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Inclusion Complex Effect on the Bioavailability of Clotrimazole from Poloxamer-based Solid Suppository

Prabagar Balakrishnan[1,2], Chung Kil Song[3], Hyun-Jong Cho[3], Su-Geun Yang[5], Dae Duk Kim[3], Chul Soon Yong[1], and Han-Gon Choi[1,4]

[1]College of Pharmacy, Yeungnam University, Gyongsan 712-749, Korea, [2]Utah-Inha DDS and Advanced Therapeutics, Incheon 406-840, Korea, [3]College of Pharmacy and Research Institute of Pharmaceutical Sciences, Seoul National University, Seoul 151-742, Korea, [4]College of Pharmacy, Hanyang University, Ansan 426-791, Korea, and [5]Department of New Drug Development and U&I DDS, School of Medicine, Inha University, Incheon 400-712, Korea

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To study the effect of β-cyclodextrin (βCD) inclusion complex on the bioavailability of clotrimazole from poloxamer-based suppository, formulations composed of P 188, propylene glycol and different molar ratio of clotrimazole-βCD inclusion complex were prepared. Clotrimazole (1%) has been formulated in a suppository using the thermo sensitive polymer P188 (70%) together with propylene glycol (30%). To increase its aqueous solubility, clotrimazole was incorporated as its inclusion complex at various molar ratios with βCD (1:0.25, 1:0.5, 1:1, and 1:2). The inclusion complex was characterized by differential scanning calorimetry (DSC), XRD and phase solubility studies. It was observed that the complexation with βCD, particularly at high molar ratio (F3 (1:1) and F4 (1:2)) decreased the release profile of clotrimazole considerably. However, suppositories containing inclusion complex at low molar ratio (F1 (1:0.25) and F2 (1:0.5)) showed excellent release profile compared to control formulation. *In vivo* study in rats at 15 mg/Kg dose showed that the F1 and F2 (82.39 ± 15.40 and 67.05 ± 8.79, respectively) significantly increased the AUC compared to that of F3 (41.48 ± 11.51), F4 (23.34 ± 8.37) and control (46.7 ± 7.87) suppositories. Thus, the suppositories containing inclusion complexes prepared at low drug to βCD molar ratio (F1) could be a potential suppository formulation to increase the bioavailability of hydrophobic drugs such as clotrimazole.

Key words: Clotrimazole, β-Cyclodextrin, Inclusion complex, Poloxamer 188, Suppository, Enhanced bioavailability

INTRODUCTION

Clotrimazole, an imidzole antimycotic agent, is a promising drug for various disease including cancer and sickle cell anemia (Thapa et al., 2009; Liu, 2010). It has been reported for its neuroprotective effect and anti-inflammatory effect in patients with rheumatoid arthritis (Thapa et al., 2008, 2009). However, due to its hydrophobic nature (Log P, 6.1) and poor water solubility (0.49 mg/L), it has been reported for significant differences in the oral bioavailability (Prabagar et al., 2007; Borhade et al., 2012). Thus, several new formulations have been tried to increase its bioavailability such as solid dispersions, microemulsion based gels, Nanoparticles, buccoadhesive tablets, inclusion complexations (Prabagar et al., 2007; Bachhav and Patravale, 2009; Tahvilian and Moosavi, 2010; Vivek et al., 2010). However, hepatotoxicity related to its oral administration overshadowed its therapeutic use (Hashem et al., 2011). Hence, to avoid its hepatotoxicity other than oral formulations such as suppositories was reported by our group (Yong et al., 2006). However, the bioavailability of the suppository formulation was not reported in our earlier study. Moreover, there are only few

Correspondence to: Han-Gon Choi, College of Pharmacy, Hanyang University, Ansan 426-791, Korea
Tel: 82-31-400-5802, Fax: 82-31-400-5958
E-mail: hangon@hanyang.ac.kr
Chul Soon Yong, College of Pharmacy, Yeungnam University, Gyongsan 712-749, Korea
Tel: 82-53-810-2812, Fax: 82-53-810-4654
E-mail: csyong@ynu.ac.kr

reports regarding the bioavailability of poorly water soluble drugs from rectal formulations and none for clotrimazole.

Cyclodextrins (CDs) also known as cyclic oligosaccharides, are produced from starch by enzymatic conversion using glucosyltransferase (Yong et al., 2007). Complexations with cyclodextrins have been reported to enhance the solubility, dissolution rate and bioavailability of poorly water-soluble drugs. β-Cyclodextrin (βCD) is one of the three natural CDs (αCD, βCD and γCD) composed of seven α(1-4)-linked α-D-glucopyranose units with a hydrophilic outer surface and a hydrophobic cavity in the center. Many of the hydrophobic drugs form inclusion complex with βCD in aqueous environment by displacing water from the hydrophobic cavity (Shahin et al., 2011). βCD has widely been used in the pharmaceutical applications as it is readily available and economically cheap, moreover the hydrophobic cavity size of βCD is suitable for a large number of hydrophobic drugs. Several methods have been used to prepare βCD-drug inclusion complexes, such as spray drying, lyophilization, kneading and coprecipitation. However, spray drying method has been widely used and have several advantages over other methods such as good yield, easy scale up, short operating and suitability (Prabagar et al., 2007).

In this study, as a base of the suppository, a mixture of poloxamer 188 (P188) and propylene glycol with the melting point of about 55°C and −10°C, respectively, was selected as they are reported for their suitable mucoadhesive force, low toxicity, less skin irritation, good drug release characteristics and compatibility with other chemicals (Choi et al., 2011; Shahin et al., 2011). The primary objective of the present study was to investigate the possibility of improving the release properties and bioavailability of clotrimazole via complexation with βCD from poloxamer based suppositories. The physicochemical properties and bioavailability in rats of clotrimazole-βCD/poloxamer based suppositories are reported.

MATERIALS AND METHODS

Clotrimazole was provided by Boryung Pharmaceutical Co. βCD and propylene glycol were purchased from Aldrich Chemical Co. Poloxamer 188 (P 188) was supplied from BASF. Semipermeable membrane tube (Spectra membrane tubing No. 1) was purchased from Spectrum Medical Industries Inc. Ethanol was obtained from Duksan Pharmaceutical Co. All other chemicals were of reagent grade and used without further purification.

Solubility diagram

The phase solubility diagram was performed according to Prabagar et al. (2007). Clotrimazole (10 mg) was added to distilled water containing various concentrations of βCD and then was shaken for 5 days at 25°C in a water bath. After equilibrium was reached, the samples were withdrawn and filtered through a 0.2 μm filter (Millipore) and suitably diluted with methanol. Drug concentration was determined by high performance liquid chromatography (HPLC). The apparent association constant (K_c) of the complex was calculated as following equation (Eq. (1)) from phase solubility slope, where the intercept is the intrinsic water solubility of drug (S_o) in the absence of βCD at 25°C.

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

Preparation of inclusion complex

A Büchi 190 nozzle type mini spray dryer (Flawil) was used for the preparation of clotirmazole-βCD inclusion complex. In brief, 0.2 g of clotrimazole and βCD at different molar ratio (1:0.25, 1:0.5, 1:1 and 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. The residue, clotrimazole-βCD inclusion complex was collected (Prabagar et al., 2007).

Preparation of poloxamer-based suppository

P 188 and propylene glycol (7:3 *w/w*) were mixed and heated up to 55°C (Yong et al., 2006). Clotrimazole or inclusion complex (1% equivalent to drug) was then slowly added to the solution with continuous agitation (Table I). The resulting solution was moved to the suppository mould and cooled down to 25°C. The prepared suppositories were kept at 4°C until further study.

Solid state characteristics

The physical state of clotrimazole in inclusion complex and suppositories was characterized by the differential scanning calorimetry (DSC Q200 v24.2 build 107, TA Instruments). The samples (about 3.00 mg) were placed in standard aluminum pans, and dry nitrogen was used as effluent gas. All samples were scanned at a temperature ramp speed of 5°C/min and at the heat flow from 0 to 180°C. Furthermore, X-ray powder scattering measurements were carried out with an X'Pert PRO diffractometer (PAN analytical) at room temperature using monochromatic CuK_α-radiation ($\lambda = 1.5406$ Å) at 30 mA and at 40 kV over a

range of 2θ angles from 10° to 50° with an angular increment of 0.02° per second.

Dissolution test

Poloxamer-based suppositories (1 g) were inserted into a semipermeable membrane tube (Table I). Both sides of the tube were tied up with a thread to prevent leakage. The semipermeable membrane tube was then placed in a dissolution tester (DST-600, Fine Chemical). Dissolution test was performed at 36.5°C using the paddle method at 100 rpm with 400 mL phosphate buffer (pH 4.4) as a dissolution medium. At predetermined time interval, 1 mL of the medium was sampled and filtered. The filtrate was analyzed by HPLC (Prabagar et al., 2007).

In vivo study

In vivo experiments

Male Sprague-Dawley rats weighing 250 ± 20 g were fasted for 24 h prior to the experiments but allowed free access to water. Thirty rats were divided into five groups. The rats in each group were administered with control suppository [clotimazole/P188 and propylene glycol at 1:2.3 ratios (1/99%)] and inclusion complex suppositories (F1-F4), respectively.

Administration and blood-collection

Each rat, anesthetized in an ether-saturated chamber, was secured on a surgical board in the supine position with a thread. Control or inclusion complex poloxamer-based suppository was administered with a dose of 1.5 g/kg (equivalent clotrimazole 15 mg/kg) into the rectum 4 cm above the anus (Choi et al., 1998). The entrance of the anus was then blocked with a cyanoacrylate adhesive, since the suppository might be leaked out from the anus during the pharmacokinetic experiment, leading to not obtaining accurate pharmacokinetic data. The blood (0.25 mL) was collected from the right or left subclavicle vein or artery at various time intervals and centrifuged at 8000 rpm for 10 min using a centrifuge 5415C (Eppendorf).

Blood sample analysis

Plasma (0.1 mL) was mixed with 10 mL of ethanol solution containing ibuprofen (100 μg/mL), as an internal standard then 50 mL of 85% phosphoric acid was added and extracted with 500 mL of dichloromethane. It was then centrifuged at 3000 g for 2 min to separate the organic phase. After evaporation of the organic phase using centrifugal vacuum concentrator, the residue was reconstituted in 50 μL of mobile phase. Then, the resulting solution (20 μL) was analyzed by HPLC (Hitachi, Model L-7100) 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) and ultrasonically deaerated. The eluent was monitored at 230 nm with a flow rate of 1.2 mL/min (Prabagar et al., 2007).

Statistical analysis

Student's *t*-tests were performed to evaluate the significant differences between the formulations. Values are reported as mean ± S.D. and the data were considered statistically significant at $p < 0.05$.

RESULTS AND DISCUSSION

Solubility studies

Though clotrimazole has been reported for various indications, its hepatotoxicity in oral formulations limited its clinical use; therefore alternative route such as suppositories has been studied to alleviate its hepatotoxicity (Yong et al., 2006). However, its poor solubility due to its high lipophilic nature may hamper the release characteristics and bioavailability in rectal formulations. Thus, to enhance the solubility and bioavailability, inclusion complex with βCD were prepared by spray drying method. The complexing behavior of clotrimazole with βCD in water at 25°C was studied by solubility method. The solubility of clotrimazole increased in a linear fashion as a function of βCD concentration ($r^2 = 0.97$), and the resulting solubility curve can be classified as A_L (Fig. 1). The apparent association constant of clotrimazole/βCD complex was found to be 71.52 M^{-1}. The aqueous solubility of clotrimazole deter-

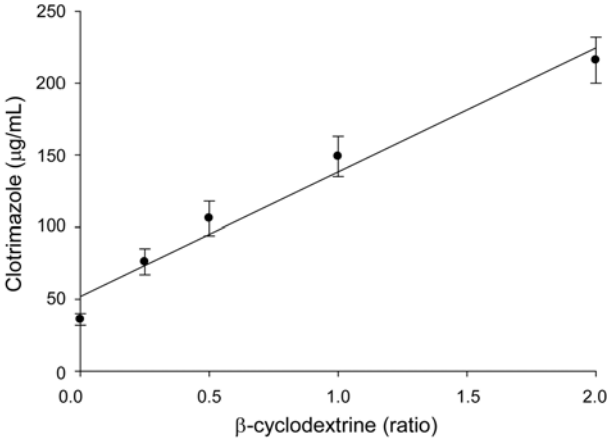

Fig. 1. Phase solubility study. Each value represents the mean ± S.D. (n = 3).

mined in this study was 0.036 mg/mL, which was consistent with our earlier report (Prabagar et al., 2007).

Complexation in the solid state

To examine the interaction of clotrimazole with βCD in the solid state, DSC and X-ray diffractometry were employed. The DSC results of the clotrimazole βCD complexes, drug and βCD, indicated the formation of inclusion complex (Fig. 2). The DSC curve of clotrimazole shows one characteristic sharp endothermic peak at around 150°C indicating the melting point of the drug. No clotrimazole melting peak or a small peak was present on the DSC of the inclusion complexes (Fig. 2A). DSC of suppository formulations showed that the melting peak of the formulations at around 35°C increase as the ratio of βCD increased (Fig. 2B). X-ray diffraction studies allow the identification of inclusion complexes of cyclodextrins, mainly based on the empirical evidence that the powder XRD patterns of these complexes should be clearly distinct from those obtained by the superimposition of the diffractograms of the individual components. Fig. 3 shows the patterns for inclusion complexes, plain βCD, and clotrimazole. The diffractograms of the inclusion complexes were clearly different from those of drug and βCD, indicating the formation of inclusion complex.

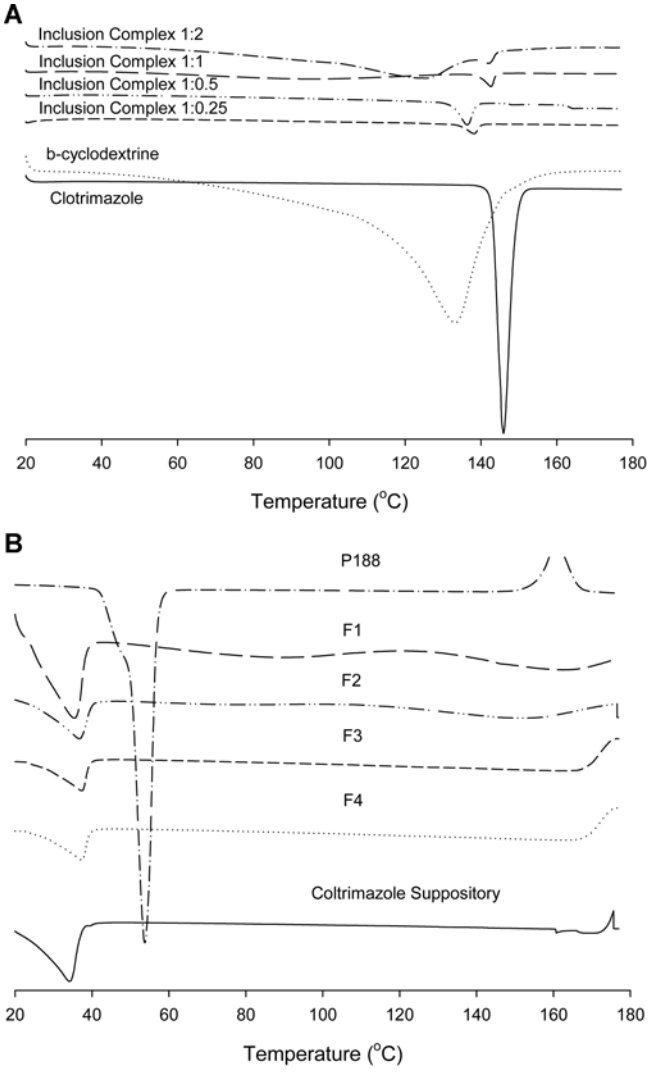

Fig. 2. Differential scanning calorimetry (DSC) study. (**A**) DSC of inclusion complexes, βCD and clotrimazole. (**B**) DSC of P188 and suppository formulations.

Dissolution studies

To test whether the ratio of drug to βCD affected the dissolution rates of clotrimazole from the suppositories, we performed the dissolution studies on the prepared four suppository formulations (Table I). Among the prepared suppositories, the suppositories containing low drug to βCD inclusion complexes (1:0.5 (F1) and 1:0.25 (F2)) showed significantly high dissolution rates of clotrimazole (Fig. 4). The results suggested that the melting point and the ratio of βCD to drug in inclusion complexes played crucial role in the dissolution of drug from the tested suppositories. The suppository formulations containing inclusion complexes of higher drug

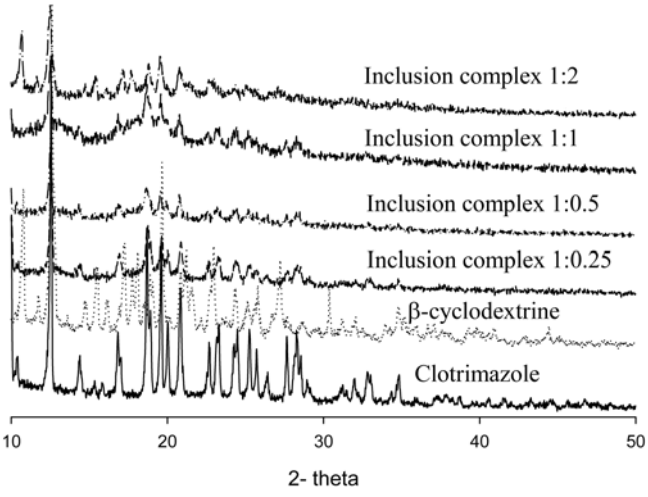

Fig. 3. X-Ray Diffraction study of clotrimazole-βCD inclusion complexes.

Table I. Formulation composition and melting point

Suppository formulations	Drug content	IC (w/w)	P188 (w/w)	PG (w/w)	Melting point (°C)
Control	1%	0	69.4	29.6	34.1
F1	1%	2% (1:0.25 IC)	68.6	29.4	35.4
F2	1%	3% (1:0.5 IC)	67.9	29.1	36.7
F3	1%	5% (1:1 IC)	66.5	28.5	37.3
F4	1%	9% (1: 2 IC)	63.7	27.3	37.5

IC: Inclusion complex, PG: Propylene glycol

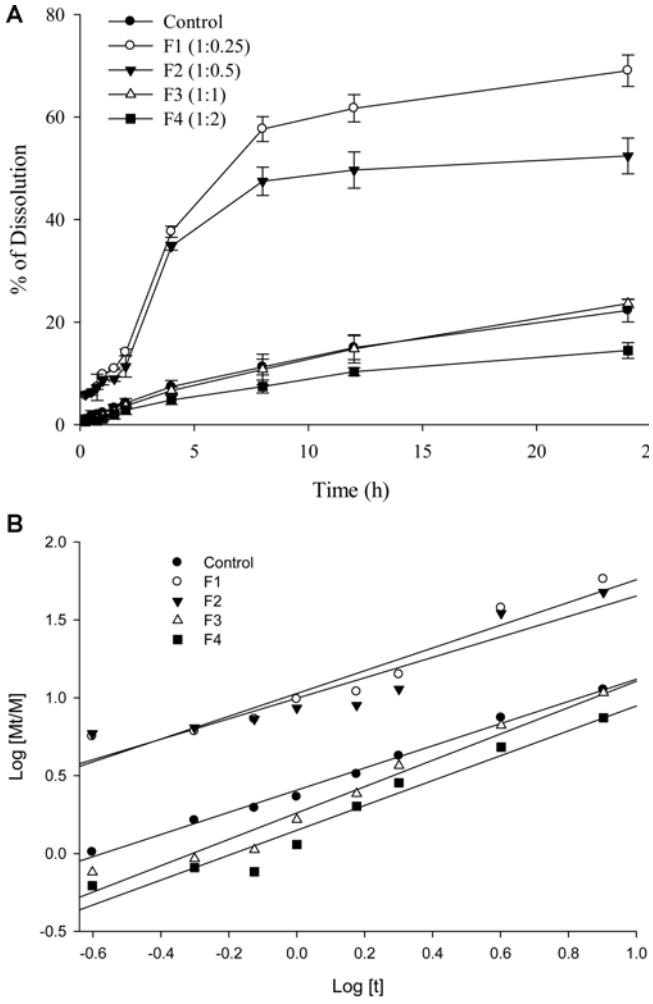

Fig. 4. Effect of different molar ratio inclusion complex on the dissolution profile of clotrimazole from poloxamer-based suppositories. (**A**) *In vitro* release profile of clotrimazole from different suppository formulations in phosphate-buffer (pH 4.4). (**B**) Dissolution kinetics of clotrimazole. Each value represents the mean ± S.D. (n = 3).

to βCD ratio (F3 and F4) showed poor dissolution profile than that of control suppositories. Inclusion complex was prepared to increase the solubility of poorly water soluble clotrimazole and it is known that inclusion complex increase the dissolution of clotrimazole (Prabagar et al., 2007). Moreover, it has also been reported that the rate of drug dissolution depends on poloxamer gel (Yong et al., 2007). However, in the present study, poloxamer alone not controls the drug dissolution from the test suppositories as the interface is eroding at a constant rate, the surface concentration of the drug is held relatively constant and the drug diffuses out at a constant rate (Xuan et al., 2011). Release from the complex is, on the other hand, a competitive process controlled by diffusion of the drug upon dilution and competitive displacement of the drug by components in the dissolution media (Xuan et al., 2011). The high ratio of drug to βCD (F3 and F4) and high melting point could be the reason for the low dissolution profile of F3 and F4 (Prabagar et al., 2007). Moreover, the higher amount of βCD in F3 and F4 could not dissolve in the water present in the immediate environment inside the semi permeable membrane, which also could have played a crucial role in the dissolution profile of F3 and F4 as a large amount of precipitation observed compared to F2 and there was no precipitation observed in F1.

To understand the dissolution mechanisms of clotrimazole, we described the dissolution rate using the following equations:

$$M_t/M = kt^n \quad (2)$$

$$\text{Log } M_t/M = \log k + n \log (t) \quad (3)$$

where Mt/M is the fraction of dissolved drug at time t, k a characteristic constant of the poloxamer-based suppository and n is an indicative of dissolution mechanism. As the k value becomes higher, the dissolution occurs faster. The n value of 1 corresponds to zero-order dissolution kinetics, $0.5 < n < 1$ means a non-Fickian dissolution model and $n = 0.5$ indicates Fickian diffusion (Higuchi model) (Xuan et al., 2011). From the plot of log(Mt/M) *vs* log(t) (Fig. 4B), kinetic parameter, n was calculated. It is clear from the n values shown in Table II that the poloxamer-based suppositories exhibited a non-Fickian diffusion mechanism (Yan et al., 2012). The k values indicate that the clotrimazole released more slowly from suppositories with higher concentration of βCD. These results suggest that the interface become more viscous with increased concentration of βCD, which decreased the diffusion of clotrimazole at the interface. This explains the low dissolution profile obtained for F3 and F4.

In vivo study

The pharmacokinetic parameters of clotrimazole were determined after rectal administration of prepared suppositories F1-4 and control (Table III). Fig. 5 shows the change of mean plasma concentration of clotrima-

Table II. Dissolution kinetic parameters

Suppository formulations	Release exponent, n	Kinetic constant, K	Correlation coefficient, r
Control	0.711	2.55	0.997
F1	0.7303	10.64	0.96
F2	0.657	9.92	0.931
F3	0.845	1.82	0.982
F4	0.798	1.41	0.973

Table III. Pharmacokinetic parameters of clotrimazole after suppository administrations in rats

Parameters	Control	F1 (1:0.25)	F2 (1:0.5)	F3 (1:1)	F4 (1:2)
AUC (μg/mL·h)	46.70 ± 7.87	82.39 ± 15.40*	67.05 ± 8.79*	41.480 ± 11.51	23.340 ± 8.37
Tmax (h)	5.20 ± 1.09	2.50 ± 0.57*	2.66 ± 0.58*	5.000 ± 2	3.250 ± 0.5
Cmax (μg/mL)	3.52 ± 0.71	7.47 ± 1.45*	5.83 ± 0.21*	3.260 ± 0.61	2.220 ± 0.85
K_{el} (h^{-1})	0.19 ± 0.01	0.16 ± 0.02*	0.14 ± 0.01*	0.129 ± 0.03	0.143 ± 0.01
$t_{1/2}$ (h)	3.45 ± 0.21	4.40 ± 0.55*	5.11 ± 0.43*	5.610 ± 1.33	4.860 ± 0.31

Each value represents the mean ± S.D. (n = 6). *$p < 0.05$ compared with control.

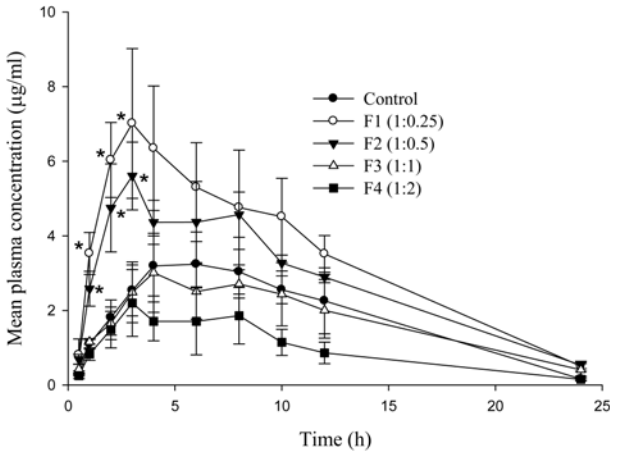

Fig. 5. Plasma concentration-time profiles of clotrimazole after rectal administration of prepared suppositories to rats. Each value represents the mean ± S.D. (n = 6). *$p < 0.05$ compared with control.

zole after rectal administration of preparations in rats. The initial plasma concentrations of clotrimazole in F1 and F2 suppository were higher compared with those of F3, F4 and control. In particular, in F1 and F2 suppository from 30 min to 4 h, the plasma concentrations of clotrimazole (0.8-7 μg/mL) at the same time interval were significantly higher than that of F3, F4 and control suppository (0.26-3.19 μg/mL) ($p < 0.05$). Our results indicated that the drug from F1 and F2 suppository could be absorbed faster than that of F3, F4 and control suppository in rats. The reason for this fast absorption could be dependent on the fast melting of F1 and F2 suppository and higher dissolution (Yong et al., 2007). The low absorption profile obtained from F3 and F4 could be due to their relatively high melting point (Table I), low dissolution profile and the presence of the high amount of βCD in F3 and F4, which may not be able to dissolve in the limited amount of water present in the immediate environment. Our results showed that the suppository formulations containing inclusion complexes prepared at low drug to βCD molar ratio showed superior in vitro and *in vivo* profile (F1 and F2) compared to formulations containing high drug to βCD molar ratio complex (F3 and F4).

Rectal irritation is one of the important limitations when it comes to suppository formulations. Though, the rectal irritation study was not performed in this study, the control suppository formulation has been reported that it did not irritate or damage the rectal tissue in rats (Yong et al., 2006); moreover, βCD has also been reported for its safety in rectal applications (Sato et al., 2010). Thus the prepared suppository formulations (F1 to F4) could be considered as safe and could be used effectively in the treatment of diseases with alleviated hepatotoxicity (Yong et al., 2006).

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