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Bioavailability of Clarithromycin Cyclodextrin Ternary Complexes Upon Oral Administration to Healthy Beagle Dogs

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The dissolution profiles of clarithromycin (CLM) and its β-cyclodextrin–citric acid ternary complexes (CTC) were examined. CTC showed an enhanced dissolution rate in pH 6.8 phosphate buffers. The relative bioavailability was evaluated by comparing area under the plasma concentration–time curve (AUC) of the pure CLM with that of its cyclodextrin–citric acid ternary complexes those were filled into hard gelatin capsules. To compare the pharmacokinetic behavior, both plasma levels of parent compound and the active metabolite 14-OH-CLM concentrations were estimated. The relative bioavailability value as the ratios of CLM of mean total AUC for CTC relative to CLM was 120.3%. The relative bioavailability value as the ratios of 14-OH CLM of mean total AUC for CTC relative to CLM was 95.3%. The results suggest that the absorption of CTC in beagle dogs was slightly improved because of the enhanced dissolution rate of CTC at pH 6.8.

Keywords clarithromycin; ternary inclusion complexes; bioavailability

INTRODUCTION

Clarithromycin (CLM) is a semi-synthetic 14-member macrolide ($C_{38}H_{69}NO_{13}$, MW 747.9) exhibiting a broad in vitro antibacterial spectrum and a variety of clinically diagnosed infections (Langtry & Brogden, 1997) in treatment. It is primarily metabolized to its biologically active 14-hydroxy-6-*O*-methylerythromycin metabolite (14-OH-CLM) in animals as well as in humans (Nakagawa, Itai, Yoshida, & Nafai 1992). However, it is a water-insoluble base (pK_a = 8.76) and with pH-dependent solubility (Ferrero et al., 1990). Several approaches have been adopted in order to overcome the solubility and bioavailability limitations of hydrophobic drugs and to guarantee drug effectiveness and safety. One of these is by enhancing solubility and, hence, bioavailability, by complexing hydrophobic drugs with cyclodextrin. The efficacy of β-cyclodextrin (βCD)-complexed CLM prepared in chloroform by coevaporation against *Mycobacterium avium* complex infections in human macrophages has been reported (Salem & Duzgunes, 2003). In the previous work we have investigated, the role of citric acid in enhancing the aqueous solubility of the complexes by adding small amount of the acid to prepare the CLM CTC system (Zhang, Chen, Zhang, Li, & Zhong, 2007), and have fully characterized complexation. Previous researches have reported a combined effect of cyclodextrin and acid or alkaline compounds on the solubility of alkaline or acidic lipophilic drugs, respectively (Redenti, Szente, & Szejtli, 2000, 2001). In some cases, this result is manifested in higher bioavailability (Piette et al., 2006).

The objective of this study is to investigate the possibility to enhance dissolution rate and bioavailability of CLM by complexing with β-cyclodextrin–citric acid ternary. The dissolution rate of CTC from cyclodextrin inclusion complexes in pH 5.0 sodium acetate buffers and 6.8 phosphate buffers were studied. The plasma levels of parent CLM were determined by using LC–MS–MS and the concentrations of its active metabolite 14-OH-CLM concentrations were estimated semi-quantitatively as equivalent. The relative bioavailability of CTC after oral administration at a dose of 75 mg/dog as CLM to beagle dogs was examined.

MATERIALS AND METHODS

Materials

CLM was provided by Jinhua Lixin Pharma Chemical Co., Ltd. (Jinhua, China). β-CD was provided by Maxdragon

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International Corporation (Guangzhou, China). Methanol was of high-performance liquid chromatography (HPLC) grade. All other chemicals were of analytical grade and were used without further purification, and deionized double-distilled water was used throughout the study.

Preparation of Lyophilized CLM–Citric Acid–β-Cyclodextrin Ternary Complexes

The ternary complexes were prepared by lyophilization reported by Zhang et al. (2007). Briefly, the amount of CLM, β-CD, and citric acid was a molar ratio of 1:1:1. β-CD was dissolved in water at 40°C and gave a clear solution. CLM was dissolved in an aqueous solution containing 0.5% (wt/vol) of citric acid. The resulting mixture was stirred on a magnetic stirrer at 40°C for 3 h to give a clear solution. The resulting solution was frozen and then lyophilized in a freeze-dryer for 48 h.

Dissolution

Capsules containing the drug equivalent to 75 mg of CLM were used in all dissolution studies. Dissolution tests, according to the USP 24 paddle method, were carried out on CTC equivalent to 75 mg CLM filled in hard capsule in 500 mL of dissolution medium maintained at 37 ± 0.5°C with a paddle speed of 50 rpm. Dissolution tests were carried out in two dissolution media of pH 5.0 sodium acetate buffer and 6.8 phosphate buffers. A 2-ml aliquot of dissolution media was taken from at 0, 10, 20, 40, 60, 90, 120, 180, and 240 min and 2 ml fresh dissolution media was replaced to maintain a constant total volume. The collected samples were assayed by using a reversed-phase HPLC-UV method according to USP 24 in which the sample solution was filtered and directly injected. Mean values were calculated based on triplicates.

Dosage Schedule and Blood Sampling

All animal studies were performed according to the Guidelines for the Care and Use of Laboratory Animals approved by the Ethics Committee of Animal Experimentation of Shenyang Pharmaceutical University. Six beagle dogs (three male and three female) with body weight 10 ± 1 kg were used. The dosage forms containing 75 mg of CLM were administered in a single-dose, randomized, open, two-crossover study. Dogs were fasted for 12 h before administration with free access to water. Each dog was given orally either reference (one pure CLM capsule) or test formulation (two capsules of CTC equivalent to 75 mg CLM). The legs of the dogs were shaven and cannulated through the cephalic vein using a 22-gauge catheter. Blood samples of 3 ml were collected into heparinized microcentrifuge tubes at predetermined times of 0, 0.5,1,1.5,2,3,4,6,8,10,12,14,and 24 h after dosing. Plasma was immediately obtained by centrifuging blood samples at 1,540 g for 10 min. The plasma samples were stored in a freezer at −20°C until analysis.

HPLC–MS–MS Assay of Plasma Samples

Chromatographic Conditions

HPLC system was equipped with a Shimadzu LC-10AD pump (Kyoto, Japan), attached to an Agilent 1,100 autosampler (Agilent, Wilminton, DE, USA). A Thermo Hypersil-Keystone-C_{18}, 5 μm, 150 × 3.0 mm column (San Jose, CA, USA) was used for separation at 20°C. The mobile phase consisted of methanol–water–formic acid (80:20:1, vol/vol/vol) with a flow-rate of 0.5 mL/min. Mass spectrometric detection was performed by a Finnigan TSQ (API II) triple-quadrupole mass spectrometer in the SRM mode using an electrospray ionization (ESI) source (San Jose, CA, USA). The spray voltage was set at 4.5 kV in the positive mode. The sheath and auxiliary gas (nitrogen) was set at 0.6 MPa and 3 L/min, respectively. The capillary temperature was 280°C. The transitions of protonated molecule for CLM at *m/z* 748 to the predominant product ion *m/z* 158, and for roxithromycin *m/z* 837 to *m/z* 158 and 14-OH-CLM *m/z* 764 to *m/z* 158 were monitored. The relative collision energy was set at 35 eV. Argon was used as the collision gas at a pressure of approximately 1.4 mTorr. The mass spectrometer was interfaced to a computer workstation running Xcalibur 1.1 software.

Sample Preparation

Frozen samples were allowed to thaw at room temperature. To 200 μL plasma samples in a glass tube, 100 μL mobile phase, 100 μL internal standard solution (4 μg/mL in water), and 200 μL of 0.1 M Na_2CO_3 solution were added. Then 2 mL of *n*-hexane–dichloromethane–isopropanol (300:150:15, vol/vol/vol) was added, the mixture was vortexed for 60 s, and centrifuged at 2,000 g for 5 min. The organic phase was transferred to a clean 10 mL glass tube and dried under a stream of nitrogen at 40°C. The extracts were reconstituted with 200 μL of the mobile phase, by vortexing for 30 s and transferred to autosampler vials. A 20-μL aliquot of the supernatant was injected onto the HPLC column. To prepare the standard calibration samples and QC samples, 100 μL internal standard roxithromycin solutions (4 μg/mL in water) and 100 μL of the standard working solutions of CLM were added to 200 μL blank plasma. The following procedures were the same as described above. Calibration standards were prepared to achieve the final standard plasma concentrations of 10, 20, 50, 150, 500, 1,000, 2,500, and 5,000 ng/mL for CLM.

Pharmacokinetic Analysis

The term T_{max} denotes the time to reach peak concentration, and C_{max} is the peak concentration, and they were obtained directly from the measured values. The elimination rate constant (K_e) was calculated from the slope of the logarithm of the plasma concentration verse time using the final four points.

The parameter $t_{1/2}$ was derived from $0.693/K_e$. The area under the plasma concentration–time curve AUC_{0-t_n} until the last sampling time (t_n) was calculated by the trapezoidal method. The area from time 0 to infinity was calculated by $AUC_{0-\infty} = AUC_{0-t} + C_t/K_e$. The relative bioavailability (F%) was calculated as $AUC_{\text{Test formulation}}/AUC_{\text{Reference}}$ formulation.

Statistical Analysis

Statistical analysis of the data obtained was done using SAS (2002 by SAS Institute Inc., Cary, NC, USA; Software Version 9.0). For comparisons of data, one-way analysis of variance (ANOVA) using comparison test and independent sample *t* test were used. The level of statistical significance was chosen as less than 0.05 ($p < 0.05$)

RESULTS AND DISCUSSION

In Vitro Characterization of CTC

The dissolution rate of CLM from CLM alone and CTC has never been previously reported. In this report, dissolution tests were carried out at pH 5.0 sodium acetate buffer and 6.8 phosphate buffers to investigate the influence of solubility on bioavailability because CLM may degrade under acidic conditions lower than pH 5.0 (Langtry & Brogden, 1997). Figure 1 shows the results of the dissolution tests of CLM from CLM alone and CTC. There were no appreciable differences between CLM and CTC at the pH 5.0 values. Satisfactory dissolution was also observed at pH 5.0 and CTC dissolved even at pH 6.8. All values of the dissolution tests at pH 6.8 and 5.0 were almost 80% at 40 min, and rapid dissolution was observed from CTC. CTC showed an enhanced dissolution rate in the basic media and may improve the absorption of CLM in the duodenum. The dissolution of CLM alone at pH 5.0 and very rapid but decreased at pH 6.8 because CLM is a weak base. It was confirmed from these results that the dissolution rate of CLM was enhanced by the ternary inclusion complexation at pH 6.8. It was reported that the organic acids promote the miconazole inclusion into CD cavity (Barillaro et al., 2004, 2007). When acid compound of flurbiprofen employed the HP-β-CD and the alkanolamines, drug solubility and dissolution rate in water were also notably improved (Maitre, Longhi, & Granero, 2007).

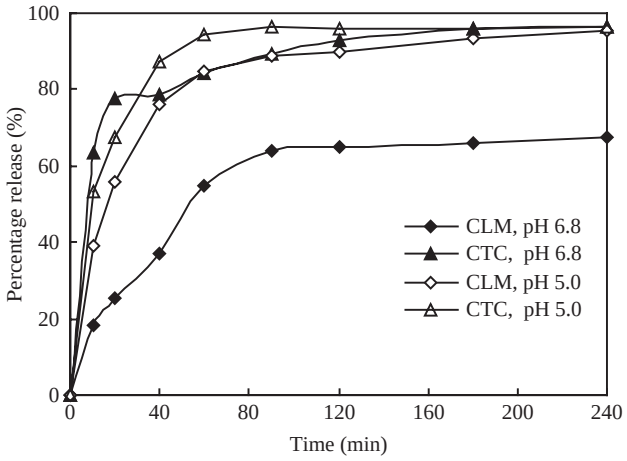

FIGURE 1. Dissolution profiles of clarithromycin (CLM) and β- cyclodextrin–citric acid ternary complexes (CTC) at dissolution media of pH 5.0 sodium acetate buffer and 6.8 phosphate buffers. Each point is the mean of three determinations.

LC–MS–MS Analysis of CLM and 14-Hydroxy-CLM in Blood Samples

The lower limit of quantification of CLM was 10 ng/mL and accuracy within 5% in terms of relative error (RE) and a precision in term of relative standard deviation (RSD) ≤ 3.6%. The lower limit of quantitation was 10.0 ng/mL when using 0.2 mL plasma, and it is sufficient for pharmacokinetic studies. Precision and accuracy were assessed by determining quality control (QC) samples at 20, 500, and 4,000 ng/mL on three different validation days. Both intra-run and inter-run precisions ranged from 2.7 to 3.3% and from 1.2 to 1.5% for each QC level, respectively. The accuracy was within 0.7%. The results, calculated using one-way ANOVA, indicated that the values were within the acceptable range and the method was accurate and precise (Shah, Midha, & Findlay, 2000). Under the present chromatographic conditions described in the experimental part, no endogenous interfering or late eluting peaks were found. Figure 2 shows the typical retention time for CLM, internal standard roxithromycin, and 14-OH-CLM was 3.34, 3.44, and 2.97 min. Figure 3 displays the product ion spectra of $[M + H]^+$ ions from CLM, 14-OH-CLM, and roxithromycin, respectively. They showed an intense ion at *m/z* 158, which was chosen in the selected reaction-monitoring (SRM) acquisition for CLM and roxithromycin, respectively. The most suitable collision energy was determined by observing the maximum response obtained for the fragment ion peak *m/z*. CLM is metabolized in rats, dogs, monkeys, and humans. In humans, the major metabolite 14-OH-CLM substantially contributes to the antibacterial activity. The quantification of metabolites is very difficult, particularly when they are not available commercially, as in the case of 14-OH-CLM. Thus, a semi-quantitative method that expressed the results in equivalents of CLM was developed for its determination according to previous report (Lerner et al., 2000).

Bioavailability of CLM Between Pure Drug and CTC

No adverse reactions were observed during or after oral administration in any of the beagle dogs. A high inter-subject variability was observed in the concentration–time curves obtained in this study. The mean serum concentration–time profile of CLM and 14-OH-CLM are presented in Figures 4 and 5 separately. The mean pharmacokinetic parameters of CLM determined by non-compartmental model after oral

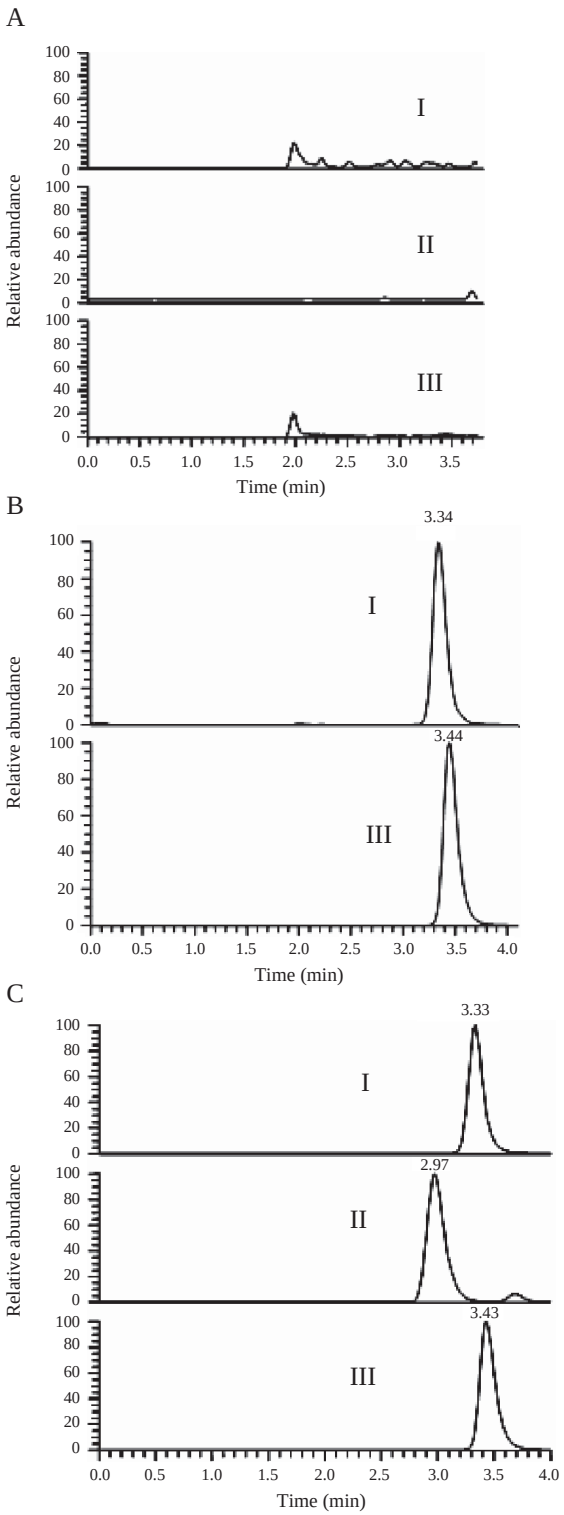

FIGURE 2. Representative selected reaction-monitoring chromatograms of clarithromycin (CLM) and roxithromycin. (A) a blank plasma sample; (B) blank plasma spiked with CLM (10 ng/mL) and roxithromycin (2,000 ng/mL); (C) a plasma sample 1.0 h after an oral administration of 75 mg CLM to beagle dog. (I) CLM; (II) 14-OH CLM; (III) Roxithromycin.

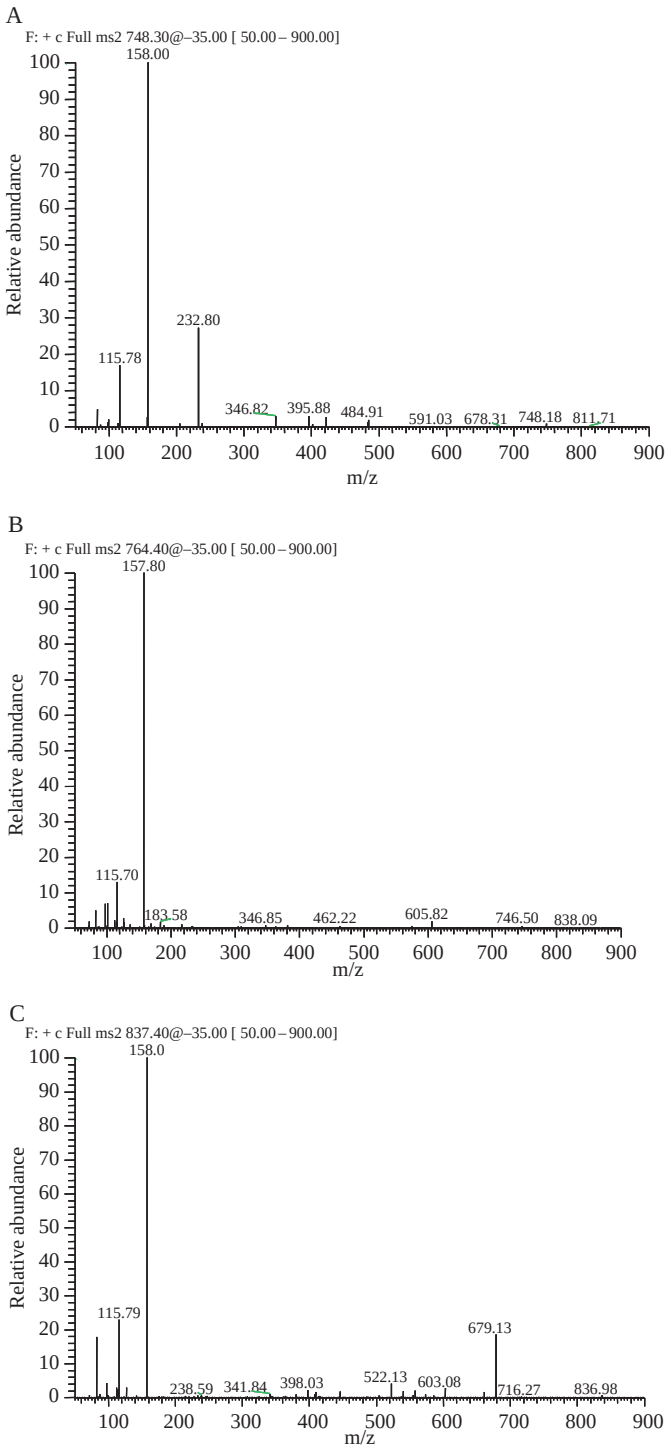

FIGURE 3. Product ion mass spectra of [M + H]+ of clarithromycin (CLM) 748→158 (A) 14-OH CLM 764→158 (B) and Roxithromycin 837→158.0 (C) in the Mobile Phase.

administration of the formulations are summarized in Table 1. The relative bioavailability of CTC calculated according to the ratio of $AUC_{0-\infty}$ of CTC relative to that of pure CLM preparation was 120.3%. However, the concentration of CLM in plasma

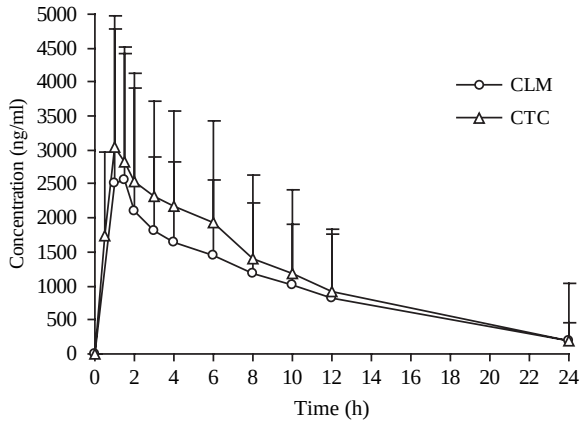

FIGURE 4. Profiles of mean plasma drug concentration-time of clarithromycin (CLM) after an oral administration of CLM and CTC capsule containing 75 mg CLM to beagle dogs under fast condition (n = 6).

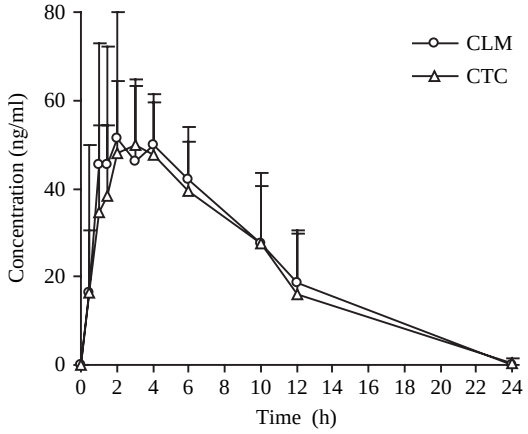

FIGURE 5. Profiles of mean plasma drug concentration-time of 14-OH clarithromycin (CLM) after an oral administration of CLM and CTC capsule containing 75 mg CLM to beagle dogs under fast condition (n = 6).

TABLE 1
Pharmacokinetic Parameters of Clarithromycin in Beagle Dogs after a Single Administration of Clarithromycin (CLM) and β-Cyclodextrin–Citric Acid Ternary Complexes (CTC) Capsule Containing 75 mg Clarit Hromycin (n = 6)

	CLM		CTC	
Parameter	*M*	*SD*	*M*	*SD*
$t_{1/2}$ (h)	5.0	1.8	4.2	1.4
K_e(1/h)	0.2	0.1	0.2	0.1
C_{max} (μg/ml)	2.7	1.9	3.2	1.8
T_{max} (h)	1.3	0.5	1.5	0.8
AUC_{0-t} (μg·h/ml)	23.6	19.4	27.7	22.3
$AUC_{0-\infty}$ (μg·h/ml)	25.4	22.1	29.1	24.4

TABLE 2
Pharmacokinetic Parameters of 14-OH-Clarithromycin in Beagle Dogs after a Single Administration of Clarithromycin (CLM) and β-Cyclodextrin–Citric Acid Ternary Complexes (CTC) Capsule Containing 75 mg Clarithromycin (n = 6)

	CLM		CTC	
Parameter	*M*	*SD*	*M*	*SD*
$t_{1/2}$ (h)	6.5	5.5	4.7	1.5
K_e(1/h)	0.2	0.1	0.2	0.0
C_{max} (μg/ml)	63.4	24.4	49.8	10.1
T_{max} (h)	3.4	2.2	4.2	2.4
AUC_{0-t} (μg·h/ml)	552.2	221.3	526.2	175.0
$AUC_{0-\infty}$ (μg·h/ml)	727.4	259.2	615.6	240.3

decreased quickly. This decrease was probably caused by a decrease of absorption of CLM in the intestinal level because of the lack of the drug in its dissolved form under the pH environment of the intestine. On the other hand, the concentration of CLM after administration of ternary complexes was relatively constant may due to a higher concentration of dissolved CLM in the intestine that is available for immediate absorption was maintained which may result from the rapid dissolution rate of CTC under pH 6.8 conditions. T_{max} of parent drug and its metabolite for CTC were delayed compared with those for CLM. When 14-OH-CLM was used as the indicator, the relative bioavailability calculated as the ratio of $AUC_{0-\infty}$ from CTC relative to that from CLM was 95.3%. Nevertheless, due to the high variability of the data, the difference between the $AUC_{0-\infty}$ of CTC and pure CLM could not be deemed as statistic significantly ($p > 0.05$). Because gastric pH influences the drug of pH-dependent solubility, the dissimilar gastric pH between the individuals may be one of the important factors contributing to the variability in the data (Zhou, Moench, Heran, & Heran, 2005). The mean pharmacokinetic parameters of metabolite 14-OH CLM determined by non-compartmental model after oral administration of the formulations are listed in Table 2. The relative amount of metabolite produced after administration, defined as the mass ratio of the metabolite to the administered parent compound, for CLM and CTC were 2.34 and 1.90%, respectively. There was no difference of the amount of metabolite between CLM and CTC after oral administration to beagle dogs at fast condition.

CONCLUSIONS

In vitro dissolution tests revealed that the ternary system gave a rapid dissolution rate at pH 6.8. The bioavailability of CLM could be improved slightly by inclusion with cyclodextrin and citric acid. No difference in the relative amount of metabolite produced after administration was observed between pure drug and its citric acid-cyclodextrin system.

However, the high inter-subject variability raised difficulties to further interpret the results. Further investigations using dose dependence as an index for drugs with poor solubility are recommended because of the dose-dependent non-linear elimination as a result of saturation of the metabolism at higher doses for CLM.

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