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Preparation, Characterization, and Bioavailability of Ursodeoxycholic Acid–Phospholipid Complex In Vivo

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The aim of this study was to prepare ursodeoxycholic acid–phospholipid complex (UDCA-PLC) to enhance oral bioavailability of UDCA, and the physicochemical properties of the complex were studied. Compared with those of UDCA tablet after oral administration in rats, the main pharmacokinetic characteristics and bioavailability of UDCA-PLC orally administered were evaluated. Tetrahydrofuran was used as a reaction medium, UDCA and phospholipids were resolved into the medium, and UDCA-PLC was formed after the organic solvent was evaporated off under vacuum condition. The physicochemical properties of the complex were evaluated using scanning electron microscopy (SEM), transmission electron microscopy (TEM), differential scanning calorimetry (DSC), X-ray diffraction, particle size distribution analysis, and *n*-octanol/water partition coefficient (*P*) study. The blood concentrations of UDCA-PLC and UDCA tablet at different time points after oral administration in rats were assayed by high-performance liquid chromatography (HPLC) after derivatization. The pharmacokinetic parameters were computed by software program 3p87. The X-ray diffraction and DSC studies showed that UDCA and phospholipids in the UDCA-PLC were combined by noncovalent bond, not forming a new compound, and *n*-octanol/water partition coefficient (*P*) of UDCA-PLC was effectively enhanced. The mean serum concentration–time curves of UDCA after oral administration of UDCA-PLC and UDCA tablet in rats were both in accordance with open two-compartment model. Pharmacokinetic parameters of UDCA tablet and the PLC in rats were T_{max} 1.9144 and 1.5610 h, C_{max} 0.0576 and 0.1346 μg/mL, and $AUC_{0-\infty}$ 4.736 and 11.437 μg h/mL, respectively. The bioavailability of UDCA in rats was significantly different ($p < .05$) compared with those of UDCA tablet after administration. The UDCA-PLC would be more prospective formulation in future.

Keywords ursodeoxycholic acid; phospholipid complex; physicochemical properties; bioavailability; derivatization

INTRODUCTION

Ursodeoxycholic acid (3a,7b-dihydroxy-5b-cholanoic acid, UDCA, see Figure 1) was discovered as the principal bile acid in the polar bear by Hammersten in 1902 (Fromm, 1984). UDCA suppresses biliary secretion of cholesterol and inhibits its intestinal absorption in humans (Hofmann, 1994; Ward, Brogden, Heel, & Speight, 1984). It is used for the dissolution of cholesterol-rich gallstones in patients with functioning gallbladders (Bachrach & Hofmann, 1982a) and in the treatment of primary biliary cirrhosis. Furthermore, it has been tried in the treatment of some chronic liver diseases associated with cholestasis such as liver disease in cystic fibrosis, cholestasis associated with pregnancy, sclerosing cholangitis, chronic active hepatitis, and viral hepatitis. UDCA has also shown some promise in the treatment of nonalcoholic steatohepatitis and refractory graft-versus-host disease of the liver in transplant patients (Parfitt et al., 1999). However, a severe limitation exists and is imputable to the poor absorption of these active constituents when administered orally or by topical application.

The bioavailability of poorly soluble drugs when administered orally as solid dosage forms is notoriously low. There are usually several factors responsible for this, but a particularly widespread problem is poor absorption due to slow and/or incomplete drug dissolution in the lumen of the gastrointestinal tract. There are

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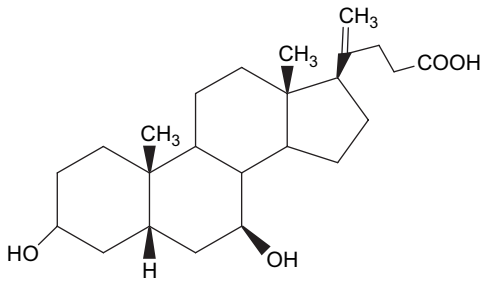

FIGURE 1. The chemical structure of ursodeoxycholic acid (UDCA).

numerous advantages of phospholipids in addition to solubility property while considering them for a carrier system. It was reported that some poorly soluble drugs combined with phospholipids could result in increase of oral bioavailability and improvement of the biological effect, such as silybin (Yanyu, Yunmei, Zhipeng, & Qineng2006), flavanolignans (Bombardelli & Piffer et al., 1987), glycyrrhetinic acid (Bombardelli & Patri et al., 1992), and bilobalide (Bombardelli & Mustich, 1991). In this article, UDCA–phospholipid complex (UDCA-PLC) was studied in order to improve oral bioavailability of UDCA.

The objectives of this study were as follows: (1) to improve oral bioavailability of UDCA—it is expected that UDCA combined with phospholipids might increase the oral bioavailability; hence, UCDA–PLC was prepared; (2) to evaluate the physicochemical characters of UDCA-PLC—using scanning electron microscopy (SEM), transmission electron microscopy (TEM), differential scanning calorimetry (DSC), X-ray diffraction, and particle size distribution analysis; the *n*-octanol/water partition coefficient (*P*) study of UDCA-PLC was performed to evaluate the solubility properties of UDCA-PLC; and (3) to study the main pharmacokinetics parameters and relative bioavailability of UDCA-PLC in rats compared with UDCA tablet, after oral administration of these two formulations. Additionally, the blood concentration of UDCA was assayed by high-performance liquid chromatography (HPLC) after derivatization because of the poor UV absorption of UDCA.

MATERIALS AND METHODS

Materials

UDCA was purchased from TianJin-Tai-ping-yang Ltd., purity 99.13%, and phospholipid was purchased from Hua-Qing-Mei-Heng Ltd., and the phosphatidyl content was approximately 82% (wt/wt). UDCA Tablet (included in Chinese pharmacopeia 2005 edition) was purchased from Jing-Wei pharmacy market. The other chemical reagents were of analytical grade or better.

Preparation of UDCA-PLC

The required amounts of UDCA and phospholipids were placed in a 100 mL round-bottom flask. They were dissolved in tetrahydrofuran and agitated at 60°C for 3 h. After tetrahydrofuran was evaporated off under vacuum at 60°C, the dried residues were gathered and placed in desiccators overnight, then crushed in the mortar, and sieved with a 100-μm mesh. The resultant UDCA-PLC was transferred into a glass bottle, flushed with nitrogen, and stored at room temperature. All the above-mentioned steps were performed under aseptic conditions.

Determination of the Content of UDCA in Phospholipid Complex

The content of UDCA in PLC was determined as follows. Approximately, 200 mg of PLC was dissolved in 10 mL of methanol, and a 20-μL aliquot of the resultant solution was injected into a HPLC system. The stationary phase, Kromasil C18 column (250 × 4.6 mm, 5 μm), was kept at 25°C. The mobile phase was acetonitrile: 0.025 mol/L phosphoric acid solution (45:55, v/v, pH = 3.0). The flow rate was 1.0 mL/min. The detector wavelength was 210 nm.

Scanning Electron Microscopy

The surface morphology of PLC powders were viewed and photographed with a Jeol scanning electron microscope (JSM-5310-LV, Jeol, Tokyo, Japan).

Transmission Electron Microscopy

Samples were prepared by dropping distilled water to PLC powders and then were swirled for 3 min. A drop of the resultant PLC dispersions was placed onto a carbon-coated copper grid, leaving a thin liquid film. The films on the grid were negatively stained by immediately adding a drop of 2.5% (wt/wt) sodium phosphotungstate (pH 6.8), removing the excess staining solution with a filter paper, which is followed by air-drying. The stained films were then viewed on a transmission electron microscope (Jeol-200 CX, Jeol, Japan) and photographed.

Particle Size Distribution Analysis

The particle size and size distribution were determined using dispersion in water at 25°C by particle size analysis. The measurements were performed using Sympatec Gmbh Nanophox (0119 P).

Differential Scanning Calorimetry

The samples sealed in the aluminum crimp cell were heated at the speed of 5°C/mL from 0 to 300°C in the atmosphere of nitrogen (Dupont 1090B, Dupont, Wilmington, USA). Peak transition onset temperature was determined by means of an analyzer. The peak transition onset temperatures of phospholipids, UDCA material, the physical mixture of phospholipids, and the UDCA–PLC were compared.

X-Ray Diffractometry

The X-ray diffractogram (D/max-r A, Rigaku Denki, Tokyo, Japan) was scanned with the diffraction angle increasing from 5° to 50°, 2θ angle, with a step angle of 0.04° and a count time of 1 s.

n-Octanol/Water Partition Coefficient (*P*) of UDCA-PLC

P of UDCA determination of UDCA material, PLC, or the physical mixture was carried out by adding excess of UDCA material, PLC, or physical mixture to a series of 10 mL water solutions (pH 1.5, 2.5, 3.5, 4.5, 5.5, 6.5, 7.5) in sealed glass containers at 25°C, respectively. Each experiment was performed in triplicate. All the 63 resultant liquids were agitated for 24 h and centrifuged to remove excessive residues (15 min 1776 g), respectively. To each liquid, 10 mL *n*-octanol was added and agitated for 24 h. Then they were centrifuged at 1776 grpm for 15 min, respectively. The resultant water phase and *n*-octanol phase were separated. The water phase and *n*-octanol phase were filtrated through a 0.45-μm membrane, respectively. The 1 mL filtrate was mixed with 9 mL of methanol, and a 20-μL aliquot of the resulting solution was injected into a HPLC system and detected as the previous description, and the concentrations of UDCA were measured, respectively.

P of UDCA determination of UDCA material, PLC, or physical mixture was calculated as follows:

$$P = \frac{C_o}{C_w}$$

where C_o was the concentration of UDCA material, UDCA-PLC, or the physical mixture in *n*-octanol; C_w was the concentration of UDCA material, UDCA-PLC, or its physical mixture in water.

Pharmacokinetics and Bioavailability Study In Vivo of UDCA-PLC Experiment Design

Male wistar rats weighing 200–220 g were obtained from National Resource Center for Rodent Laboratory Animal. Each rat was housed at ambient temperature 20–25°C and 45–55% relative humidity. Every rat was fed a standard diet and made to fast during 24 h prior to experiment.

The study was of open, single dose, randomized. Two UDCA preparations were compared in the study: a test preparation of UDCA-PLC and a reference preparation of UDCA tablet (UDCA Tablet). The rats were randomly divided into two groups (n = 6/group/time point). A sample equivalent to 20 mg/kg of UDCA of PLC suspended in 2 mL of water was orally administered to one group of rats. The suspensions of UDCA Tablet equivalent to 20 mg/kg of UDCA were orally administered to another group of rats.

Under ether anesthesia, jugular vein blood samples were collected from both groups of rats into centrifuge tubes at predetermined time points (0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 10, 12, 14 h) after oral administration of UDCA preparations at a dose equivalent to 20 mg/kg of UDCA. Blood samples were allowed to clot at room temperature for about 1 h and centrifuged at 8,000 rpm for 10 min, and the serum was separated and kept at –20°C prior to analysis.

Sample Preparation and Solid Phase Extraction

Cholic acid (CA, 500 10^{-4} mM, i.e. 60 nmol of internal standard) in methanol was transferred into a 9 mL tube and evaporated gently (45°C, stream of nitrogen) to dryness. A serum sample (1 mL) containing UDCA was then added and the tube content mixed and diluted with 5 mL of the phosphate buffer (pH 7.5). SPE columns (Supelclean LC-18) were activated on the SPE vacuum manifold with 2 mL of methanol followed by 3 mL of ultrahigh-quality (UHQ) water (flow rate 3 mL/min). The serum spiked with the internal standard and diluted with the phosphate buffer was passed through SPE column with a flow rate of 1 mL/min. After washing the SPE column with 2 mL of UHQ water and 2 mL of 20% methanol (flow rate of 3 mL/min), the captured analytes (UDCA and CA) were eluted from the column with 3 mL of methanol (flow rate 1 mL/min). The methanolic eluate was evaporated (45°C, stream of nitrogen) to dryness.

Derivatization Procedure

2-Bromo-2-acetonaphthone (130 mL of 10^{-3} M) and *N*,*N*-di-isopropylethylamine (260 μL of 10^{-3}, both in acetonitrile solutions) were added into the tube with the dried serum eluate. The mixture was allowed to react at 60°C for 20 min. Following reaction, the remaining acetonitrile was evaporated (45°C, stream of nitrogen) and the dry residue (a mixture of UV-absorbing naphthacyl esters of bile acids, the derivatizing agent, and the amine) was reconstituted in 200 mL of the mobile phase and transferred into a vial containing a glass insert (230 mL volume). One hundred microliters of the sample was then injected into the chromatographic column.

Chromatography

Concentrations of UDCA in the sample were determined using a HPLC apparatus (Agilent, CA, USA) equipped with Kromasil C18 column (250 × 4.6 mm, 5 μm) and C-18 precolumn. The mobile phase was acetonitrile/H_2O (65/35). The flow rate was set at 1.0 mL/min, the column temperature was room temperature, the volume of the injected sample was 20 μL, and the detector wavelength was 245 nm.

Pharmacokinetics and Statistical Analysis

The pharmacokinetic parameters were evaluated. The zero-order moment area under the blood concentration–time curve was calculated by the trapezoidal rule. The pharmacokinetic parameters and AUC_{0-24} were computed by software program 3p87. The differences among pharmacokinetic parameters in test groups were estimated by multiple comparison tests with SPSS software.

RESULTS AND DISCUSSION

Preparation of UDCA-PLC

UDCA-PLC was prepared according to different quantity ratio of phospholipids and drugs, such as 1, 2, 3, and 4. The results showed that when the ratio was more than 3, the appearance of resultant materials appeared viscous and it was not easy that resultant

materials were prepared to other preparations, but when the ratio was lower than 3, the complex ratio of PLC was poor. To get the best quality, at last we prepared UDCA-PLC in terms of the quantity ratio 3. The content of UDCA in the PLC was 24.92% (wt/wt).

Scanning Electron Microscopy

The surface morphology of UDCA-PLC as examined by SEM is shown in Figure 2. PLCs were made up of phospholipids and drugs and appeared as spherical particles. When at × 5,000 magnification, we could conclude that phospholipids did not exit on the appearance of drugs but drugs uniformly dispersed in phospholipids and formed the structure of spherical particles.

Transmission Electron Microscopy

The TEM images of PLCs after slightly shaking in distilled water are shown in Figure 3. After slightly shaking in distilled water, we could see that there were some particles suspended in the water and infusible particles still exited in the solution. For PLCs, the TEM morphology was that of spherical particles like vesicles.

Particle Size Distribution

Figure 4 presents the size distribution of PLCs after slightly shaking in distilled water. All particles were below 1,300 nm, whereas 50% of the particles in PLCs were in the range 206-451 nm and 90% were in the range 206–717 nm.

Differential Scanning Calorimetry

Figure 5 shows the DSC curves of UDCA material, phospholipids, UDCA-PLC, and the physical mixture of UDCA

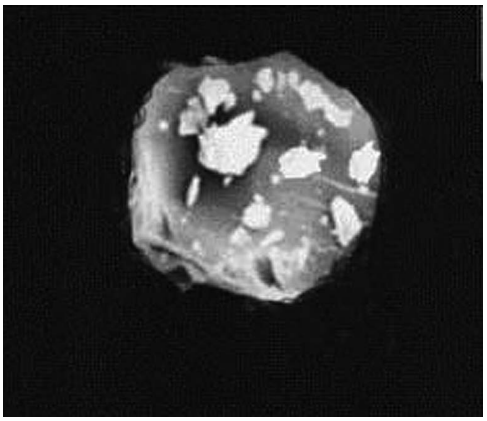

FIGURE 2. Scanning electron micrographs of ursodeoxycholic acid–phospholipid complex (UDCA-PLC) at × 100,000 magnification.

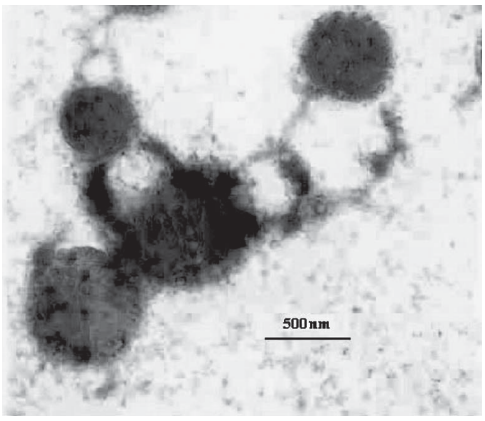

FIGURE 3. Transmission electron micrographs of ursodeoxycholic acid–phospholipid complex (UDCA-PLC) after slightly shaking in distilled water at × 10,000 magnification.

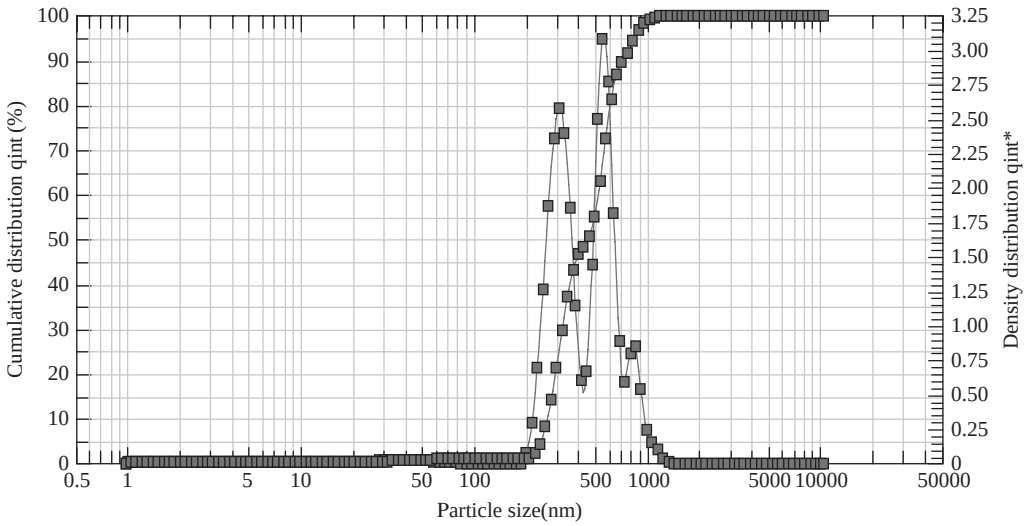

FIGURE 4. Size distribution of ursodeoxycholic acid–phospholipid complex (UDCA-PLC) after slightly shaking in distilled water.

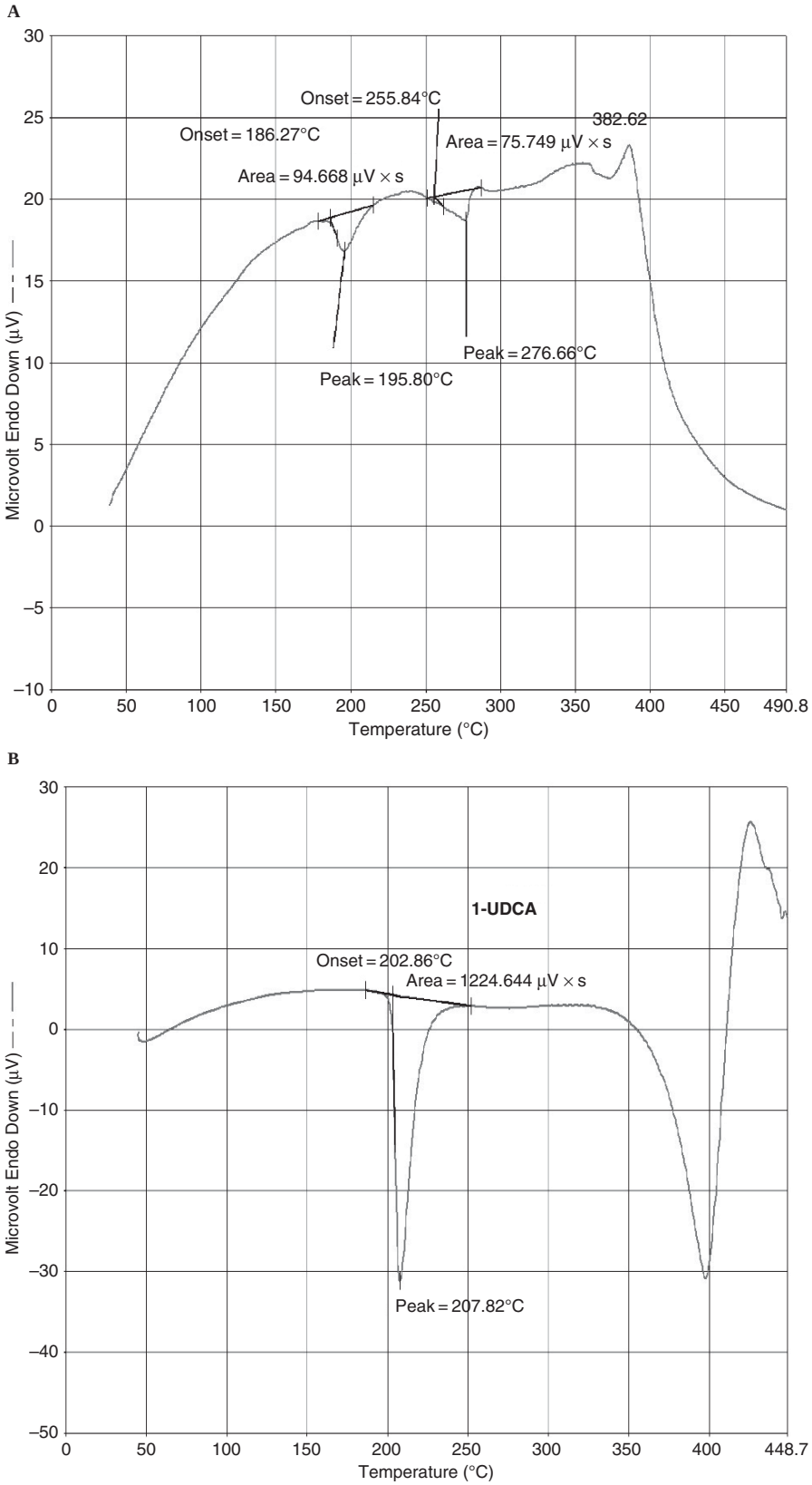

FIGURE 5. Differential scanning calorimetry (DSC) thermograms of phospholipids complex (A), UDCA (B), phospholipids (C), and physical mixture (D).

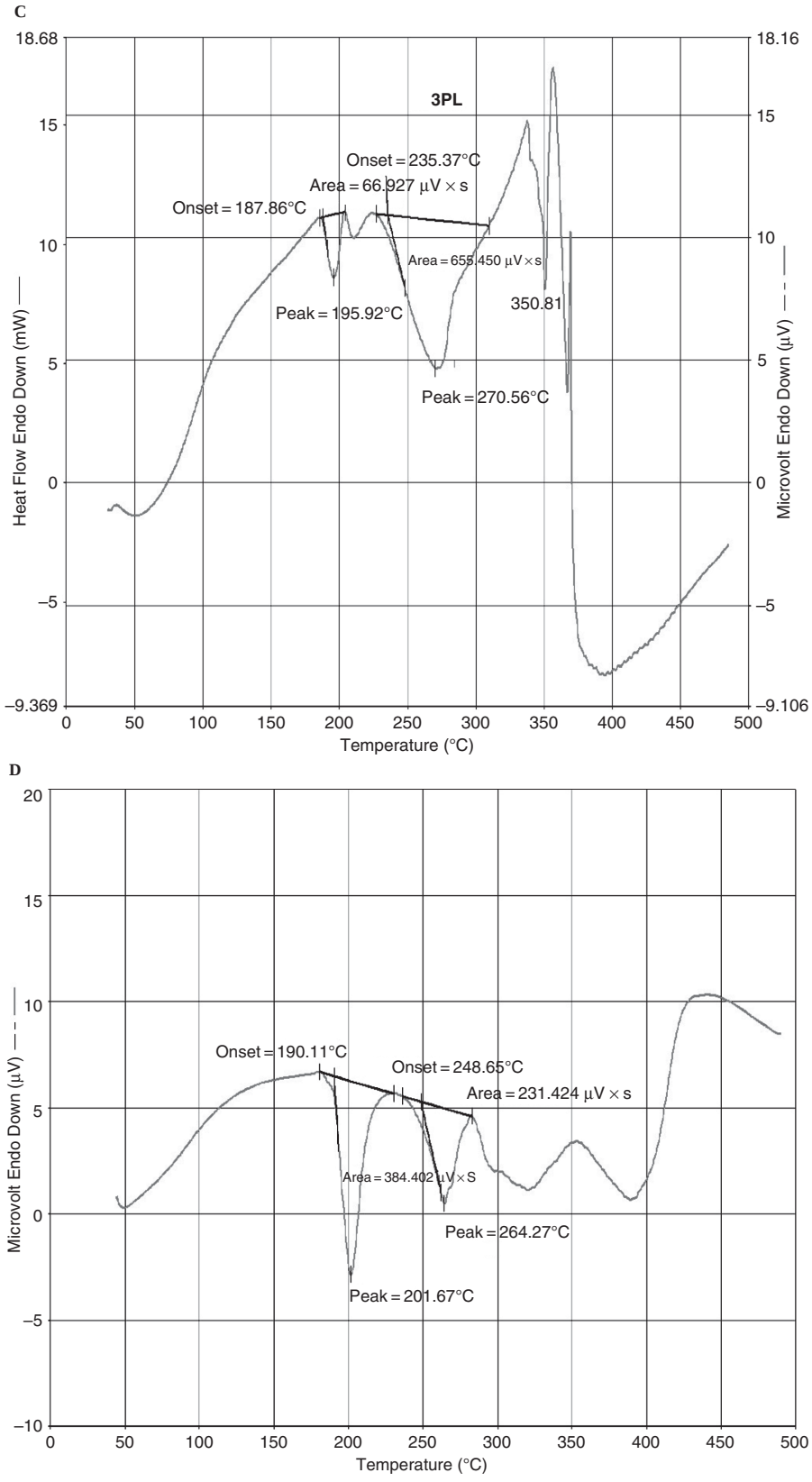

FIGURE 5. *(Continued)*

and phospholipids. DSC of PLCs showed the endothermal peaks of UDCA and phospholipid were disappeared and the phase transition temperature was lower than the phase transition temperature of phospholipids; it was considered that UDCA and phospholipids should have some interaction, such as the combination of hydrogen bonds or van der Waals force (Venema & Weringa, 1988). After the combination of UDCA and the phospholipid molecule polarity parts, the carbon–hydrogen chain in phospholipids could turn freely and enwrap the phospholipids molecule polarity parts, which made the sequence decrease between phospholipids aliphatic hydrocarbon chains, made the second endothermal peak of phospholipids disappear, and depressed the phase transition temperature (Lasonder & Weringa, 1990).

X-Ray Diffractometry

Figure 6 shows the powder X-ray diffraction patterns of UDCA material, phospholipids, their physical mixture, and the complex. The UDCA material powder diffraction pattern shown in Figure 6(C) displayed partial sharp crystalline peaks, which is the characteristic of a molecule with some crystallinity. In contrast, phospholipids shown in Figure 6(B) were amorphous, lacking crystalline peaks. Compared with that of the physical mixture, the crystalline peaks had disappeared in the complex shown in Figure 6(A). This suggested that UDCA in the phospholipids lipid matrix was either molecularly dispersed or amorphous form. However, as seen in Figure 6(D), some crystalline drug signal was still detectable in the physical mixture of UDCA and phospholipids.

n-Octanol/Water Partition Coefficient (P) of UDCA-PLC

Table 1 shows the *n*-octanol/water partition coefficient (*P*) of UCDA, the physical mixture, and UCDA-PLC at different pH values. The data showed that UCDA-PLC markedly increased the solubility of UDCA, and *P* of UCDA-PLC in *n*-octanol and water was slightly increased with increasing of pH, and it was up to about 10 multiples more than that of UCDA material at pH 7.5. These were due to the strong dispersibility or/and amorphous form of the PLCs, and polar group of UDCA was masked by phospholipids. However, *P* of UCDA in the physical mixture was about 1.5 multiples more than that of UCDA material; it was the reason that the phospholipids slightly improved the solubility of UDCA in the physical mixture by means of its solubilization effect.

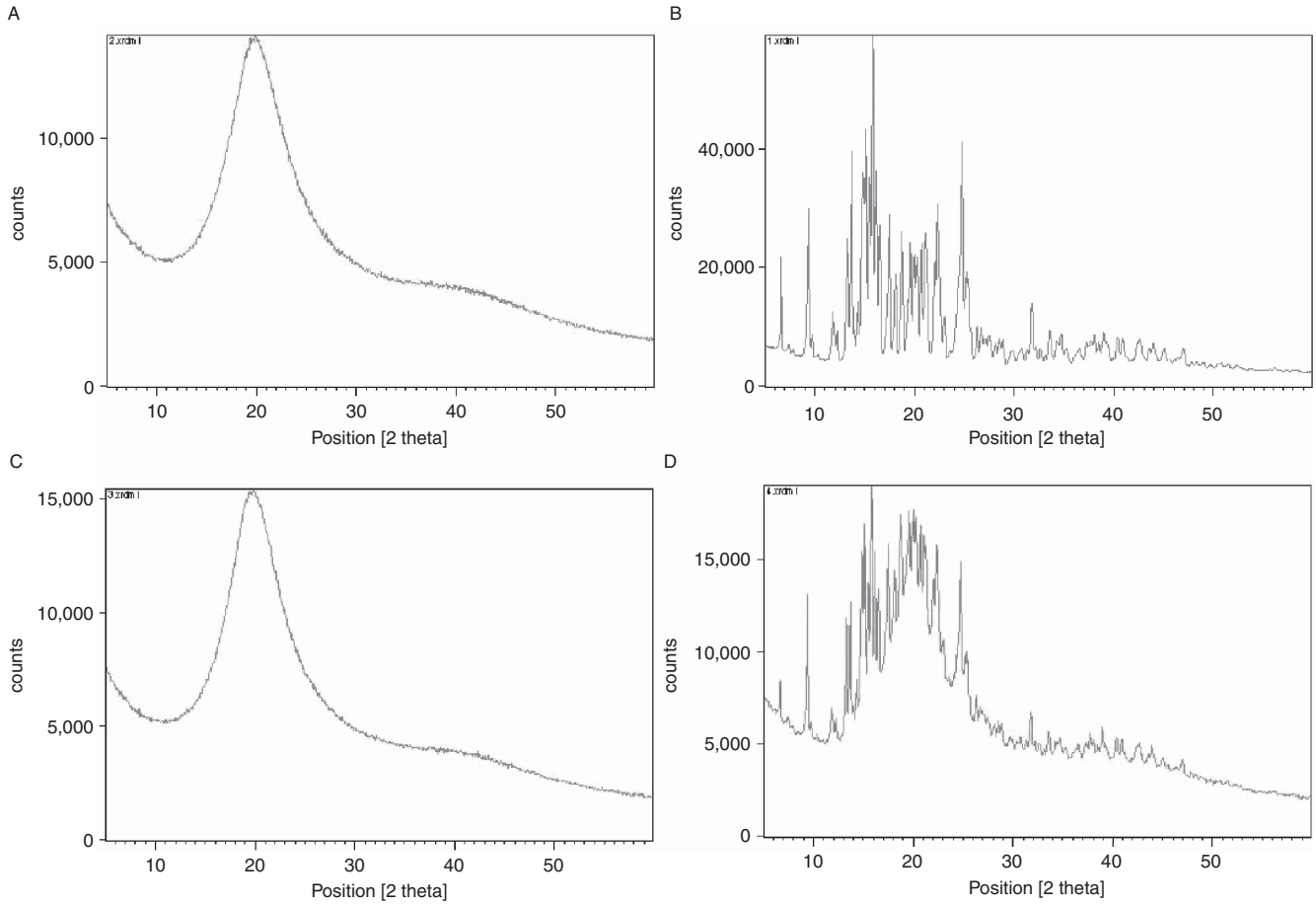

FIGURE 6. X-ray diffractometry spectra of (A) complex, (B) ursodeoxycholic acid (UDCA), (C) phospholipids, and (D) physical mixture.

TABLE 1
n-Octanol/Water Partition Coefficient (*P*) of UCDA, Physical Mixture, and UCDA-PLC at Different pH Values

Samples	pH	C_w (mg/mL)	C_o (mg/mL)	P (C_o/C_w)
	1.5	0.128 ± 0.098	0.227 ± 0.035	1.773
	2.5	0.125 ± 0.086	0.244 ± 0.074	1.952
	3.5	0.143 ± 0.034	0.243 ± 0.057	1.699
UDCA	4.5	0.145 ± 0.057	0.243 ± 0.067	1.676
	5.5	0.148 ± 0.097	0.247 ± 0.038	1.669
	6.5	0.154 ± 0.086	0.211 ± 0.078	1.370
	7.5	0.157 ± 0.067	0.230 ± 0.083	1.465
Physical	1.5	0.487 ± 0.076	1.341 ± 0.075	2.754
mixture	2.5	0.476 ± 0.084	1.409 ± 0.037	2.960
	3.5	0.469 ± 0.039	1.448 ± 0.028	3.087
	4.5	0.473 ± 0.059	1.436 ± 0.063	3.036
	5.5	0.482 ± 0.065	1.437 ± 0.052	2.981
	6.5	0.484 ± 0.098	1.450 ± 0.084	2.996
	7.5	0.476 ± 0.056	1.447 ± 0.057	3.040
UDCA-PLC	1.5	0.394 ± 0.050	4.568 ± 0.063	11.594
	2.5	0.393 ± 0.185	4.593 ± 0.057	11.687
	3.5	0.394 ± 0.044	4.627 ± 0.052	11.744
	4.5	0.376 ± 0.050	4.583 ± 0.057	12.189
	5.5	0.354 ± 0.015	4.591 ± 0.036	12.969
	6.5	0.343 ± 0.089	4.588 ± 0.073	13.376
	7.5	0.367 ± 0.088	4.825 ± 0.067	13.147

Values are mean ± *SD* (n = 3).

Pharmacokinetics and Bioavailability Study of UDCA-PLC In Vivo

Solid phase extraction (SPE) methods have been routinely used for sample preparation from various body fluids containing bile acids (Setchell & Matsui, 1983). Bile acids do not show significant UV absorption. Neither do they have fluorescent or electrochemical properties suitable for their sensitive and selective detection. UV detection at 200 nm is nonspecific because of biological matrix interference. Precolumn labeling of bile acids with either achromophore or fluorophore is usually necessary (Lingemans & Underberg, 1990).

According to the literary sources (Cooper & Anders, 1974; Gatti, Roda, Cerre, Bonazzi, & Cavrini, 1997) and in our experience, the most effective way to remove UDCA from blood samples is SPE of a diluted serum. For the enhancing of the detector response and the specificity of the UDCA determination, a precolumn derivatization procedure based on the esterification of the carboxylic group with 2-bromo-2%-acetonaphthone (UV-absorbing chromophore) was developed. The derivatization procedure was performed in acetonitrile in the presence of di-isopropylethylamine (a scavenger of protons).

Under these conditions, the typical HPLC analyses are shown in Figure 7. The analytical procedure involving the SPE, derivatization, and HPLC analysis of the derivatized UDCA and CA (internal standard) is accurate and precise. The calibration curve was found to be linear $y = 0.9444x + 0.1495$ (r = .9992, where x is the concentration ratio of UDCA to CA and y is the corresponding peak–area ratio UDCA/CA) in the 0.0086–0.146 μg/mL range. The accuracy and precision of UDCA determination (using CA as the internal standard) in spiked serum samples were found to be 102.96 and 7.6%, respectively. The mean UDCA plasma recovery was 93.6 ± 1.24%. The relative *SD* (*RSD*) in days were 3.18, 3.52, and 3.29%, respectively, the *RSD* intra-days were 3.41, 3.74, and 3.45%, respectively, which showed the recoveries and *RSD* in days or intra-days were satisfying, and the lowest detection limit was 8 ng/mL.

The mean serum concentrations of UDCA-PLC and UDCA Tablet are shown in Figure 8, after oral administration, respectively. The pharmacokinetic parameters such as C_{max}, T_{max}, AUC_{0-24}, and $AUC_{0-\infty}$ are given in Table 2. The results suggested that the main pharmacokinetic parameters of UDCA-PLC were significantly different from those of UDCA Tablet. The C_{max} was 0.1346 μg/mL after oral administration of UDCA-PLC with a T_{max} of about 1.5610 h. However, the average value of C_{max} was 0.0576 μg/mL after oral administration of UDCA Tablet with a T_{max} of about 1.9144 h. The bioavailability ($AUC_{0-\infty}$, 11.437 ± 0.464 μg h/mL) of UDCA-PLC in vivo was significantly increased compared to that ($AUC_{0-\infty}$, 4.736 ± 0.417 μg h/mL) of UDCA Tablet. All formulations of UDCA-PLC showed higher C_{max} and higher $AUC_{0-\infty}$ values than those of reference. The relative bioavailability of UDCA-PLC ($AUC_{0-\infty}$) compared with that of UDCA t ($AUC_{0-\infty}$) was 241%. The increase of the bioavailability of UDCA-PLC after oral administration might be due to the following reasons: Phospholipid is an important component of cell membrane, having the actions of keeping cell membrane fluidity. Compared with that of UDCA, the P of UCDA-PLC in *n*-octanol–water was significantly increased. The lipophilicity of UCDA-PLC was effectively increased. In this case, improved bioavailability can be achieved by the use of delivery systems, which can enhance the rate and/or the extent of drug solubilizing into aqueous intestinal fluids. Phospholipids play a major role in drug delivery technology (Yanyu et al., 2006).

In addition, it is shown in Figure 8 that the presence of secondary peaks in the serum concentration–time profile was typical for drugs with enterohepatic recirculation and endogenous UDCA levels made calculating parameters of $t_{1/2}$ and *ke* impossible. These are consistent with those from previous study (Bachrach & Hofmann, 1982b).

CONCLUSION

In this study, UCDA-PLC was successfully prepared by a simple method. DSC and X-ray diffractometry curves of PLCs showed that UDCA and phospholipids might combine and

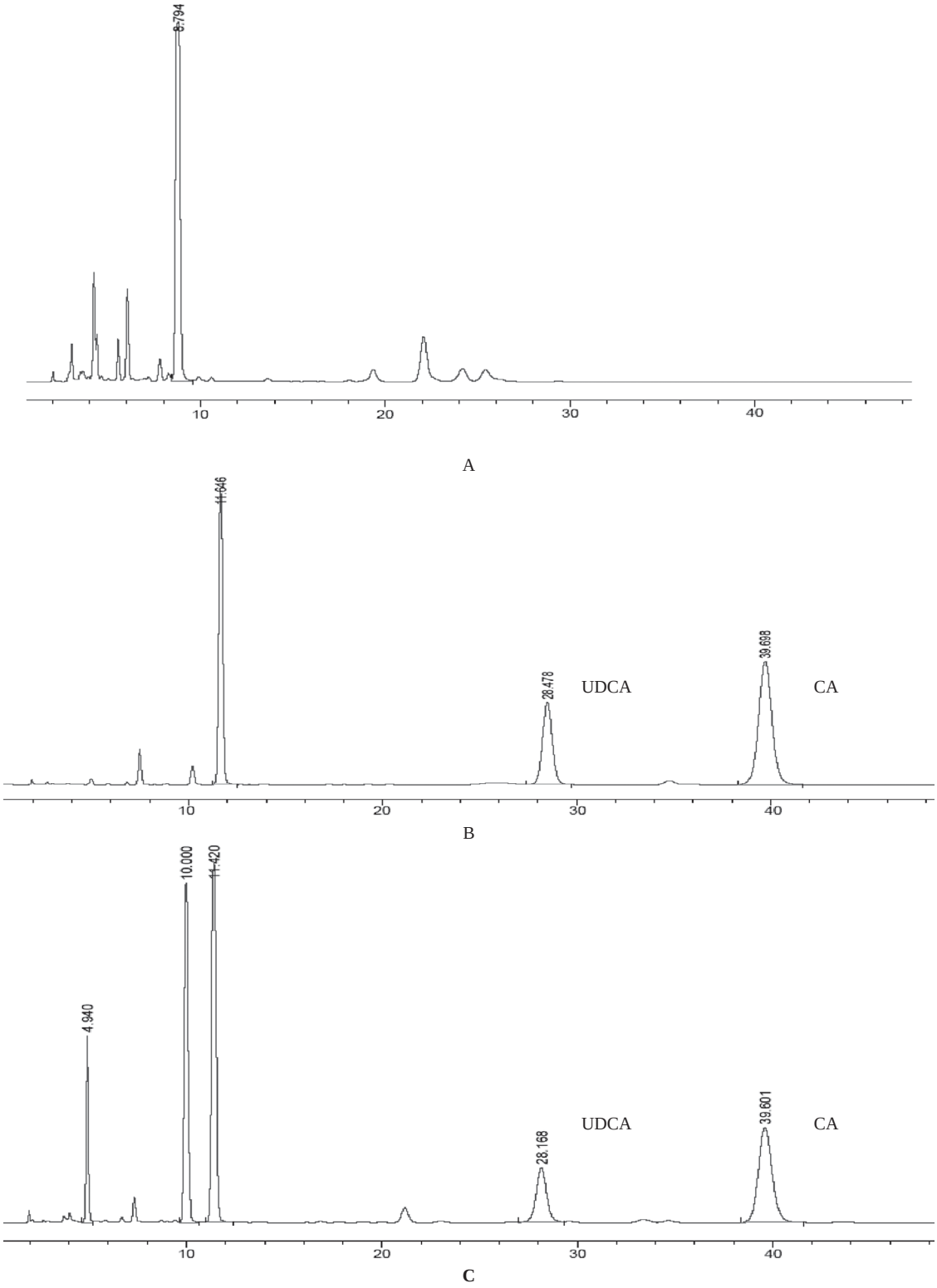

FIGURE 7. Typical high-performance liquid chromatography (HPLC) of blank rat serum (A); blank rat serum spiked with ursodeoxycholic acid (UDCA) and internal standard (B); a sample after oral administration of UDCA–phospholipid complex (UDCA-PLC) (C).

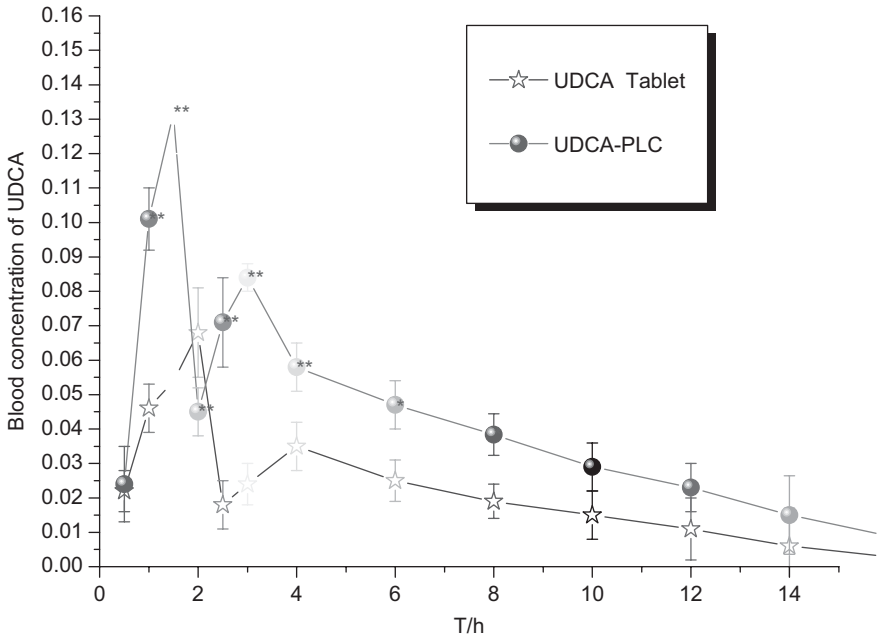

FIGURE 8. Mean serum concentration–time curve (μg/mL) of ursodeoxycholic acid (UDCA) in rats after oral administration of UDCA–phospholipid complex (UDCA-PLC) (●) and UDCA Tablet (☆) equivalent to 20 mg/kg of UDCA, respectively. Values are mean ± *SD* (*n* = 6/group/time point). $^{**}p < .01$, $^{*}p < .05$.

TABLE 2
The Main Pharmacokinetic Parameters of UDCA-PLC and UDCA Tablet in Rats (*n* = 6/Group/Time Point), Respectively

Parameters	UDCA Tablet	UDCA-PLC
AUC_{0-24} (μg h/mL)	3.308 ± 0.859	7.846 ± 0.518**
$AUC_{0-\infty}$ (μg h/mL)	4.736 ± 0.417	11.437 ± 0.464**
V (c) (mL/kg)	70.197 ± 2.218	79.326 ± 3.183
C_{max} (μg/mL)	0.0576 ± 0.0137	0.1346 ± 0.0313*
CL (mL/h)	38.63 ± 4.762	71.82 ± 3.321

Values are mean ± *SD*. $^{**}p < .01$ and $^{*}p < .05$ are statistical significances with the UDCA phospholipid complex versus UDCA Tablet.

form some kind of bond, such as hydrogen bonds or van der Waals force. The *n*-octanol/water partition coefficient (*P*) of UDCA-PLC studies showed UCDA-PLC significantly increased the hydrophilicity and lipophilicity of UDCA, and *P* of UCDA-PLC in *n*-octanol and water was about 10 multiples more than that of UCDA material. The blood concentration of UCDA was precisely assayed by HPLC after precolumn derivatization. Compared with UDwt, the PLC can markedly improve the bioavailability of UDCA in vivo of rats. Further studies about the absorbed mechanism of UDCA-PLC would be performed through small intestine and therapeutic evaluation in vivo. The UDCA-PLC would be more prospective preparation in future.

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