Absorption, Biliary Excretion, and Metabolism of a New Cholelitholytic Agent, Ursodeoxycholy *N*-Carboxymethylglycine and Its Esters in Rats

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Intestinal absorption, biliary excretion and metabolism of a calcium gallstone dissolving agent, [11,12-³H]ursodeoxycholy *N*-carboxymethylglycine (UDC-CMG) and its monoethyl, diethyl and dipivaloyloxyethyl esters (UDC-CMG-Et, UDC-CMG-Et₂ and UDC-CMG-PV₂) were studied in bile duct cannulated rats.

Biliary recovery of [³H]-labeled UDC-CMG, UDC-CMG-Et and UDC-CMG-Et₂ after intraduodenal administration were 65%, 80%, 98%, respectively. Radio-thin layer chromatography analysis of the bile revealed that UDC-CMG didn't undergo any biotransformation during administration and excretion. About 80% and 20% of radioactivity recovered in the bile was identified as UDC-CMG-Et and UDC-CMG, respectively, after intraduodenal administrations of [³H]UDC-CMG-Et₂ and [³H]UDC-CMG-Et. The administered intact UDC-CMG-Et₂ was not found in the bile.

Intraduodenally administered [³H]UDC-CMG-PV₂ was rapidly recovered in the bile. The total recovery rate was 78% within a 24 h period. More than 80% of the radioactivity recovered in the bile was found as UDC-CMG. Lesser amounts of the monopivaloyloxyethyl ester of UDC-CMG were also found, but intact UDC-CMG-PV₂ was not detected in the bile as in the case of UDC-CMG-Et₂.

Among the esters of UDC-CMG investigated in the present studies, only UDC-CMG-PV₂ was excreted in the bile mainly as the perhydrolyzed form, UDC-CMG. These results suggest the usefulness of UDC-CMG-PV₂ as the pro-drug in calcium gallstone dissolution therapy.

Keywords — bile acid derivative; ursodeoxycholy *N*-carboxymethylglycine; calcium gallstone; cholelitholytic agent; intestinal absorption; biliary excretion; prodrug; bile duct cannulated rat

Introduction

Gallstone is known to be classified in two groups. One is cholesterol stone which consists mainly of cholesterol and the other is pigment stone which consists mainly of calcium bilirubinate. Recently, nonsurgical approaches, such as stone dissolution by oral administration of bile acids,^{1,2)} instillation of solvents into the biliary system,^{3,4)} or exocorporeally induced shock waves to disintegrate gallstones,⁵⁾ have been pursued with great enthusiasm.

These treatments, however, are effective only if stones are composed mainly of cholesterol without significant admixtures of calcium salts or pigments.⁶⁾ Furthermore, it is pointed out

that during the bile acid administration therapy, calcification of outer shells of gallstone occurs in high incidence. The calcification seems to be an important cause of resistance to bile acid therapy.⁷⁾ Usually calcium in gallstones is present as calcium carbonate, calcium phosphate, calcium bilirubinate or calcium salt of fatty acids. Solubilization of these insoluble calcium salts seems to be very important in gallstone dissolution therapy.

Ethylenediaminetetraacetic acid (EDTA) and citrate are known as calcium chelators, and instillation of these compounds have been shown to solubilize bile duct, pancreatic, and renal stones.⁸⁻¹⁰⁾ Cholic acid and ursodeoxycholic acid in combination with EDTA have been used

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for dissolution and disintegration of calcium bilirubinate stones in bile duct.¹¹⁻¹³ These agents, however, can be used only in instillation therapy but not in oral administration therapy. Delivering chelating agents into the bile after oral administration seemed to be useful in dissolution of gallstones which contain insoluble calcium salts. Recently, iminodiacetate and aspartate of chenodeoxycholic acid (CDCA) or ursodeoxycholic acid (UDCA), which have two carboxyl groups at the end of the side chain, were synthesized.^{14,15} These dibasic bile acid derivatives have calcium chelating activity and could be expected as therapeutic agents for dissolution of calcium slat stones. Lack *et al.*, however, have found that the introduction of an additional negative charge on the side chain of naturally occurring monobasic bile salts caused a marked reduction in ileal bile salt transport.¹⁶⁻¹⁸ To improve intestinal absorption of the dibasic bile acid derivatives, we synthesized the monoethyl ester (UDC-CMG-Et), diethyl ester (UDC-CMG-Et₂), dipivaloyloxyethyl ester (UDC-CMG-PV₂) of ursodeoxycholyl *N*-carboxymethylglycine (UDC-CMG) (Fig. 1). To exert chelating activity, these esters should be hydrolyzed to return the dibasic bile acid, UDC-CMG, after intestinal absorption. Therefore, we investigated the intestinal absorption, biliary excretion, and metabolism of these esters in bile duct cannulated rats.

UDC-CMG: $R_1 = R_2 = H$;
 UDC-CMG-Et: $R_1 = C_2H_5$, $R_2 = H$;
 UDC-CMG-Et₂: $R_1 = R_2 = C_2H_5$;
 UDC-CMG-PV₂: $R_1 = R_2 = CH(CH_3)COOC(CH_3)_3$

Fig. 1. Chemical Structure of UDC-CMG and Its Esters

Materials and Methods

1) General — Thin-layer chromatography (TLC), radio-TLC analysis and determination of radioactivity were carried out as described previously.¹⁹⁾

Nuclear magnetic resonance (NMR) spectra were recorded with a JEOL JMN-FX-400 spectrometer in pyridine-*d*₅ with tetramethylsilane as an internal standard.

“The usual work-up” refers to dilution with water, acidification with 1N HCl, extraction with ethyl acetate, washing to neutrality, drying over anhydrous Na₂SO₄, filtration, and evaporation of the organic solvent under reduced pressure to dryness.

2) Ursodeoxycholyl *N*-Carboxymethyl Diethyl Ester (UDC-CMG-Et₂) — To a solution of carboxymethylglycine diethyl ester (4.3 g) and triethylamine (5.0 ml) dissolved in 140 ml of ethyl acetate 2-ethoxy-1-ethoxycarbonyl-2-dihydroquinoline (4.1 g) and UDCA (5 g) were added with stirring at room temperature. Then the reaction mixture was refluxed overnight. The usual work-up gave an amorphous powder (6.9 g, yield 96%) of UDC-CMG-Et₂.

NMR: 0.71 (3H, s, 18-CH₃), 0.96 (3H, s, 19-CH₃), 0.96 (3H, d, $J = 6.6$ Hz, 21-CH₃), 1.26 and 1.29 (each 3H, t, $J = 7.1$ Hz, -CH₂-CH₃), 3.48 (2H, m, 3β- and 7α-H), 4.17 and 4.23 (each 2H, q, $J = 7.1$ Hz, -CH₂-CH₃), 4.12 (2H, m, N-CH₂-C=O), 4.29 (2H, s, N-CH₂-C=O).

3) Ursodeoxycholyl *N*-Carboxymethylglycine Monoethyl Ester (UDC-CMG-Et) — UDC-CMG-Et₂ (1 g) was partially hydrolyzed with 5% K₂CO₃ with stirring overnight at room temperature. The usual work-up gave a residue which was purified by a reversed phase column chromatography (LiChroprep Si 60, 40–60 μm; Merck). Evaporation of the solvent of the appropriate fractions gave an amorphous powder (360 mg, yield 38%) of UDC-CMG-Et.

NMR: 0.71 (3H, s, 18-CH₃), 0.96 (3H, s, 19-CH₃), 0.96 (3H, d, $J = 6.4$ Hz, 21-CH₃), 1.26 and 1.29 (each 3H × 1/2, t, $J = 7.1$ Hz, -CH₂-CH₃)*, 3.48 (2H, m, 3β- and 7α-H), 4.17

* The signals were observed at two positions by nonequivalency of the protons.

and 4.23 (each 2 H $\times$ 1/2, q, J = 7.1 Hz, $\text{CH}_2\text{-CH}_3$)*, 4.12 (2H, m, N- $\text{CH}_2\text{-C=O}$), 4.26 (2H, s, N- $\text{CH}_2\text{-C=O}$).

4) Ursodeoxycholyl *N*-Carboxymethylglycine (UDC-CMG) — UDC-CMG-Et₂ (2 g) was hydrolyzed with 5% methanolic KOH with stirring at room temperature for 2 h. The usual work-up resulted in a residue which was purified by reversed phase chromatography to give an amorphous powder (1.2 g, yield 64%) of UDC-CMG.

NMR: 0.71 (3H, s, 18- CH_3), 0.96 (3H, s, 19- CH_3), 0.96 (3H, d, J = 7.4 Hz, 21- CH_3), 3.48 (2H, m, 3 β - and 7 α -H), 4.09 and 4.14 (each 1H, d, J = 17.4 Hz, N- $\text{CH}_2\text{-C=O}$), 4.24 (2H, s, N- $\text{CH}_2\text{-C=O}$).

5) Ursodeoxycholyl *N*-Carboxymethylglycine Dipivaloyloxyethyl Ester (UDC-CMG-PV₂)

— Diisopropylethylamine (70 μ l) and UDC-CMG (100 mg) were dissolved in 20 ml of CHCl_3 and pivaloyloxyethyl chloride (120 mg) was added to the solution. After refluxing for 7 d, the usual work-up resulted in a residue which was purified by silica gel (10 g) chromatography to give an amorphous powder (62.4 mg, yield 42%) of UDC-CMG-PV₂.

NMR: 0.71 (3H, s, 18- CH_3), 0.96 (3H, s, 19- CH_3), 0.96 (3H, d, J = 6.6 Hz, 21- CH_3), 1.18, 1.19, and 1.20 (3H $\times$ 3, 3H $\times$ 2, 3H, respectively, s, -C(CH_3)₃ $\times$ 2), 1.46 and 1.47

(each 3H, d, J = 5.3 Hz, -O-CH- CH_3), 3.48 (2H, m, 3 β and 7 α -H), 4.01—4.37 (4H, m, N- $\text{CH}_2\text{-C=O}$), 6.79—6.85 (2H, m, -O-CH-O).

6) Preparation of Labeled Compounds — [11,12-³H]Labeled UDC-CMG, UDC-CMG-Et, UDC-CMG-Et₂ and UDC-CMG-PV₂ were prepared from [11,12-³H]UDC (New England Nuclear Corp. 0.083 MBq/mg), in the same manner as described above for the unlabeled UDC-CMG, UDC-CMG-Et, UDC-CMG-Et₂ and UDC-CMG-PV₂, respectively. Each synthetic labeled compound was purified by reversed phase chromatography. The radioactive purity of each substance was checked by radio-TLC and shown to be greater than 98%.

To a solution of each labeled compound dissolved in EtOH was added a drop of Tween 80 (Kanto Chemical Co., Inc., Tokyo, Japan) and the solvent was removed under N₂ stream. Then the residue was dispersed in distilled water just before administration.

7) Experiments with Bile Duct Cannulated Rats — Wistar strain male rats (Hiroshima Experimental Animal Center, Hiroshima, Japan), weighing 180—220 g, were fasted for a whole night prior to experiments but water was allowed freely. Animals were anesthetized with pentobarbital (30 mg/kg), and their bile ducts were cannulated with polyethylene tubing (0.28 mm i.d.).

A single dose of each [³H]labeled bile acid

Fig. 2. Biliary Recovery of Radioactivity in the Bile Duct Cannulated Rats after Intraduodenal Administration of Labeled UDC-CMG and Its Esters

Each point represents the mean of three experiments.

○, UDC-CMG; ●, UDC-CMG-Et; □, UDC-CMG-Et₂; ■, UDC-CMG-PV₂; △, CDCA.

Fig. 3. Biliary Recovery of Radioactivity in the Bile Duct Cannulated Rats after Intravenous Administration of Labeled UDC-CMG and Its Esters

Each point represents the mean of three experiments.

○, UDC-CMG; ●, UDC-CMG-Et; ■, UDC-CMG-PV₂; △, CDCA.

derivative (0.17 MBq/2mg/rat) and [24- ^{14}C]-CDCA (0.019 MBq/55 μg /rat, New England Nuclear Corp.), which was added as an internal standard, were injected simultaneously into the duodenum of bile duct cannulated rat. The animals were placed in restraining cages, and free access to water. Bile samples were collected for 24 h.

A single dose of [^3H]labeled UDC-CMG, UDC-CMG-Et and UDC-CMG-PV₂ was infused into the femoral vein of the bile duct cannulated rats for 20 min and bile samples were collected for 120 min. [^{14}C]CDCA was also infused simultaneously as an internal standard.

Results and Discussion

Tritium labeled UDC-CMG or its ester was administered into bile duct cannulated rats. Carbon-14 labeled CDCA was given simultaneously as an internal standard in all experiments. Recovery of radioactivity in the bile is shown in Fig. 2 and 3. After intraduodenal administration of CDC-CMG and its esters, biliary recoveries of radioactivity varied from one another, whereas these compounds administered intravenously were recovered rapidly and quantitatively in the bile as in the case of CDCA administration. These results indicate that the structural difference of the bile acid derivatives affects on rate of their intestinal absorption but not on rate of the hepatic and biliary transports.

After intraduodenal administration of [^3H]CDC-CMG, a remarkable biliary excretion of radioactivity was not observed in the first 4 h period but 58% of the administered radioactivity was recovered in the bile following 20 h period. The maximum excretion was observed between 4 and 7 h period. The result indicates that the absorption of CDC-CMG containing two negative charges on its side chain from the proximal intestinal tract was almost negligible and occurred after UDC-CMG reached to the distal intestinal tract, in which only the singly charged species of the dibasic bile acid derivative could be absorbed by a process of active transport. The insufficient absorption of UDC-CMG is in good agreement with those observed in the case of other dibasic bile acids.^{16,17)}

When [11,12- ^3H]UDC-CMG-Et, like the naturally occurring bile acids, possessing a negative charge on the side chain, was administered intraduodenally into bile fistula rats, biliary recovery of radioactivity was faster than that of UDC-CMG but was much slower than that of CDCA. The maximum biliary excretion of the radioactivity was observed between 1 and 4 h periods and the cumulative recovery rate was 80% within the 24 h period. The most likely explanation for the impaired recovery of the monobasic bile acid derivative is that before the intestinal absorption, a part of UDC-CMG-Et undergoes hydrolysis to form UDC-CMG which is not absorbed efficiently from the intestinal tract.

When UDC-CMG-Et₂ was administered intraduodenally, radioactivity was recovered rapidly and almost quantitatively in the bile as in the case of CDCA administration. Cumulative recovery rate was more than 97% within a 24 h period. The result suggests that the neutral derivative itself is absorbed efficiently from the proximal intestinal tract by a simple passive diffusion mechanism.

After intraduodenal administration of [^3H]CDC-CMG-PV₂ to bile fistula rats, radioactivity was rapidly recovered in the first 3 h bile sample, but recovery rate of radioactivity was decreased during the following hours. Thus,

Fig. 4. Typical Radiochromatograms of Biliary Metabolites after Intraduodenal and Intravenous Administration of [11,12- ^3H]UDC-CMG-Et₂ and [11,12- ^3H]UDC-CMG-PV₂ in Rats

1, UDC-CMG; 2, monoesters of UDC-CMG; 3, diesters of UDC-CMG. Solvent system: ethyl acetate-acetic acid-water (80:15:10) (v/v/v).

TABLE I. Distribution of Radioactivity in the Biliary Bile Acids Following i.d. and i.v. Administration of Labeled UDC-CMG and Its Esters to Bile Duct Cannulated Rats

Compound administered	Route	Percentage of radioactivity in the bile		
		UDC-CMG-Et ₂	UDC-CMG-Et	UDC-CMG
UDC-CMG	i.d.	N.D.	N.D.	100.0
	i.v.	N.D.	N.D.	100.0
UDC-CMG-Et	i.d.	N.D.	82.4 ± 6.7	17.6 ± 7.6
	i.v.	N.D.	90.9 ± 2.2	9.1 ± 2.2
UDC-CMG-Et ₂	i.d.	N.D.	81.3 ± 4.3	18.7 ± 5.0

Each value means the average of two or three experiments. i.d., intraduodenally; i.v., intravenously. N.D., not detected.

TABLE II. Distribution of Radioactivity in the Biliary Bile Acids Following i.d. and i.v. Administration of Labeled UDC-CMG-PV₂ to Bile Duct Cannulated Rats

Route	Percentage of radioactivity in the bile		
	UDC-CMG-PV ₂	UDC-CMG-PV	UDC-CMG
i.d.	N.D.	16.6 ± 2.3	83.4 ± 2.3
i.v.	N.D.	38.8 ± 4.9	61.2 ± 4.9

Each value means the average of three experiments. i.d., intraduodenally; i.v., intravenously. N.D., not detected.

cumulative recovery rate was 80% within a 24 h period. Compared to the diethyl ester, UDC-CMG-Et₂, which is absorbed almost quantitatively from the intestine, the decrease in the intestinal absorption of the dipivaloyloxyethyl ester, UDC-CMG-PV₂, may result from a part of UDC-CMG-PV₂ undergoing hydrolysis to form the less absorbable dibasic acid, UDC-CMG.

The labeled compounds excreted in the bile were analyzed by means of radio-TLC. Figure 4 shows typical chromatograms of the labeled compounds recovered from the bile of rats after intraduodenal administration of [11,12-³H]-UDC-CMG-Et₂ and [11,12-³H]-UDC-CMG-PV₂, respectively. The distribution of radioactivity in the biliary labeled compounds after intraduodenal and intravenous administration is shown in Table I and II. When UDC-CMG was given into bile duct cannulated rats, the administered bile acid was the only radioactive compound recovered in the bile.

When [³H]UDC-CMG-Et was administered intraduodenally, more than 80% of the radioactivity recovered in the bile was found as the administered compound, UDC-CMG-Et. The

remainder (17%) of the biliary radioactivity was identified as UDC-CMG. When [11,12-³H]-UDC-CMG-Et was given intravenously to bile duct cannulated rats, a part (9%) of the radioactivity recovered in the bile was identified as UDC-CMG. It seems, therefore, likely that hydrolysis of the monoethyl ester, UDC-CMG-Et to the dibasic acid, UDC-CMG occur during both the intestinal and hepatic passages.

After intraduodenal administration of [11,12-³H]UDC-CMG-Et₂ to bile duct cannulated rats, no radioactive UDC-CMG-Et₂ was recovered in the bile. Radioactive compounds excreted in the bile were identified as UDC-CMG-Et and UDC-CMG. The results suggest that during absorption and excretion UDC-CMG-Et₂ is hydrolyzed quickly and efficiently to form UDC-CMG-Et, a part of which is further hydrolyzed to form UDC-CMG.

After intraduodenal and intravenous administration of UDC-CMG-PV₂ to bile duct cannulated rats, the major metabolite recovered in the bile was identified as UDC-CMG. A lesser amount of the monopivaloyloxyethyl ester of UDC-CMG (UDC-CMG-PV) was also present, but the administered compound, UDC-CMG-

PV₂ was not recovered in the bile. The result indicates that UDC-CMG-PV₂ was hydrolyzed to form the perhydrolyzed form, UDC-CMG rather than the partially hydrolyzed form, UDC-CMG-PV during the passage throughout the liver.

The present results confirm previous studies which showed that bile acid derivatives possessing two negative charges on its side chain were poorly absorbed from the intestine.^{17,18)} The present results also indicate that intestinal absorption of UDC-CMG is enhanced by the conversion of the dibasic bile acid into the monoethyl and diethyl esters. However, these ethyl esters, UDC-CMG-Et and UDC-CMG-Et₂ were not hydrolyzed completely to form UDC-CMG during absorption and excretion.

Needless to say, the agent having calcium chelating activity is UDC-CMG but not its monoethyl and diethyl esters, hence high biliary concentration of UDC-CMG is required for the dissolution of calcium gallstones. Thus, we synthesized the dipivaloyloxyethyl ester of UDC-CMG, UDC-CMG-PV₂ which was absorbed more efficiently than UDC-CMG intact and hydrolyzed easily to give a sufficient biliary concentration of UDC-CMG. These data indicate that UDC-CMG-PV₂ could be used as a pro-drug in calcium gallstone dissolution therapy.

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