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RESEARCH PAPER

Oral Delivery of Ionic Complex of Ceftriaxone with Bile Acid Derivative in Non-human Primates

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ABSTRACT

Purpose Since the absorption of ceftriaxone (CTO) in the intestine is restricted by its natural physiological characteristics, we developed a series of small synthetic compounds derived from bile acids to promote the absorption of CTO in the gastrointestinal tract.

Methods Several bile acid derivatives were screened by measuring water solubility and partition coefficient of their complexes with CTO. The pharmacokinetic parameters of the selected CTO/HDCK ionic complex in monkeys were evaluated. The absorption pathway of CTO/HDCK complex was evaluated using Caco-2 cells and MDCK cells transfected with ASBT gene.

Results HDCK enhanced the apparent membrane permeability of CTO 5.8-fold in the parallel artificial membrane permeability assay model. CTO/HDCK complex permeated Caco-2 cell *via* transcellular pathway, and interaction of the HDCK complex with ASBT was important to enhance uptake. When CTO/HDCK (equivalent to 50 mg/kg of ceftriaxone) formulated with lactose, poloxamer 407 and Labrasol was orally administered to monkeys, its maximum plasma concentration was 19.5 ± 1.8 μg/ml and oral bioavailability $28.5 \pm 3.1\%$.

Conclusions The CTO/HDCK formulation could enhance oral bioavailability of CTO in non-human primates. This oral formulation could be an alternative to injectable CTO with enhanced clinical effects.

KEY WORDS ceftriaxone · deoxycholic acid · enhancer · ionic complex · oral delivery

ABBREVIATIONS

ASBT	apical sodium bile acid transporter
AUC	area under the curve
C_{max}	the maximum plasma concentration
CTO	sodium ceftriaxone
DAPI	4,6-diamidino-2-phenylindole
DOCA	deoxycholic acid
HDCK	an oral drug carrier derived from deoxycholic acid
HPMCP	hydroxypropyl methylcellulose phthalate
IVF	*in vitro* fertilization
KRICT	Korea Research Institute of Chemical Technology
MDCK	Madin-Darby canine kidney
PAMPA	parallel artificial membrane permeability assay
THF	tetrahydrofuran
T_{max}	the time to reach the peak concentration

INTRODUCTION

Bile acids have been recognized as absorption enhancers, which could increase permeation of drugs and facilitate

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their oral, buccal, transdermal, ocular, nasal, rectal or pulmonary delivery. As bile acids are efficiently absorbed *via* the apical sodium bile acid transporter (ASBT) in the ileum, ASBT may also serve as a target for enhancing oral bioavailability of poorly permeating compounds that are chemically conjugated or physically complexed with bile acid (1,2). In addition, bile acids can increase the oral absorption of drugs by masking the hydrophilic surface, and also solubilize fatty acids through the micelle formation (3,4). Recently, owing to this absorbability and amphiphilicity, bile acids have been put to use in the oral delivery of various drugs. For example, cholylsarcosine, an absorption enhancer as well as a non-toxic bile salt derivative, enhanced absorption of peptide drugs such as octreotide and desmopressin (5,6). The oral administration of acyclovir valylchenodeoxycholate, a conjugate of valacyclovir and chenodeoxycholate, resulted in a 2-fold increase in the oral bioavailability of acyclovir in rats (7). Deoxycholic acid derivative, as a permeation enhancer, improved the bioavailability of orally administered insulin (8,9) and heparin (10–12).

One of the well-known antibiotics in need of an oral dosage form is ceftriaxone. Ceftriaxone (CTO), a third-generation cephalosporin having a broad spectrum of activity against Gram-positive and Gram-negative aerobic and some anaerobic bacteria, has been indicated for the treatment of a range of infections caused by susceptible microorganisms, including infections of the lower respiratory tract and central nervous system (13,14). Although CTO has the advantage of superior efficacy and a long elimination half-life that permits once daily administration, its outpatient use was limited due to the lack of an oral formulation (15,16). The availability of new oral forms of therapy may provide an impetus for expanding the usage of CTO through community-based prescribing, as well as for following up on parenteral antibiotics using oral therapy (the so-called 'step-down therapy') (17,18). Consequently, it has multiple benefits such as cost saving, potential early discharge from the hospital, and the reduced risk of parenteral complications (19,20). However, switching from intravenous or intramuscular to oral formulation is confronted with a difficulty because CTO is hydrophilic and has a low octanol/water partition coefficient (log $K=-2.10\pm0.19$) (21). Due to the poor passage of CTO through epithelial membranes, many efforts have been made to find improved methods and carriers for enhancing the absorption from the small intestine (22–24).

Based on our previous results that the physically associated cationic analogue of bile acid could increase the lipophilicity of CTO and potentially target ASBT, we have synthesized a permeation-enhancer library based on bile acid derivatives (25). The purpose of the present study was to select an optimal enhancer and optimize its composition for improving the intestinal absorption of CTO *in vivo*. In this study, HDCK, an oral drug carrier derived from deoxycholic acid, showed desirable physicochemical properties and higher permeation enhancement than previously reported carriers did. The physical complex of CTO with HDCK was formulated with a solubilizer and excipients in order to completely dissolve the complex in water and to promote the stability and dispersibility of the complex in solution. We prepared the physical ionic complex of CTO and HDCK, and evaluated its permeability *in vitro* using parallel artificial membrane permeability assay (PAMPA), Caco-2 cells, and ASBT gene transfected MDCK cells. Finally, pharmacokinetic parameters of the optimized formulation of CTO/HDCK complex were evaluated in non-human primates.

MATERIALS AND METHODS

Materials

Sodium ceftriaxone (CTO) was obtained from the Chong Kun Dang Pharmaceutical Corp. (Seoul, Korea). Ethyl chloroformate, *N*-methylmorpholine, deoxycholic acid (DOCA), N_ε-Boc-L-lysine methyl ester hydrochloride (H-Lys(Boc)-OMe·HCl), lithium aluminum hydride ($LiAlH_4$), acetyl chloride, phalloidin-tetramethylrhodamine B isothiocyanate and 4',6-diamidino-2-phenylindole (DAPI) were purchased from Sigma-Aldrich Co. (St. Louis, MO), and tetrahydrofuran (THF), chloroform and methanol from Merck (Darmstadt, Germany). Madin-Darby Canine Kidney (MDCK) cell line was purchased from ATCC (Manassas, VA). For oral formulations, PEG-8 caprylic/capric glyceride (Labrasol) was obtained from Gattefossé (Lyon, France). Polyethylene polyoxypropylene block copolymer (poloxamer 407) was from BASF (Ludwigshafen, Germany). Lactose was purchased from DMV International (Veghel, Netherlands).

Synthesis of Absorption Enhancer, HDCK

HDCK was synthesized as follows (Fig. 1). Ethyl chloroformate (6.4 ml) and *N*-methylmorpholine (7.4 ml) were added in a dropwise manner to deoxycholic acid (26 g) in THF (800 ml) in ice bath. The mixture was stirred under the same condition for another 30 min, followed by further reacting at room temperature for 2 h. Both H-Lys(Boc)-OMe·HCl (20 g) and *N*-methylmorpholine (7.4 ml) were added and the mixture was refluxed for 2 h. The reaction mixture was cooled to room temperature and then stirred overnight. The precipitates were removed by filtration, and the solvent was removed by evaporation. The residues were purified by column chromatography, thereby obtaining Lys(Boc)DOCA. Lys(Boc)DOCA (24 g) was dissolved in THF (560 ml) and cooled down to 0°C in an ice bath. $LiAlH_4$ (32 g) was slowly added. Temperature was increased up to room

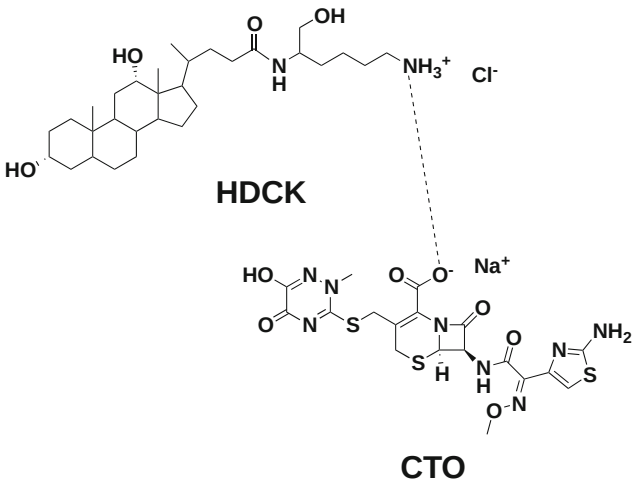

Fig. 1 Structural diagram of the electrostatic interactions between CTO and HDCK.

temperature, and the reaction mixture was refluxed overnight. Upon completion of the reaction, the temperature dropped to 0°C in ice bath, and both 24 ml of distilled water and 48 ml of 15% NaOH were added. The reaction temperature was raised up to room temperature again, and the reaction mixture was stirred until a white solid appeared. After removing the white solid by filtration, the solvent was evaporated. Acetyl chloride (23.4 ml) was added to methanol (100 ml) in ice bath, and the resulting mixture was allowed to stir for 30 min. The above residue was added to this solution and stirred at room temperature overnight. After the solvent was removed by evaporation, the residue was dissolved in water and washed with chloroform three times. The aqueous layer was lyophilized, resulting in a white powder (HDCK). ^{1}H NMR (DMSO-d6); δ 0.57 (3H, s), 0.82 (3H, s), 0.90 (2H, d, J=6.3 Hz), 1.11–1.83 (31H, m), 1.95–2.19 (1H, m), 2.71 (2H, dt, J=6.0, 12.9 Hz), 3.29–3.44 (1H, m), 3.77 (1H, brs), 4.09–4.37 (11H, m), 7.94 (3H, brs).

Preparation of CTO/HDCK Complex

The oral CTO formulation was prepared by making a physical ionic complex of CTO with HDCK. Briefly, CTO (10 mg) and HDCK (9.1 mg) were dissolved in 1 ml of distilled water. The CTO/HDCK complex was then formed by the addition of HDCK solution to CTO solution over vortex mixing. Precipitates were centrifuged and lyophilized at −80°C to obtain as a white powder. Both water solubility and the partition coefficient in octanol/water of CTO/HDCK complex were measured. The formation of CTO/HDCK complex was confirmed by comparing the crystalline character of the physical mixture and the complex by powder X-ray diffraction (D5005, Bruker, Germany). X-ray powder diffraction patterns were recorded on a Rigaku-D/MAX-IIIV diffractometer using Ni-filtered Cu-Kα radiation, a voltage of 40 kV and a 40 mA current. The scanning rate was 0.02° s^{-1} over a 2θ range of 3–40°.

In Vitro Permeability of CTO/HDCK Complex

The penetration enhancing activity of the selected enhancer, HDCK, was evaluated *in vitro*, using the BD Gentest™ Pre-Coated PAMPA Plate system. CTO, the CTO/HDCK complex, and the CTO/HDCK complex containing lactose and poloxamer 407 were dissolved in buffer (PBS, pH 7.4), respectively. The CTO/HDCK complex lyophilized powder with lactose and poloxamer 407 were also dissolved in 1% solution of Labrasol in PBS (pH 7.4). Each solution (300 μl, equivalent to 200 μM of CTO) was then carefully added into each well of the donor plate. PBS buffer (200 μl, pH 7.4) was added to each well of the acceptor plate. Then, the acceptor plate was placed on the compound-filled donor plate by slowly lowering the pre-coated PAMPA plate. The assembled plate was then incubated at room temperature for 5 h without agitation and placed into a sealed container with wet paper towels to avoid evaporation. After incubation, the pre-coated PAMPA plate and the donor plate were separated, and the sample concentrations in the donor and the acceptor plates were determined by HPLC. Each experiment was performed in triplicate and the mean volume of the three samples was used in the data analysis.

The permeability of each group was calculated using the following formula (26):

$$P = -\ln\left[1 - C_A(t)/C_{equilibrium}\right]/[A \times (1/V_D + 1/V_A) \times t]$$

where P is permeability in units of cm/s, $C_A(t)$=compound concentration (mM) in the acceptor well at time t, V_D= donor well volume (0.3 ml), V_A=acceptor well volume (0.2 ml), A=filter area (0.3 cm^2), t=incubation time (18000 s), $C_D(t)$=compound concentration (mM) in donor well at time t, and $C_{\text{equilibrium}} = [C_D(t) \times V_D + C_A(t) \times V_A]/(V_D + V_A)$.

Optical Analysis of CTO Permeation Through Caco-2, MDCK, and ASBT-Transfected MDCK Cell Monolayer

To evaluate the penetration route of CTO/HDCK complex, Caco-2 cells were treated with fluorescein isothiocyanate conjugated CTO (FITC-CTO) and FITC-CTO/HDCK complex (0.05 mg/ml) for 1 h at 37°C, respectively. The tight junction marker, phalloidin, was used to differentiate the paracellular route from transcellular route of drug absorption. MDCK cells were stably transfected with apical

sodium bile acid transporter (ASBT) genes using Lipofectamine 2000® in order to observe the interaction between bile acid transporter and CTO/HDCK complex, according to the procedure described in the literature (27). MDCK cells transfected with ASBT (MDCK-ASBT) were seeded at the density of 2×10^5 cells/well to *in vitro* fertilization (IVF) culture dishes (SPL Life Sciences, South Korea) and grown until a confluent monolayer was formed. The cells were equilibrated for 30 min and washed three times with Hanks' balanced salt solution (HBSS). The cells were treated with 200 μl of FITC-CTO/HDCK complex (0.1 mg/ml) for 1 h at 37°C. Subsequently, after washing the cells three times with HBSS, the cells were fixed with 200 μl of 4% cold paraformaldehyde solution in PBS for 20 min at room temperature. Then, the samples were treated for 40 min with 0.3% Triton X-100 in blocking solution made of 10% normal goat serum in PBS. The samples were treated with anti-human ASBT antibody (Sigma-Aldrich) at a dilution of 1:1000 in the diluted blocking buffer overnight. The primary anti-human ASBT antibody was stained with Alexa flour 546 labeled secondary antibody (Invitrogen, CA) at 10 μg/ml. The nucleus was counterstained by DAPI (Sigma-Aldrich) for 5 min. After several washings with PBS, the cells were fixed on glass slides and covered with a glass cover slip using Mounting® medium (Sigma-Aldrich). The fluorescence images were then observed using a Carl Zeiss LSM Meta 710 inverted confocal laser scanning microscope (Carl Zeiss, Germany).

Absorption of Orally Administered CTO/HDCK Complex in Monkeys

In order to prepare enteric-coated capsules for *in vivo* monkey experiment, CTO/HDCK complex (190.8 mg) lyophilized with lactose (10 mg) and poloxamer 407 (10 mg) was granulated with either 1 mg of Labrasol (formulation A) or well suspended with 300 mg of Labrasol and 200 mg of water (formulation B). A capsule enteric-coated with hydroxypropyl methylcellulose phthalate (HPMCP) was filled with such granule formulation (formulation A) and suspension formulation (formulation B) of CTO/HDCK, respectively, and then sealed with 10% HPMCP solution. Male cynomolgus monkeys (4.5–5.0 kg, Korea Research Institute of Chemical Technology, KRICT, Daejeon, Korea) were fasted for 12 h before the drug administration. The prepared capsules were orally administered with 10 ml of water. In addition, CTO (1 mg/kg) in PBS solution (pH 7.4) was administered intravenously in order to determine its oral bioavailability. After oral or intravenous administration of drugs, the blood samples (450 μl) were collected from the vein and mixed with 50 μl of sodium citrate (3.8% solution), followed by centrifugation at 2,500*g* for 15 min at 4°C. The plasma samples were separated and stored at −70°C. All the animal experiments were carried out in accordance with the procedures outlined in the Guide for the Care and Use of Laboratory Animals and approved by KRICT.

The concentration of CTO was determined by HPLC using Agilent 1200 series (Agilent, Palo Alto, CA). Chromatographic separation was achieved using a Luna C18 column (250×4.6 mm, 5 μm, Phenomenex) with the injection volume of 50 μl. The mobile phase consisted of a 40:60 (v/v) mixture of 25 mM potassium phosphate buffer (pH 7.0) and acetonitrile containing 0.5% hexadecyltrimethylammonium bromide. The flow rate was set to 0.8 ml/min, and UV detection wavelength was 280 nm. To determine the plasma concentration of CTO, a polypropylene centrifuge tube (2 ml) was filled with exactly 200 μl of thawed plasma, then mixed with 200 μl of distilled water and 800 μl of acetonitile. The tube was vortexed for 1 min and then centrifuged at 12,000*g* for 10 min at 4°C. The supernatant was filtered with a PVDF syringe filter (Millex AP, Millipore, 0.45 μm). The filtrate was analyzed by HPLC as describe above.

Pharmacokinetic and Statistical Analysis

The pharmacokinetic parameters were obtained by non-compartmental analysis from WinNonlin (Pharsight Corporation, Mountain View, CA). Statistical analysis was done by student's *t*-test, where *P*-values less than 0.05 were considered significant. All results were expressed as mean± standard deviation (SD).

RESULTS

Characterization of HDCK and CTO/HDCK Complex

The water solubility of CTO/enhancer complex was greatly influenced by the characteristics of chemical structure of the enhancer, and the ability of a bile acid to enhance the CTO permeation also depended on its hydroxyl groups. HDCK was selected as an optimal enhancer from the library of bile acid derivatives, with the water solubility of CTO/HDCK complex being 0.98±0.04 mg/ml. Octanol/water partition coefficient ($K_{o/w}$) of CTO/HDCK was increased by as much as 0.42±0.02, compared to that of CTO ($K_{o/w}$= 0.01±0.00) (9,25). Furthermore, HDCK was composed of the hydrophobic part of deoxycholic acid and the positive charge of lysine, thereby facilitating ion-pairing interactions with negatively charged CTO. The evidence of CTO/HDCK ionic complex formation in a solid state was obtained by X-ray powder diffraction. X-ray diffraction patterns of CTO, HDCK, CTO/HDCK powder mixture, and CTO/HDCK complex were presented in Fig. 2.

Notable diffraction peaks were observed for CTO within 10–30° 2θ range with characteristic peaks displaying crystallinity at 11.18°, 12.56°, 18.92°, 21.24°, 22.74°, 23.80°, 25.20°, and 28.28°. On the other hand, HDCK did not exhibit crystalline diffraction patterns compared to CTO. In the case of the powder mixture of CTO and HDCK, the X-ray pattern appears to be a simple superimposition of the CTO pattern and the HDCK pattern, and the characteristic peaks of ceftriaxone were still detectable. However, the crystalline peaks disappeared in the CTO/HDCK complex. The disappearance of crystallinity in the X-ray diffraction pattern confirmed drug amorphization due to the complex formation of ceftriaxone and HDCK.

In Vitro Permeability of CTO/HDCK Complex

To investigate the effect of HDCK on the permeability of CTO, we measured the permeabilities of CTO, CTO formulated with solubilizer, CTO/HDCK complex, and CTO/HDCK complex with solubilizer in a PAMPA model, respectively, as shown in Fig. 3. CTO alone demonstrated a low permeability (P=0.16±0.14×10^{-6}cm/s) through the PAMPA artificial membrane, and there was little effect of formulation with lactose, poloxamer 407 and Labrasol on CTO permeability (P=0.40±0.14×10^{-6}cm/s). Whereas, the complex formation with HDCK significantly enhanced the permeability of CTO (P=0.93±0.08×10^{-6}cm/s, p<0.05), and further increased permeability was observed in CTO/HDCK complex formulated with lactose, poloxamer 407, and Labrasol (P=1.96±0.21×10^{-6}cm/s) by more than 10-fold increase compared to CTO alone. Since the artificial membrane in PAMPA was used for screening enhancers that tend to permeate by the passive transcellular mechanism, the highly enhanced permeability of CTO/HDCK complex was an indication that passive permeation of CTO was considerably improved by forming complex with HDCK. The CTO/HDCK complex was pre-formulated with lactose, poloxamer 407 and Labrasol because the CTO/HDCK complex itself tended to form particles in water at a high concentration due to the hydrophobic nature of HDCK.

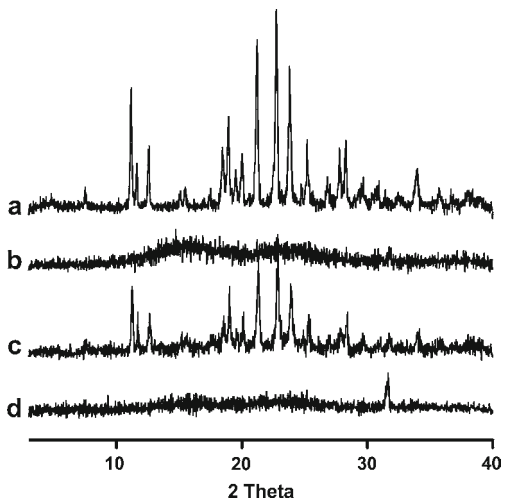

Fig. 2 X-ray diffraction patterns of (**a**) CTO, (**b**) HDCK, (**c**) the powder mixture of CTO and HDCK, and (**d**) the CTO/HDCK complex.

Absorption of CTO/HDCK Complex Mediated by Apical Sodium Bile Acid Transporters (ASBT)

The cellular absorption of CTO/HDCK complex was revealed by its penetration in Caco-2 cells. CTO alone failed to be absorbed in cells, whereas the CTO/HDCK complex was observed to penetrate mostly by means of transcellular route rather than a paracellular pathway (Fig. 4). The merge image at a high magnification indicated that HDCK enhanced transepithelial transport of CTO without disrupting the tight junction structure. To evaluate the interaction between CTO/HDCK complex and ASBT, the uptake of CTO/HDCK complex was compared between ASBT gene-transfected MDCK (MDCK-ASBT) cell lines and the non-transfected MDCK cell lines (Fig. 5). The CTO/HDCK complex was highly uptaken in MDCK-ASBT cells that expressed ASBT. On the other hand, the CTO/HDCK complex was slightly uptaken in MDCK

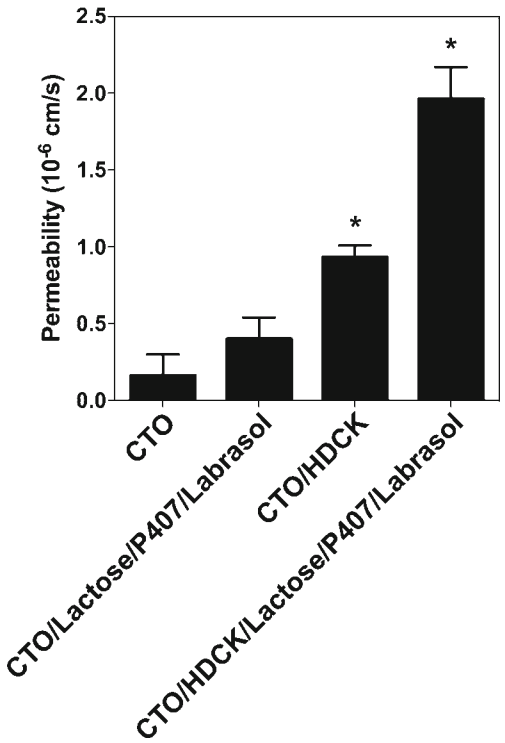

Fig. 3 *In vitro* permeability (*P*) of ceftriaxone and CTO/HDCK complex. Each value represents the mean ± SD (n=5). * p < 0.01 *vs*. CTO.

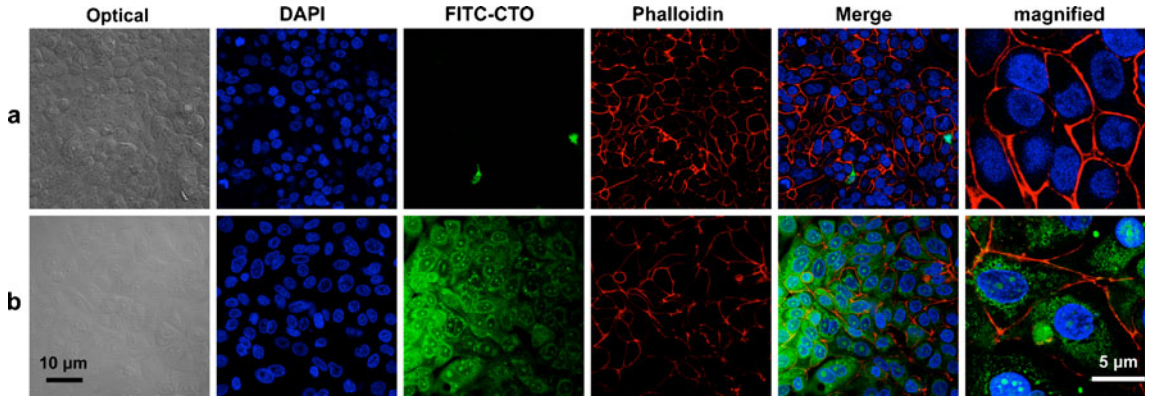

Fig. 4 Confocal laser scanning microscopic images of Caco-2 cell monolayers. Cells were treated with 0.05 mg/ml of (**a**) FITC-CTO and (**b**) FITC-CTO/enhancer complex for 1 h at 37°C.

cells that did not expressed ASBT. Therefore, complexed HDCK strongly interacted with ASBT, and it might enhance the CTO absorption in the gastrointestinal tract.

Oral Administration of CTO/HDCK Complex in Monkeys

To assess the oral absorption of CTO/HDCK complex in monkeys, the plasma concentration of CTO at each sampling time was measured after a single oral administration of the capsule formulation of CTO/HDCK complex. The mean plasma concentration-time profiles following oral administration are shown in Fig. 6 and the estimated pharmacokinetic parameters are summarized in Table I. The oral administration of 100 mg of CTO yielded the maximum plasma concentration (C_{max}) of only 3.5 ± 0.2 μg/ml and the oral bioavailability of 6.7 ± 0.7%, indicating its poor intestinal permeability in monkeys. On the other hand, upon administration of CTO/HDCK complex (equivalent to 100 mg of CTO), lactose (10% w/w of CTO), poloxamer 407 (10% w/w of CTO), and Labrasol (1% w/w of CTO), a 6.2-fold increase in C_{max}, 2.7-fold increase in area under the concentration-time curve from zero to 10 h ($AUC_{0-600min}$), and 2.7-fold increase in oral bioavailability were observed compared to CTO alone.

The pharmacokinetic parameters for CTO/HDCK complex were significantly changed with the increase of the Labrasol content. When CTO/HDCK was formulated with 1% of Labrasol and 60% Labrasol aqueous solution, C_{max} were 21.7 ± 3.7 and 26.3 ± 6.9 μg/ml, respectively. Their $AUC_{0-600min}$ values were 4410.1 ± 572.7 and 5676.4 ± 1531.8 μg/(ml/min) and the oral bioavailabilities were 18.0 ± 2.3 and 23.2 ± 6.2%, respectively ($p < 0.05$). Therefore, the oral absorption of CTO in monkeys was enhanced

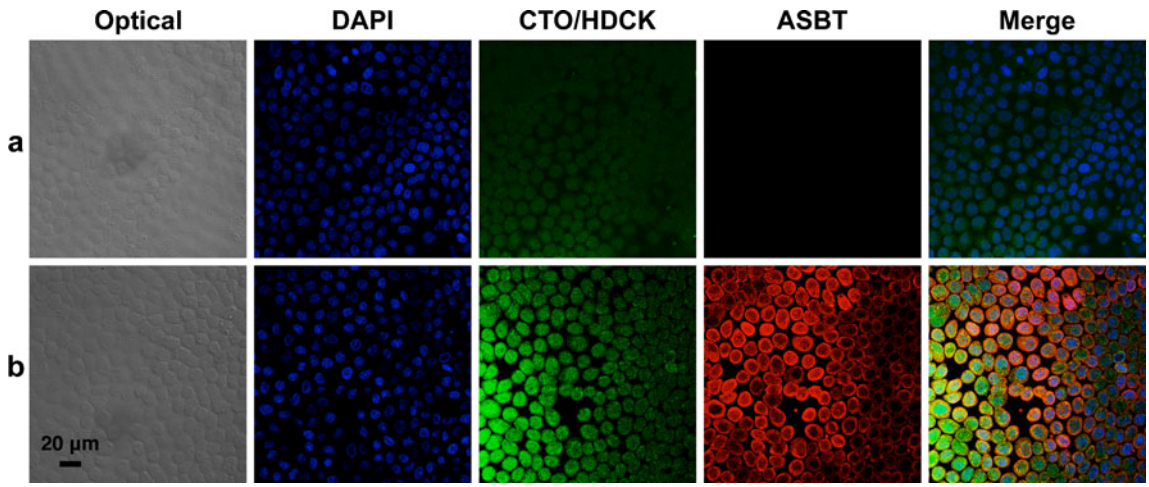

Fig. 5 Confocal laser scanning microscopic images of (**a**) MDCK cells and (**b**) MDCK-ASBT cells. Cells were treated with 200 μl of FITC-CTO/enhancer complex (0.1 mg/ml) for 1 h at 37°C (scale bar=20 μm).

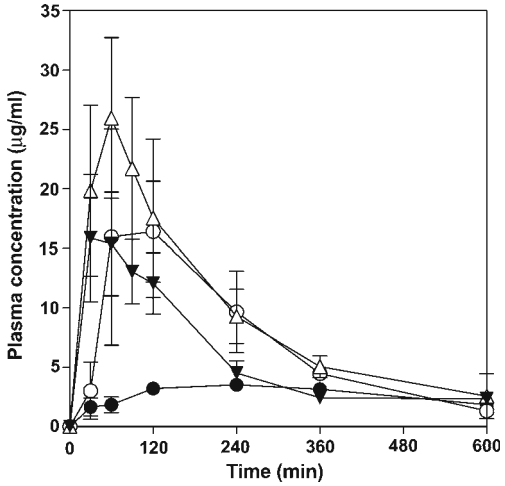

Fig. 6 The plasma concentration of ceftriaxone in monkeys after oral administration; 100 mg of CTO (●), formulation A: the granules formed with CTO/HDCK complex (equivalent to 100 mg of CTO), lactose (10% w/w of CTO), poloxamer 407 (10% w/w of CTO), and Labrasol (1% w/w of CTO) (○), formulation B: CTO/HDCK complex equivalent to 100 mg of CTO with lactose (10% w/w of CTO) and poloxamer 407 (10% w/w of CTO) suspended in 60% Labrasol aqueous solution (300% w/w of CTO) (△), and formulation B: CTO/HDCK complex equivalent to 50 mg of CTO with lactose (10% w/w of CTO) and poloxamer 407 (10% w/w of CTO) suspended in 60% Labrasol aqueous solution (300% w/w of CTO) (▼). The data are plotted as mean ± SD (n=4).

by the complex formation with HDCK and it was further enhanced with the increased Labrasol content.

Two different doses of the CTO (50 and 100 mg/kg) complexed with HDCK were used for the evaluation of the dose-dependency. It was observed that C_{max} after a single oral administration of enteric capsules containing the CTO/HDCK complex at the concentration equivalent to 50 mg and 100 mg of CTO with lactose (10% w/w of CTO) and poloxamer 407 (10% w/w of CTO) suspended in 60% Labrasol aqueous solution (300% w/w of CTO) were 19.5 ± 1.8 and 26.3 ± 6.9 μg/ml, respectively. Their $AUC_{0-600min}$ values were 3497.3 ± 374.4 and 5676.4 ± 1531.8 μg/(ml/min) and the oral bioavailabilities were 28.5 ± 3.1 and 23.2 ± 6.2%, respectively. It showed that the increased amount of CTO in dose induced the significant enhancement of C_{max} in plasma and $AUC_{0-600min}$ ($p<0.05$), but oral bioavailability of CTO/HDCK complex was not proportional to the dose. The time to reach the peak plasma concentration (T_{max}) following oral administration of CTO/HDCK varied approximately from 45 min to 96 min according to the Labrasol content and the dose of CTO, and the plasma levels of CTO did not fall below the lower limit of quantification until 10 h after administration.

DISCUSSION

CTO has been classified as a Biopharmaceutics Classification System (BCS) Class III compound that possesses high water solubility and poor intestinal permeability. Several attempts were made to improve the oral bioavailability of CTO by means of permeation enhancers. Our approach relied on the development of bile acid-based delivery agent that is ionically complexed with CTO to enhance its oral absorption. Bile acids are amphiphilic, and the polar and nonpolar domains are separated along the longitudinal axis of the bile acid molecules. The facial amphiphilicity of the bile acids influences the way in which they organize in solution and presumably plays a role in their ability to promote the absorption of polar drugs across membranes. Following the work on drug carriers for oral delivery based on bile acid molecules that could form complexes with drug molecules, we synthesized cationic analogues of bile acids and evaluated their physicochemical and permeation-enhancing properties. Among a series of compounds, we selected HDCK as the optimal enhancer for the oral delivery of CTO, and assessed the *in vivo* performance of HDCK. HDCK ionically complexed with CTO significantly enhanced the oral absorption of CTO. The physically associated HDCK could be considered to be enhancing drug partition into the cell membranes. In the PAMPA system, when the CTO/HDCK complex was formed, its permeability was increased compared to CTO, which ultimately resulted in the enhanced permeation across the PAMPA

Table I Pharmacokinetic Parameters of Ceftriaxone After Oral Administration of Enteric Coated Capsules Containing Free Ceftriaxone or CTO/HDCK Complex Formulation to Monkeys

Capsule formulation	Dose of ceftriaxone (mg/monkey)	T_{max}[a] (min)	C_{max}[b] (μg/ml)	$AUC_{0-600min}$[c] (μg•min/ml)	F[d] (%)
Ceftriaxone	100	240.0 ± 0.0	3.5 ± 0.2	1640.0 ± 170.8	6.7 ± 0.7
Formulation A	100	96.0 ± 32.9	21.7 ± 3.7	4410.1 ± 572.7	18.0 ± 2.3
Fromulation B	100	50.0 ± 17.3	26.3 ± 6.9	5676.4 ± 1531.8	23.2 ± 6.2
Formulation B	50	45.0 ± 17.3	19.5 ± 1.8	3497.3 ± 374.4	28.5 ± 3.1

Each value represents the mean ± SD (n=4). [a] T_{max}, the time to reach the peak concentration; [b] C_{max}, the peak concentration; [c] $AUC_{0-600min}$, area under the concentration-time curve from zero to 10 h; [d] F, bioavailability. Bioavailability was calculated by the following equation: $\left[\left(AUC_{0-600\,min,\,oral}/Dose_{oral}\right)/\left(AUC_{0-600\,min,\,iv,\,1\,mg}/1\text{ mg of CTO}\right)\times 100\right]$

membrane. However, PAMPA has limitations in that it is a non-cell-based model, lacking transporter- or pore-mediated permeability and that it provides only predictions about the passive transcellular permeability.

The route of drug absorption was confirmed in a Caco-2 cell model. The CTO/HDCK complex was mostly transported through the transcellular pathway. The driving force of this pathway was attributed to the interaction of HDCK and ASBT that is located on the apical membrane of cell monolayer. The postulated mechanism underlying the pathway was that HDCK would enhance the concentration gradient of CTO by interacting with ASBT at the site of absorption. The increase in gradient thus allows the drug transcellular absorption without having to disrupt the tight junction structure. CTO/HDCK complex was slightly uptaken into the non-transfected MDCK cells due to the increase in its lipophilicity. This also suggested that the mechanism of HDCK's action as an enhancer was unique and different from other tight junction openers.

The oral bioavailability of CTO in monkeys was not increased proportionally to the dose at 100 mg/kg of CTO complexed with HDCK. This suggests that the gastrointestinal fluids were saturated and the fraction of dose absorbed was decreased when the dose for levels higher than critical dose was used (28). In other words, the nonproportional absorption of 50 and 100 mg doses indicates that dose-dependent absorption was a passively uptaken CTO. However, these concepts were based on the assumption that drug permeability is constant along the intestinal lumen and that carrier-mediated transport is ruled out. Since the absorption of CTO/HDCK complex was not only due to passive permeation but also due to the interaction with ASBT, saturation of carrier-mediated absorption also might be involved.

The selection of formulation composition is also critical for *in vivo* performance. To promote the stability and dispersity of CTO/HDCK in solution and optimize the formulation, pharmaceutically acceptable excipients such as lactose and poloxamer 407 were used. In addition, since the complexation with HDCK enhanced the lipophilicity of CTO and decreased its solubility in water, Labrasol, one of the widely used solubilizers with good miscibility was chosen to emulsify the complex in solution in the small intestine. We observed a concentration-dependent enhancing effect of Labrasol on the intestinal absorption of CTO complexed with HDCK.

The jejunum in the small intestine is the ideal region for disintegrating an oral dosage form of CTO and thus enteric-coated oral formulations were prepared for monkey experiments. The polymer used in the present study was HPMCP, which is insoluble in acidic gastric fluids and only permits the release of the tablet contents in neutral or alkaline media. After the oral administration of CTO/HDCK complex (100 mg/head) with lactose (10% w/w of CTO) and poloxamer 407 (10% w/w of CTO) in 60% Labrasol aqueous solution to monkeys, the oral bioavailability of CTO was increased by 3.5-fold, and the maximum concentration in plasma was 26.3 ± 6.9 μg/ml. It is reported that the time above minimum inhibitory concentration (MIC) is related to efficacy of CTO, and the maximal efficacy is attained when the time above MIC is held for 60–70% of the time before the next dosing (29). MIC of CTO was reported to range from 0.016 to 1.0 μg/ml against *Escherichia coli*, *Haemophilus influenzae*, *Klebsiella pneumoniae*, *Neisseria gonorrhoeae*, *Streptococcus pneumoniae*, and *Branhamella catarrhalis*, and our results showed that the concentration in the plasma remained above MIC for 10 h (30). Thus, according to the present monkey pharmacokinetic results, CTO/HDCK oral formulation is expected to achieve positive pharmacodynamic outcome in clinical study.

CONCLUSION

The drug carrier for oral delivery, HDCK, has been designed, characterized, and validated for enhancing permeation and oral absorption of CTO. Results from this study demonstrated that the oral bioavailability of CTO was enhanced by CTO/HDCK complex formulated with a solubilizer. Taking advantage of the long time above MIC, an oral product given once or twice daily may deliver adequate therapeutic levels of CTO. Enteric coating formulations for CTO/HDCK complex would allow optimal delivery of CTO to the desired site of the intestine. It is anticipated that this oral drug carrier might be a potential candidate for the oral delivery of ceftriaxone, which could improve patient acceptability and therapeutic effect.

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