compression. Finally, the bioavailability studies of the prepared ODTs were performed by oral administration to rabbits. The ODTs showed a higher *in vitro* dissolution rate and bioavailability compared with the commercial tablets. It is evident from the results herein that the developed ODTs provide a promising drug delivery system in drug development, owing to their excellent performance of a rapid onset of action, improved bioavailability, and good patient compliance.

KEY WORDS: bioavailability; clozapine; hydroxypropyl-β-cyclodextrin; inclusion complexes; orally disintegrating tablets.

INTRODUCTION

Clozapine (CLZ), a potent antipsychotic with favorable pharmacodynamic properties, is widely used to suppress both the positive and the negative symptoms of schizophrenia, mania, and neuroleptic responses (1). However, CLZ can be classified as a class II drug according to the Biopharmaceutics Classification System, owing to its good permeation properties through biological membranes and low aqueous solubility. Its poor aqueous solubility may represent an obstacle for absorption and its bioavailability is limited by the hepatic metabolism (2,3). What is more, patients especially the psychotics find it difficult to swallow conventional solid dosage forms and, therefore, refuse to take their medications. This is also the case when patients are traveling or having no access to water, which may result in a high incidence of noncompliance and ineffective therapy (4).

Orally disintegrating tablets (ODTs) have been widely used to improve patient compliance and curative effects with an outstanding performance. ODTs have unique properties that involve the rapid disintegration and/or dissolution in contact with saliva and produce a suspension that can be easily swallowed by the patient without the need for water. Besides, a part of the dissolved drug can be absorbed directly through the pre-gastric route from the mouth, pharynx, and esophagus to produce a rapid pharmacological action or to improve bioavailability for drugs that are susceptible to hepatic first-pass metabolism (5,6).

Cyclodextrins (CDs) are cyclic oligosaccharides with hydrophobic internal cavities and hydrophilic outer surfaces. They have been well known to increase the solubility, stability, and bioavailability of hydrophobic drugs by the formation of inclusion complexes (7,8). Hydroxypropyl-β-cyclodextrin (HP-β-CD) is a hydroxypropylated derivative of β-cyclodextrin that has attracted growing interest because of its improved inclusion complexing ability, greater water solubility, and lesser toxicity (9,10). Literature lacks any data about the CLZ-CD systems and no related dosage forms are available on the market. Therefore, it is valuable to develop CLZ/HP-β-CD ODTs, providing a new oral solid dosage form with rapid onset, high bioavailability, and good patient compliance.

In the present study, we first prepared CLZ/HP-β-CD inclusion complexes by an evaporation method to improve the solubility and dissolution rate of CLZ. Phase solubility studies, differential scanning calorimetry (DSC), X-ray powder diffraction (XRD), and Fourier transform infrared spectroscopy (FTIR) were used to evaluate the complexation of CLZ with HP-β-CD and the formation of true inclusion complexes. Subsequently, characterization and dissolution studies were carried out to evaluate the ODTs prepared by direct compression. Finally, the bioavailability studies of the prepared ODTs were performed by oral administration to rabbits.

[1] School of Pharmacy, China Pharmaceutical University, Nanjing, 210009, People's Republic of China.
[2] To whom correspondence should be addressed. (e-mail: zong216@yahoo.com.cn)

MATERIALS AND METHODS

Materials

CLZ was purchased from Hubei Hengshuo Chemical Co., Ltd. (Wuhan, China). HP-β-CD was kindly donated by ISP Technologies, Inc. (Calvert City, Kentucky, USA). Mannitol (Pearlitol® 200 SD) and sodium carboxymethyl starch (Glycolys® STD) were obtained from Roquette Pharm. Co. (Lestrem, France). The other excipients used were microcrystalline cellulose (Ceolus™ KG-1000, Asahi Kasei Chemicals Corp., Tokyo, Japan) and magnesium stearate (Shanhe Pharmaceutical Excipients Co., Ltd, Anhui, China). All other chemicals were of reagent grade and used without further purification.

Animals

Six healthy male New Zealand white rabbits (1.8–2.3 kg) were obtained from the Laboratory Animal Center of China Pharmaceutical University (Nanjing, China). The study and all procedures concerning animals were carried out according to the regulations for animal care and use of China Pharmaceutical University. All procedures with animals were reviewed and approved by the Animal Ethics Committee of China Pharmaceutical University.

Analytical Methods

UV spectroscopy was used to determine CLZ in *in vitro* dissolution study in our research. The calibration curve of CLZ in phosphate buffer of pH 6.8 at 292 nm exhibited an excellent linearity over a concentration range of 1 to 20 μg/mL with a correlation coefficient of 0.9999.

The concentration of CLZ was detected by HPLC analysis in *in vivo* bioavailability studies and endogenous components produce no interference in the chromatograms. All the tests were carried out by six replicate determinations to validate the method. The calibration curve of CLZ was linear over the range from 20 to 1,000 ng/mL with the determination coefficient of 0.9999. The calibration curve had the regression equation of $Y=725.05X+0.11$, where Y was the concentration of CLZ, and X was the peak area ratio of CLZ to internal standard. The precision of the assay was estimated by performing the quality control (QC) samples with low, middle, and high concentrations. The intra-day precision (RSD) was always below 5.9% and the inter-day precision (RSD) was below 6.5%. The extraction recoveries of CLZ at three QC levels were within the range 75~90%. The lowest limit of CLZ quantification in plasma was 10 ng/mL.

Phase Solubility Studies

Phase solubility studies were performed according to the method reported by Higuchi and Connors (11). Briefly, excess amounts of CLZ were added to 10 mL of distilled water or acetate buffer solution of pH 4.5 or phosphate buffer solution of pH 6.8 containing serial concentrations of HP-β-CD (0–25 mM) in stoppered glass bottles. The suspensions were vigorously shaken at 25±1°C to reach equilibrium. Samples were done subsequently by filtering through a 0.45-μm microporous membrane and appropriately diluted. The CLZ concentration was determined by UV analysis. Each experiment was carried out in triplicate. The phase solubility diagrams were drawn according to the concentration of HP-β-CD and the totally dissolved CLZ. The apparent stability constants (K_s) were calculated from the diagrams according to the following equation:

$$K_s = \frac{\text{Slope}}{S_0(1 - \text{Slope})}$$

where S_0 is the CLZ solubility in the absence of HP-β-CD.

Preparation of Solid Inclusion Complexes

Products of CLZ/HP-β-CD inclusion complexes were prepared in a 1:1 molar ratio based on the results from the phase solubility studies by an evaporation method (12). The stoichiometric proportion of the drug and HP-β-CD were dissolved in ethanol and further sonicated for 30 min. The obtained clear solution was evaporated until nearly dry. The solid residue was further dried with a vacuum dryer at 40°C for 24 h then milled and passed through an 80-mesh sieve and stored in a desiccator at room temperature.

Characterization of CLZ/HP-β-CD Inclusion Complexes

Differential Scanning Calorimetry

DSC analysis was performed using a DSC-204 differential scanning calorimeter (NETZSCH, Germany). All accurately weighed samples were placed in sealed aluminum pans and heated at a constant rate of 10 K/min between 30°C and 300°C temperature ranges under an air flow.

X-ray Powder Diffraction

XRD patterns were collected on a Rigaku-D/MAX-2500PC diffractometer. The samples were irradiated with Ni-filtered Cu-Kα radiation, at a voltage of 40 kV, and a current of 40 mA. The scanning rate employed was $0.02°s^{-1}$ over a 2θ range of 3–50°.

Fourier Transform Infrared Spectroscopy

The FTIR spectra were recorded on a FTIR spectroscopy (PerkinElmer Spectrum One, Waltham, Massachusetts, USA) by KBr disc technique in the scanning range of 4,000–400 cm^{-1}.

Solubility Studies

Solubility studies were performed by adding excess amounts of CLZ and CLZ/HP-β-CD inclusion complexes to 10 mL of distilled water in sealed glass vials vigorously shaken at 25±1°C. After 48 h, aliquots were withdrawn, filtered, and spectrometrically measured at 292 nm for drug concentration. The experiments were carried out in triplicate.

Preparation of ODTs

Tablets (140 mg) containing 12.5 mg of CLZ (F1) or its equivalent amount of CLZ/HP-β-CD inclusion complexes

(F2) and suitable excipients were prepared by direct compression using a GLZP-10A rotary tablet press equipped with 8.5 mm flat facetted punches. The hardness was set to 30 N/mm using a 78X-2 hardness tester. The compositions of the tablets are given in Table I.

Characterization of the Prepared ODTs

Formulated tablets based on formulations F1 and F2 were subjected to different physical characterization studies. Twenty tablets were finely powdered; powder sample equivalent to 12.5 mg of CLZ was accurately weighed and analyzed for drug content by UV spectroscopy. The uniformity of the drug content in tablets was evaluated by determining the contents of ten tablets individually. The weight variation was determined on 20 tablets using an electronic balance. The crushing strength of the ten tablets was measured using the 78X-2 hardness tester. Friability of the tablets was calculated as the percentage weight loss of 50 tablets after rotation for 4 min at 25 rpm using a CJY-300C tablet friability tester. The *in vitro* disintegration time (DT) was noted using a stopwatch when the tablet completely disintegrated into fine particles, which was placed in the center of a Petri dish (10 cm diameter) filled with 10 mL of distilled water (13). And the wetting time (WT) was measured according to the reported method (14). A tablet was put on a twice-folded piece of tissue paper (10×12 cm) in a small culture dish (6.5 cm in diameter) containing 5 mL of distilled water with methylene blue. The time required for the upper surface of the tablet to become blue was taken as the WT.

In Vitro Dissolution Studies

In vitro dissolution studies of tablet formulations were performed according to the China Pharmacopoeia 2010 using the paddle method at 50 rpm in a 1,000-mL phosphate buffer of pH 6.8 maintained at 37±0.5°C. Aliquots of the solution (5 mL) were withdrawn at predetermined time intervals and replaced with an equal volume of fresh medium. The concentration of the CLZ was determined according to the UV method. All the studies were carried out six times.

In Vivo Bioavailability Studies

Drug Administration

Six healthy male New Zealand white rabbits were housed under standard conditions and allowed free access to food and water. All rabbits were dosed following an overnight fasting; food was returned 2 h after dosing. The study was conducted according to a two-period, two-sequence crossover design at intervals of 2 weeks. Six rabbits were randomly divided into two groups. One group received the commercial tablets (containing 50 mg CLZ), whereas the other group received ODTs (F2, containing 50 mg CLZ). During the drug administration, an operator placed each rabbit in a body-restraint device, which exposed the animal's head, and lifted apart the gums with a wooden tongue depressor. Subsequently, ODTs were placed in the rabbit's mouth, wetting with 2 mL of water. At the same time, the operator applied a gentle tension to restrain the mouth of the rabbit for 1 min in order to ensure the complete disintegration of the ODTs as well as the prevention of chewing from the rabbit. For the other group of rabbits, the operator placed the commercial tablets at the back of the pharynx and gave 2 mL of water to facilitate swallowing and to prevent the tablets from sticking to the animal's throat.

Rabbit blood samples were obtained from the ear marginal vein before administration and at predetermined time intervals after administration (0.5, 0.75, 1, 1.5, 2, 3, 4, 5, 6, 8, 10, 12, 20, and 24 h for commercial tablets after drug administration and 0.1, 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 5, 6, 8, 10, 12, 20, and 24 h for the ODTs after drug administration) and then stored in heparinized tubes.

Table I. Composition of Tablet Formulations Containing Different Ingredients

Tablet code	F1	F2
CLZ	12.5	–
Inclusion complexes (equal to 12.5 mg of CLZ)	–	67.2
Ceolus™ KG-1000	21	21
Glycolys® STD	11.2	11.2
Pearlitol® 200 SD	94.6	39.9
Magnesium stearate	0.7	0.7

All quantities in milligrams
CLZ clozapine

Preparation and Analysis of Blood Samples

All samples were immediately centrifuged at 3,000 rpm for 10 min, after which the plasma layers were collected and stored at −20°C until HPLC analysis. Frozen plasma samples were thawed at ambient temperature. Plasma (0.1 mL) was thoroughly mixed with a 10-μL perphenazine solution (8,000 ng/mL) as an internal standard and 1 mL of ether. This mixture was vortexed for 2 min and centrifuged at 4,000 rpm for 10 min to precipitate the proteins. The supernatant was removed, transferred to a test tube, and evaporated to dryness under nitrogen in a 40°C water bath. The residue was reconstituted in 100 μL of the mobile phase, vortex mixed for 2 min, centrifuged at 15,000 rpm for 10 min, and the clear supernatant (20 μL) was injected into the HPLC system.

The HPLC system was performed on a Shimadzu chromatographic system equipped with an LC-10ATvp solvent delivery pump, SPD-10Avp UV detector, and Class VP Chromato software. CLZ was analyzed using an ODS C18 column (150×4.6 mm i.d., 5 μm, Shimadzu, Japan) at room temperature. The mobile phase consisted of 75:25 (*v*/*v*) methanol–water with 0.03 mol/L ammonium acetate. The analysis was run at a flow rate of 1.0 mL/min, and detection wavelength was at 254 nm.

Pharmacokinetic and Statistical Analysis

The pharmacokinetic parameters of CLZ were calculated by non-compartmental pharmacokinetic models using Kinetica® software (version 4.4.1). The maximum CLZ concentration (C_{max}) and corresponding peak time (T_{max}) were obtained directly from the plasma concentration–time profiles. The area under the plasma concentration–time curve from time zero to the last measured concentration (AUC_{0-t}) was calculated by the trapezoidal rule. The terminal elimination rate constant (λ_z) was estimated by linear regression of the terminal portion of the log plasma concentration *vs* time curve, and the elimination half-life was calculated. $AUC_{0-\infty}$ was the corresponding area

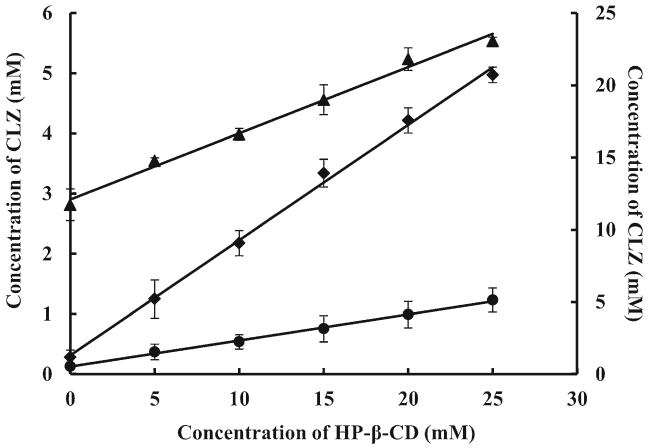

Fig. 1. Phase solubility diagrams of CLZ/HP-β-CD system in water (*black circle*), pH 6.8 phosphate buffer (*black square*) [*left y-axis*], and pH 4.5 in acetate buffer (*black triangle*) [*right y-axis*]. *Error bars* indicate the SD

extrapolated to infinity calculated by $AUC_{0-t}+C_t/\lambda_z$, where C_t was the last measurable drug concentration. All data are expressed as mean±standard deviation (SD). The differences in the average of data were compared by simple analysis of variance using the software SPSS Statistics (version 17.0, Chicago, USA), in which $p<0.05$ was considered to be significant.

C_{max}, AUC_{0-t}, and T_{max} were considered as primary variables to assess the bioequivalence between the CLZ/HP-β-CD ODTs and the commercial tablets. The logarithmic transformation of the C_{max} and AUC_{0-t} values were subjected to the analysis of variance and one-sided *t* tests. The formulations were considered bioequivalent if the 90% confidence intervals (90% CI) for the ratio of C_{max} and AUC_{0-t} fell within 80–125% (15).

RESULTS

Phase Solubility Studies

Figure 1 shows the phase solubility diagrams of CLZ/HP-β-CD in three different aqueous solutions. The apparent solubility of the CLZ increased linearly with the concentrations of HP-β-CD. Therefore, all of them can be considered as A_L-type diagrams, indicating the formation of the 1:1 inclusion complexes in the systems. The K_s values and the corresponding phase solubility diagrams are summarized in Table II.

Inclusion Complexes Characterization

Differential Scanning Calorimetry

Figure 2 illustrates the DSC profiles of pure components, their physical mixtures, and inclusion complexes. The curve of physical mixture systems can be regarded as the superimposition

Table II. The Stability Constants (K_s) of CLZ in Different Solutions

Medium	Linear equation	K_s (M^{-1})	r^2
pH 4.5	$y=0.4584c+12.093$	72.21	0.9911
pH 6.8	$y=0.1915c+0.3116$	847.35	0.9970
Distilled water	$y=0.0433c+0.1282$	360.83	0.9976

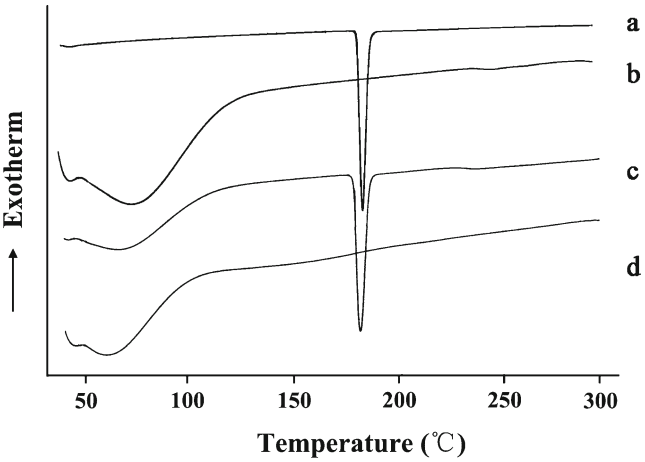

Fig. 2. DSC curves of *a* CLZ, *b* HP-β-CD, *c* physical mixtures of CLZ and HP-β-CD, and *d* CLZ/HP-β-CD inclusion complexes

of those of the pure components, as the endothermic peak of CLZ (180.0°C) and HP-β-CD dehydration peaks (80°C) are clearly distinguishable and almost do not change. However, the complete disappearance of the CLZ endothermic peak was observed in CLZ/HP-β-CD inclusion complexes.

X-ray Powder Diffraction

Figure 3 shows the XRD patterns of CLZ, HP-β-CD, CLZ/HP-β-CD inclusion complexes, as well as their physical mixtures. The diffraction pattern of CLZ exhibited a series of sharp high intense peaks, suggesting the crystalline character of the drug. The absence of any peaks with a diffuse hollow pattern in HP-β-CD revealed its amorphous nature. The XRD pattern of the investigated physical mixtures corresponded to the superposition of those of the pure components. Drug crystallinity peaks were

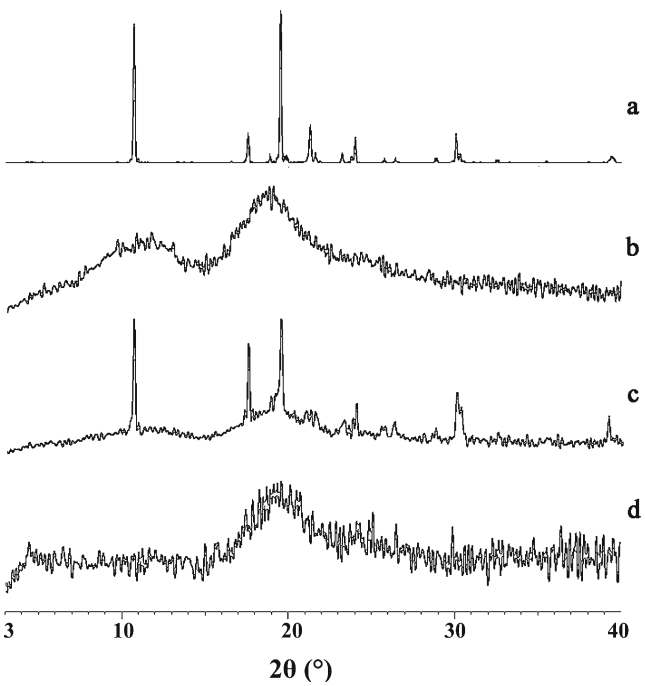

Fig. 3. XRD patterns of *a* CLZ, *b* HP-β-CD, *c* physical mixtures of CLZ and HP-β-CD, and *d* CLZ/HP-β-CD inclusion complexes

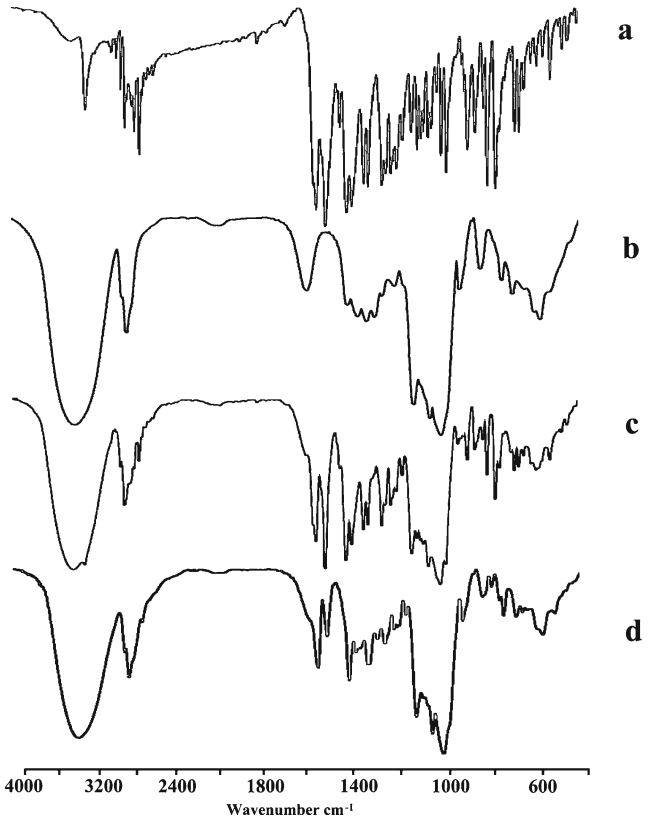

Fig. 4. FTIR spectra of *a* CLZ, *b* HP-β-CD, *c* physical mixtures of CLZ and HP-β-CD, and *d* CLZ/HP-β-CD inclusion complexes

still detectable, indicating no formation of new structures and crystalline CLZ highly dispersed in the amorphous carrier. However, a total drug amorphization was recorded for inclusion complexes, indicating the formation of inclusion complexes.

Fourier Transformation Infrared Spectroscopy

Figure 4 shows the FTIR spectra of CLZ, HP-β-CD, the physical mixtures, and the inclusion complexes. The FTIR spectrum of the physical mixtures showed the main absorption bands of the drug and the HP-β-CD, but some peaks were slightly shifted showing some signs of interaction between the CLZ and HP-β-CD. In case of spectra of inclusion complexes, the peaks appeared at 2,844.4 cm^{-1}, 2,799.4 cm^{-1} (C–C stretching due to a CH_2 group), and 1,200~1,000 cm^{-1} (C–N stretching due to the tertiary amine) and disappeared completely while the intensity of other bands was decreased sharply but still observed.

Solubility Studies

The solubility of raw CLZ and CLZ/HP-β-CD inclusion complexes was 13.36±1.56 and 1,015.38±1.06 μg/mL, respectively. The inclusion complexes were clearly more soluble than CLZ alone, and the improvement was almost 76 times.

Characterization of the Prepared ODTs

The properties of the prepared ODTs (F1, F2) such as weight variation, hardness, friability, drug content, DT, and WT met the China Pharmacopoeia 2010 requirements as shown in Table III. Content uniformities were found to be good where the percentages of both of the drug contents were more than 98%.

In Vitro Dissolution Studies

The dissolution profiles for CLZ commercial tablets, CLZ ODTs, and CLZ/HP-β-CD ODTs are shown in Fig. 5. It is evident that the inclusion complexes remarkably improved the dissolution rate of CLZ compared to CLZ alone. CLZ dissolved from formulation F1 at 1 and 10 min was 2.8±2.2% and 9.5±1.3%, respectively, while that of the formulation F2 was found to be 53.2±2.3% and 88.2±1.8%, respectively. There was a 19-fold and a 9.3-fold increase in the dissolution at 1 and 10 min in comparison with that of formulation F1 and also a 7.6-fold and a 3.9-fold in comparison with that of the commercial tablets, significantly improving the dissolution of the drug.

In Vivo Bioavailability Studies

Figure 6 shows the mean serum concentration–time profiles of the CLZ after oral administration of the commercial tablets and the CLZ/HP-β-CD ODTs. The relevant pharmacokinetic parameters are listed in Table IV. The plasma levels of CLZ after administration of the ODTs were clearly faster and higher than those of the commercial tablets. In particular, the T_{max} after the administration of commercial tablets was observed at 2.42 h; however, the ODTs resulted in the rapid appearance of CLZ in plasma, attaining the T_{max} after 0.67 h. In addition, the value of C_{max} for the ODTs (841.53±165.28 ng/mL) was 1.4-fold in comparison with that of the commercial tablets (590.35±83.18 ng/mL). The differences between the two formulations for C_{max} and T_{max} were statistically significantly different ($p<0.05$). The relative bioavailability of the ODTs/the commercial tablets for AUC_{0-t} and $AUC_{0-\infty}$ was 116.85% and 115.60%, respectively. The analysis of variance suggested no period effects on log-transformed C_{max} and AUC_{0-t}; however, significant formulation effects were detected for C_{max} and AUC_{0-t}. The 90% CIs of the ratios of the test ODTs/the commercial tablets for the log-transformed C_{max} and AUC_{0-t} were 121.9% to 135.3% and 99.6% to 138.3%, respectively; all of which did not fell within the predefined bioequivalence criteria of 80% to 125%.

Table III. Characterization of the ODTs

Tablet code	Weight variation (mg)	Drug content (%)	Friability (% loss)	Hardness (N/mm)	DT (s)	WT (s)
F1	140.5±1.3	98.66±0.35	0.68±0.22	31.2±1.3	14.6±1.8	32.1±0.6
F2	140.5±0.8	98.66±0.40	0.63±0.07	31.4±0.8	15.3±1.5	36.5±1.2

Data represent mean value±SD

DISCUSSION

The present study is the first report that used cyclodextrin complexation technique to improve the solubility and dissolution rate of CLZ, aiming at developing fast and efficient ODTs. The phase solubility study is very useful for investigating inclusion complexation of poor water-soluble drugs with CDs in solutions. In this study, the K_s in the pH 4.5 buffer (72.21 M^{-1}) is 12-fold smaller than that in the pH 6.8 buffer (847.35 M^{-1}), indicating that the ability of inclusion between the CLZ and HP-β-CD is smaller in acidic media than that in neutral media. Since CLZ is a weak basic drug (pK_a=7.5), its ionized form of high solubility is predominant in acidic media. These ionized molecules with lower hydrophobicity should produce weaker interactions with the hydrophobic cavities of HP-β-CD compared with the unionized ones (16). It might be concluded that the ionization of CLZ plays an important role in complexation. Similar observations have been reported by other authors (17,18). K_s value is an index used to evaluate the capability of CDs to form inclusion complexes with a drug. If the K_s is too small, there is little improvement in the solubility of the drug. On the other hand, if the K_s is greater than 10,000 M^{-1}, indicating strong complexation, the inclusion complexes cannot dissociate easily and not be absorbed, finally resulting in decreased bioavailability compared with drugs dosed alone (19). From the K_s values derived from the phase diagrams, it can be concluded that the complexation between the CLZ and HP-β-CD is weak in a pH 4.5 acetate buffer and moderate in a pH 6.8 phosphate buffer. These results indicated that dissociation of the CLZ from the complexes would not be a problem in such conditions.

The formation of inclusion complexes between CLZ and HP-β-CD was confirmed by DSC, XRD, and FTIR studies. It was observed in the DSC profiles that the CLZ endothermic peak completely disappeared in the CLZ/HP-β-CD inclusion complexes, indicative of the complete amorphization of the system and/or the inclusion complexes formation (20,21). XRD studies indicated the crystalline nature of CLZ and amorphous nature of HP-β-CD. The diffractograms of inclusion complexes showed complete reduction in crystallinity and displayed a typical diffuse pattern, indicating a total drug amorphization. Lack of crystallinity is an added evidence for the formation of the inclusion complexes (22). Accordingly, taking into account the results of the DSC analysis, we can assume the formation of new solid phases, probably due to the formation of inclusion complexes. What is more, in the spectra of the inclusion complexes, the characteristic bands of the CLZ appeared at 2,844.4 cm^{-1}, 2,799.4 cm^{-1} (C–C stretching due to a CH_2 group), and 1,200~1,000 cm^{-1} (C–N stretching due to the tertiary amine) disappeared completely while the intensity of other bands decreased sharply but were still observable, suggesting that the piperazine side chain was embedded into the cavity. These results served to confirm the existence of strong interaction between the CLZ and HP-β-CD and the formation of true inclusion complexes.

The hardness of the prepared ODTs was maintained in the range of 30~40 N/mm and the friability was found to be below 1%, indicating the sufficient structural integrity to withstand handling without substantial breakage. On the other hand, ODTs of CLZ/HP-β-CD inclusion complexes exhibited short DT (15.3±1.5 s) and WT (36.5±1.2 s), reflecting rapid disintegration and dissolution in the mouth. The dissolution studies of ODTs showed a significant increase of CLZ dissolution rate in the case of inclusion complexes. This fact could be attributed to the formation of soluble inclusion complexes, amorphization of the drug and, consequently, reduction of the crystallinity, and solubility increase (23).

The oral absorption of CLZ was evaluated using the rabbit as the animal model, due to its convenient size, easy

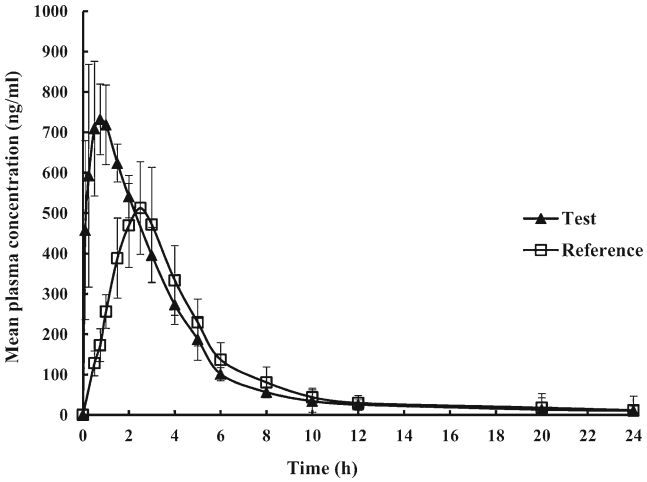

Fig. 6. Mean plasma concentration profiles of the CLZ after oral administration of the reference products (commercial CLZ tablets) and the test formulations (CLZ/HP-β-CD ODTs) in rabbits. Mean± SD, n=6. *Error bars* indicate the SD

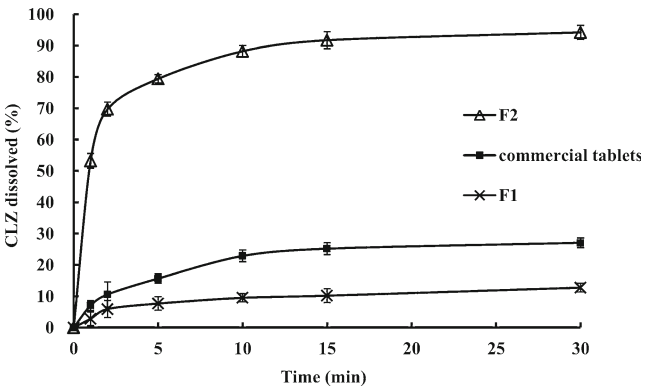

Fig. 5. Dissolution profiles of CLZ from tablets. *Error bars* indicate the SD

Table IV. Pharmacokinetic Parameters Following Oral Administration of the Reference Products (Commercial CLZ Tablets) and the Test Formulations (CLZ/HP-β-CD ODTs) in Rabbits

Parameter	Reference	Test
C_{max} (ng/mL)	590.35±83.18	841.53±165.28*
T_{max} (h)	2.42±0.58	0.67±0.26*
AUC_{0-t} (ng·h/mL)	2,489.57±356.29	2,909.10±444.16
$AUC_{0-\infty}$ (ng·h/mL)	2,639.00±383.96	3,050.69±525.27
$T_{1/2}$ (h)	9.36±2.61	8.50±2.78
MRT (h)	6.98±1.53	5.31±1.81

Data represent mean value±SD, n=6
MRT mean residence time
*p<0.05, significantly different from the corresponding values of the commercial tablets

handling, and the nonkeratinized oral mucosa that closely resembles human sublingual and buccal tissues (24,25). The concentration–time profiles of CLZ showed a faster and higher absorption in rabbits of the ODTs compared with the commercial tablets. These results could be due to the rapid disintegration and dissolution of the drug in the saliva, resulting in very fast absorption from the mouth mucosa, which may result in the reduction of hepatic first-pass effects of CLZ. On the other hand, only the free form of the drug can pass through the lipid barrier of the gastrointestinal tract. After oral administration of cyclodextrin complexes, the drug absorption is largely dependent upon the magnitude of K_s as well as the solubility and dissolution rate of the inclusion complexes. The inclusion complexes with a high magnitude of K_s might lead to a retarding effect on drug dissociation from the cyclodextrins, resulting in a reduction of bioavailability (26,27). In the present study, since the K_s value of complexes is relatively low, it seems very unlikely that it has a negative influence in complexes dissociation. Thus, we can conclude that the higher solubility, dissolution rate, and the suitable K_s magnitude of the complexes are conducive to improving the oral bioavailability of CLZ.

CONCLUSION

The CLZ ODTs containing CLZ/HP-β-CD inclusion complexes were prepared by direct compression in this study. The prepared ODTs exhibited short DT (15.3±1.5 s) and WT (36.5±1.2 s) and rapid drug dissolution, improving the solubility and dissolution rate of CLZ. The *in vivo* studies in rabbits suggested a faster absorption rate and a higher absorption extent of CLZ/HP-β-CD ODTs in comparison with the commercial tablets. Hence, this "patient-friendly dosage form" could be a useful alternative to the commercial tablet with regards to its improved patient compliance, rapid onset of action, and increase in bioavailability.

ACKNOWLEDGMENTS

The authors are grateful to the College Students Innovation Project for the R&D of Novel Drugs (program no. J1030830) for supporting the present research work.

REFERENCES

1. Sweetman SC. Martindale: the complete drug reference. 36th ed. London: Pharmaceutical Press; 2009. p. 768–71.
2. Lindenberg M, Kopp S, Dressman JB. Classification of orally administered drugs on the World Health Organization Model list of Essential Medicines according to the biopharmaceutics classification system. Eur J Pharm Biopharm. 2004;58:265–78.
3. Gerald KM. AHFS drug information. Bethesda: American Society of Health-System Pharmacists Press; 2004. p. 2273–85.
4. Seager H. Drug delivery products and the Zydis fast dissolving dosage forms. J Pharm Pharmacol. 1998;50:375–82.
5. Badgujar BP, Mundada AS. The technologies used for developing orally disintegrating tablets: a review. Acta Pharm. 2011;61:117–39.
6. Goel H, Rai P, Rana V, Tiwary AK. Orally disintegrating systems: innovations in formulation and technology. Recent Pat Drug Deliv Formul. 2008;2:258–74.
7. Szejtli J. Introduction and general overview of cyclodextrin chemistry. Chem Rev. 1998;98:1743–54.
8. Uekama K, Hirayama F, Irie T. Cyclodextrin drug carrier systems. Chem Rev. 1998;98:2045–76.
9. Misiuk W, Zalewska M. Investigation of inclusion complex of trazodone hydrochloride with hydroxypropyl-β-cyclodextrin. Carbohydr Polym. 2009;77:482–8.
10. Gould S, Scott RC. 2-Hydroxypropyl-β-cyclodextrin (HP-β-CD): a toxicology review. Food Chem Toxicol. 2005;43:1451–9.
11. Higuchi T, Connors KA. Phase solubility techniques. Adv Anal Chem Instrum. 1965;4:117–212.
12. Yadav VR, Suresh S, Devi K, Yadav S. Effect of cyclodextrin complexation of curcumin on its solubility and antiangiogenic and anti-inflammatory activity in rat colitis model. AAPS PharmSciTech. 2009;10:752–62.
13. Jain SK, Shukla M, Shrivastava V. Development and *in vitro* evaluation of ibuprofen mouth dissolving tablets using solid dispersion technique. Chem Pharm Bull. 2010;58:1037–42.
14. Rajitha K, Shravan KY, Adukondalu D, Ramesh G, Madhusudan Y. Formulation and evaluation of orally disintegrating tablets of buspirone. Int J Pharm Sci Nanotechnol. 2009;1:372–4.
15. US Food and Drug Administration. *In vivo* bioequivalence guidances. Pharmacopeial Forum. 1993;19:6501–8.
16. Loftsson T, Brewster ME. Pharmaceutical applications of cyclodextrins. 1. Drug solubilization and stabilization. J Pharm Sci. 1996;85:1017–25.
17. Zornoza A, Martín C, Sánchez M, Vélaz I, Piquer A. Inclusion complexation of glisentide with α-, β- and γ-cyclodextrins. Int J Pharm. 1998;169:239–44.
18. Fernandes CM, Teresa Vieira M, Veiga FJ. Physicochemical characterization and *in vitro* dissolution behavior of nicardipine-cyclodextrins inclusion compounds. Eur J Pharm Sci. 2002;15:79–88.
19. Carrier RL, Miller LA, Ahmed I. The utility of cyclodextrins for enhancing oral bioavailability. J Control Release. 2007;123:78–99.
20. Cirri M, Righi MF, Maestrelli F, Mura P, Valleri M. Development of glyburide fast-dissolving tablets based on the combined use of cyclodextrins and polymers. Drug Dev Ind Pharm. 2009;35:73–82.
21. Cirri M, Rangoni C, Maestrelli F, Corti G, Mura P. Development of fast-dissolving tablets of flurbiprofen-cyclodextrin complexes. Drug Dev Ind Pharm. 2005;31:697–707.
22. Williams RO, Mahaguna V, Sriwongjanya M. Characterization of an inclusion complex of cholesterol and hydroxypropyl-beta-cyclodextrin. Eur J Pharm Biopharm. 1998;46:355–60.
23. Veiga F, Fernandes C, Maincent P. Influence of the preparation method on the physicochemical properties of tolbutamide/cyclodextrin binary systems. Drug Dev Ind Pharm. 2001;27:523–32.
24. Dali MM, Moench PA, Mathias NR, Stetsko PI, Heran CL, Smith RL. A rabbit model for sublingual drug delivery: comparison with human pharmacokinetic studies of propranolol, verapamil and captopril. J Pharm Sci. 2006;95:37–44.
25. Odou P, Barthelemy C, Chatelier D, Luyckx M, Brunet C, Dine T, *et al.* Pharmacokinetics of midazolam: comparison of sublingual and intravenous routes in rabbit. Eur J Drug Metab Pharmacokinet. 1999;24:1–7.
26. Nie S, Fan X, Peng Y, Yang X, Wang C, Pan W. *In vitro* and *in vivo* studies on the complexes of vinpocetine with hydroxypropyl-β-cyclodextrin. Arch Pharm Res. 2006;30:991–1001.
27. Bekers O, Uijtendaal EV, Beijnen JH, Buit A, Under-berg WJM. Cyclodextrins in the pharmaceutical field. Drug Dev Ind Pharm. 1991;17:1503–49.