Metabolism of sulfonate analogs of ursodeoxycholic acid and their effects on biliary bile acid composition in hamsters

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Abstract The metabolism of sodium 3α,7β-dihydroxy-5β-cholane-24-sulfonate and sodium 3α,7β-dihydroxy-24-nor-5β-cholane-23-sulfonate was studied in hamsters. In bile fistula animals these sulfonate analogs of ursodeoxycholic acid were absorbed mainly from the terminal ileum and secreted rapidly into the bile without biotransformation or conjugation. After oral administration, the sulfonate analogs were excreted in the feces at the same rate as chenodeoxycholic acid and its metabolic products. The intestinal microorganisms transformed chenodeoxycholic acid largely into lithocholic acid; the sulfonate analogs were completely resistant to biotransformation. After a 2-week feeding period, the sulfonate analogs of ursodeoxycholic acid accounted for 24.0% and 16.9% of total biliary bile acids. These sulfonates did not affect the proportions of the natural bile acids in the bile, and the ratio of glycine-conjugated bile acids to taurine-conjugated bile acids was not altered by feeding the sulfonates. In contrast, when ursodeoxycholic acid was fed, the proportions of the natural bile acids and the glycine/taurine ratio were changed. These results suggest that the sulfonate analogs had no profound effect on endogenous bile acid metabolism and did not cause a depletion of the hepatic taurine pool during enterohepatic circulation. The sulfonates had no effect on intestinal cholesterol absorption and serum cholesterol levels. **—Mikami, T., K. Kihira, S. Ikawa, M. Yoshii, S. Miki, E. H. Mosbach, and T. Hoshita.** Metabolism of sulfonate analogs of ursodeoxycholic acid and their effects on biliary bile acid composition in hamsters. *J. Lipid Res.* 1993. **34:** 429–435.

Supplementary key words taurine-conjugated bile acids • glycine-conjugated bile acids • bile fistula

Chenodeoxycholic acid (CDCA) and ursodeoxycholic acid (UDCA) are currently used as therapeutic agents for the dissolution of cholesterol gallstones (1, 2). These compounds are absorbed from the small intestine, conjugated with glycine or taurine by the liver, and undergo enterohepatic circulation. However, during enterohepatic circulation the conjugates are hydrolyzed and 7-dehydroxylated to lithocholic acid (LCA) by the action of anaerobic microorganisms. LCA exhibits hepatotoxicity (3, 4) and may act as a promoter of colon cancer (5). It is known that bacterial 7-dehydroxylation takes place mainly with unconjugated bile acids (6) and the reaction requires the presence of a free carboxyl group in the side chain (7). There have been reports that examined the metabolism of sarcosine (N-methylglycine) conjugated bile acids resistant to deconjugation–dehydroxylation (8–10). We hypothesized that bile acid analogs possessing a sulfonic acid group at the terminus of the bile acid side chain instead of a carboxylic acid group would resist bacterial dehydroxylation. To test this hypothesis we synthesized the sulfonate analogs of certain bile acids (11, 12). In the hamster the sulfonate analog of CDCA, sodium 3α,7α-dihydroxy-5β-cholane-24-sulfonate (CDC-SO_3) showed complete resistance to bacterial 7-dehydroxylation (13). The toxicity of UDCA is lower than that of CDCA (14), and recent studies have shown a protective effect of UDCA on the hepatocyte (15). This prompted us to investigate the metabolism of the sulfonate analogs of UDCA, sodium 3α,7β-dihydroxy-5β-cholane-24-sulfonate (UDC-SO_3) and sodium 3α,7β-dihydroxy-24-nor-5β-cholane-23-sulfonate (NUDC-SO_3) (**Fig. 1**), and study their effects on biliary bile acids and cholesterol metabolism.

MATERIALS AND METHODS

Bile acids

UDCA and hyodeoxycholic acid were commercial products. Ursodeoxycholyltaurine (UDC-tau), hyodeoxy-

Abbreviations: CDCA, chenodeoxycholic acid; UDCA, ursodeoxycholic acid; LCA, lithocholic acid; CDC-SO_3, sodium 3α,7α-dihydroxy-5β-cholane-24-sulfonate; UDC-SO_3, sodium 3α,7β-dihydroxy-5β-cholane-24-sulfonate; NUDC-SO_3, sodium 3α,7β-dihydroxy-24-nor-5β-cholane-23-sulfonate; UDC-tau, ursodeoxycholyltaurine; TLC, thin-layer chromatography; HPLC, high performance liquid chromatography.

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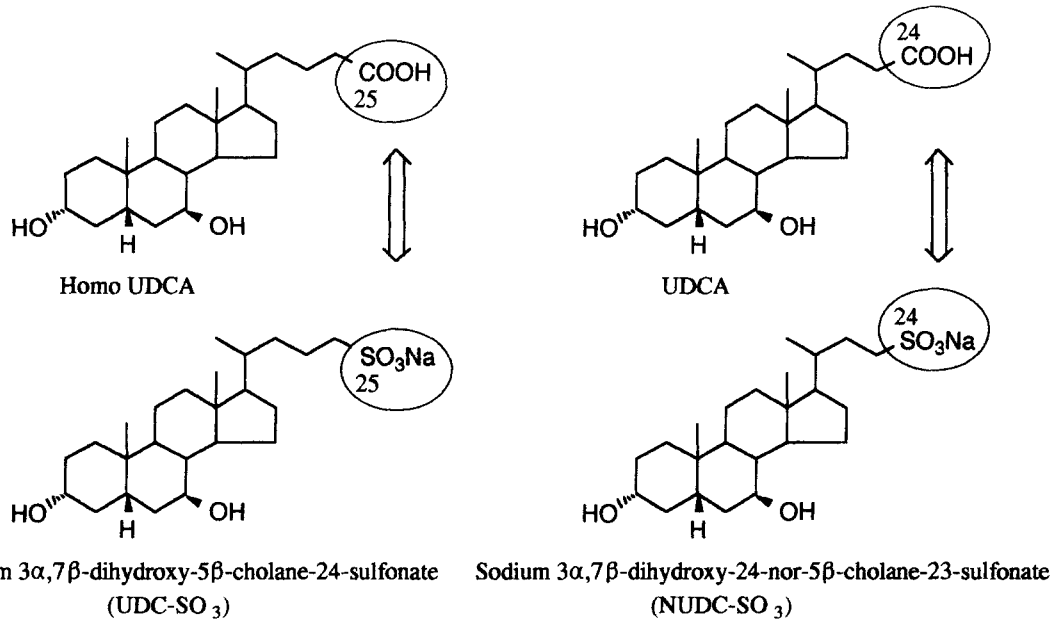

Fig. 1. Structures of sodium 3α,7β-dihydroxy-5β-cholane-24-sulfonate (UDC-SO_3) and sodium 3α,7β-dihydroxy-24-nor-5β-cholane-23-sulfonate (NUDC-SO_3).

cholyltaurine, and hyodeoxycholylglycine were prepared according to the method reported previously (16). [24-^{14}C]CDCA was purchased from Daiichi Kagaku Yakuhin, Tokyo. [11,12-^{3}H]UDCA was purchased from NEN Research Products, Boston, MA. Sodium [11,12-^{3}H]3α,7β-dihydroxy-5β-cholane-24-sulfonate ([^{3}H]UDC-SO_3) and sodium [11,12-^{3}H]3α,7β-dihydroxy-24-nor-5β-cholane-23-sulfonate ([^{3}H]NUDC-SO_3) were prepared from [11,12-^{3}H]UDCA according to the method reported previously (11, 12). Radiopurity of [^{14}C]CDCA, [^{3}H]UDC-SO_3 and [^{3}H]NUDC-SO_3 were found to be greater than 99%.

Radio-thin layer chromatography (radio-TLC)

The samples were chromatographed on precoated silica gel G sheets (0.2 mm thickness, Merck). Chloroform–methanol–acetic acid–water 13:4:2:1; CMAW and isooctane–isopropanol–acetic acid 30:10:1; S-9 were used as the solvent systems for radio-TLC. Spots were visualized with 10% phosphomolybdic acid in ethanol and heating at 110°C for 5 min. The sheet was cut into 1 cm sections and the radioactivity of the sections was counted with a liquid scintillation counter (LSC-3500 Aloka, Tokyo) in a toluene-based scintillator.

High performance liquid chromatography (HPLC)

HPLC was carried out on a Shimadzu LC-4A chromatograph (Shimadzu, Kyoto, Japan) equipped with a Shimadzu HPLC fluorescence monitor RF-530 (excitation wavelength 370 nm; emission wavelength 470 nm), (Shimadzu, Kyoto, Japan). An Inertsil ODS-2 column (5 μm, 4.6 × 250 mm, Gasukuro Kogyo) was used at ambient temperature.

Analysis of biliary bile acid composition

The gallbladder bile was extracted with ethanol (2 ml) and the solvent was evaporated under a stream of nitrogen. The residue was dissolved in methanol (2 ml) and to a 10 μl aliquot of this solution was added hyodeoxycholyltaurine and hyodeoxycholylglycine (1 μg each) as internal standards. The solution was evaporated under a stream of nitrogen and the residue was treated with 1-anthroyl nitrile to form the 3-O-anthroyl esters and separated on PHP-LH-20 into glycine and taurine conjugates according to the method reported previously (17). Each fraction was dissolved in methanol (400 μl) and a 5–10 μl aliquot was analyzed by HPLC using the following conditions: solvent system, 0.3% potassium phosphate buffer (pH 7.0)–methanol 1:9; flow rate, 1.0 ml/min.

Intestinal absorption and hepatic metabolism of UDC-SO_3 and NUDC-SO_3

Male golden Syrian hamsters (Hiroshima Experimental Animal Center, Hiroshima) weighing about 100 g were anesthetized with sodium pentobarbital (Nembutal, Dinapot Co., Tokyo) and their bile ducts were cannulated with polyethylene tubing (PE-10, 0.28 mm i.d.). The jejunum or the ileum was tied to make a 10 cm loop. A solution (0.5 ml) of [^{3}H]UDC-SO_3 (0.5 mg, 1.75×10^2 kBq/mg) or [^{3}H]NUDC-SO_3 (0.5 mg, 2.10×10^2 kBq/mg) and [^{14}C]CDCA (0.5 mg, 19.7 kBq/mg) in saline was in-

jected into the ileal or jejunal loops of three hamsters, and bile samples were collected every 0.5 h for 4 h. Biliary bile acids were extracted with ethanol and the radioactivity was analyzed by radio-TLC.

Metabolism of UDC-SO_3 and NUDC-SO_3 by intestinal microorganisms

Three hamsters weighing about 100 g were fed 0.1% UDC-SO_3 or 0.1% NUDC-SO_3 in a commercial rodent chow for 2 weeks. On the 7th day, 1 ml of an emulsion consisting of Tween 80, saline, [^{14}C]CDCA, and [^{3}H]UDC-SO_3 or [^{3}H]NUDC-SO_3 (1 mg each) was administered intragastrically. Feces were then collected every day for 1 week. Fecal bile acids were extracted with boiling ethanol for 8 h and the radioactivity was analyzed by radio-TLC.

Effect of UDC-SO_3 and NUDC-SO_3 on biliary bile acid composition

Four hamsters weighing about 80 g each were fed the commercial rodent chow, containing 0.1% UDC-SO_3, 0.1% NUDC-SO_3, 0.1% UDC-tau, or 0.1% UDCA for 2 weeks. After fasting the animals for 24 h, the gallbladder was resected under ethyl ether anesthesia and the bile was immediately extracted with ethanol. The bile extract was derivatized as described above and analyzed by HPLC.

Effect of UDC-SO_3 and NUDC-SO_3 on cholesterol metabolism

Four hamsters were fed rodent chow containing 0.1% cholesterol (CH diet), plus 0.1% UDC-SO_3, 0.1% NUDC-SO_3, 0.1% UDC-tau, or 0.1% UDCA for 2 weeks. On the 11th day, 1.5 ml of an emulsion consisting of Tween 80, saline, [1,2-^{3}H]cholesterol (32 kBq) and [4-^{14}C]sitosterol (3.2 kBq) was administered intragastrically. Feces were collected daily for 3 days and fecal neutral sterols were extracted with n-hexane for 6 h with a Soxhlet apparatus. Radioactivity in feces was assayed by liquid scintillation counting in a toluene-based scintillator. Cholesterol absorption (%) was calculated from the radioactivity using [4-^{14}C]sitosterol as a nonabsorbable marker as reported previously (18, 19).

On the 14th day, the hamsters were fasted for 24 h and a blood sample was collected from the heart under diethyl ether anesthesia. Serum free and total cholesterol concentrations were determined using an enzymatic kit (BMY reagent and Monotest cholesterol, Boehringer-Mannheim GmbH, West Germany).

RESULTS

[^{3}H]UDC-SO_3 or [^{3}H]NUDC-SO_3 was injected into the ileal loop of three hamsters using [^{14}C]CDCA as a reference bile acid. The recovery of radioactivity is shown in **Fig. 2.** When UDC-SO_3 and NUDC-SO_3 were administered into the ileal loop, they were efficiently absorbed from the ileum, extracted by the liver, and rapidly secreted into the bile at the same rate as [^{14}C]CDCA, simultaneously injected as a reference bile acid. In contrast, the absorption of UDC-SO_3 and NUDC-SO_3 from the jejunum was slower than from the ileum as shown in **Fig. 3.** Only 54% (UDC-SO_3) and 42% (NUDC-SO_3) of the administered radioactivity was recovered in the bile within 4 h following injection into the jejunal loop.

The radioactivity recovered in the bile within 4 h after the injection of [^{3}H]UDC-SO_3 or [^{3}H]NUDC-SO_3 into the ileal loop was analyzed by radio-TLC. UDC-SO_3 and NUDC-SO_3 remained unchanged while CDCA was excreted in the form of its glycine and taurine conjugates.

The metabolism of UDC-SO_3 and NUDC-SO_3 by intestinal microorganism was examined by feeding these sulfonate analogs. Three hamsters ate an average of 10 g/day of a diet containing 0.1% UDC-SO_3 or 0.1% NUDC-SO_3, about 10 mg of sulfonates per day as calculated from the food intake. All hamsters were healthy throughout the experimental period and gained similar amounts of weight. On the 7th day of feeding, 1 mg each of [^{3}H]UDC-SO_3 or [^{3}H]NUDC-SO_3 and [^{14}C]CDCA was administered intragastrically. The cumulative fecal excretion of ^{3}H and ^{14}C is shown in **Fig. 4.** Radioactivity from administered [^{3}H]UDC-SO_3 or [^{3}H]NUDC-SO_3 was recovered in feces to the same extent as [^{14}C]CDCA.

Radio-TLC of the fecal radioactive compounds (7 day collection) is shown in **Fig. 5.** The results showed that CDCA was largely metabolized to LCA during its passage through the intestinal tract. In contrast, UDC-SO_3 and NUDC-SO_3 were not metabolized during enterohepatic circulation.

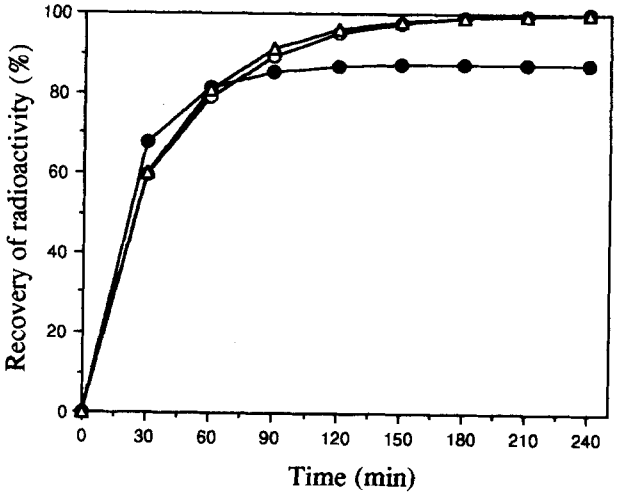

Fig. 2. Cumulative biliary recovery of radioactivity in bile fistula hamsters after intraileal administration of [^{3}H]UDC-SO_3 (○), [^{3}H]NUDC-SO_3 (△), and [^{14}C]CDC (●).

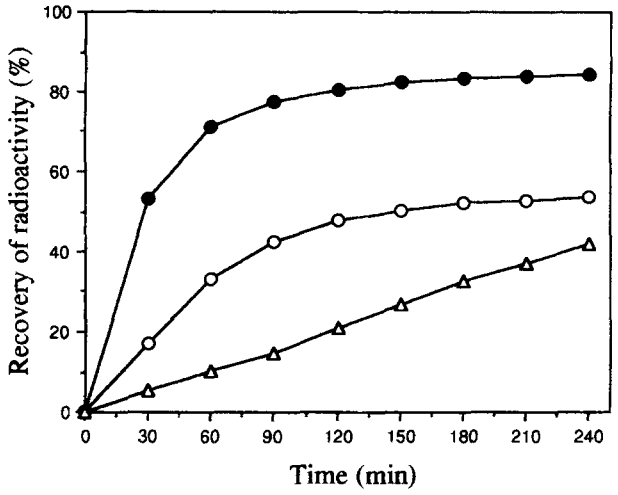

Fig. 3. Cumulative biliary recovery of radioactivity in bile fistula hamsters after intrajejunal administration of [^{3}H]UDC-SO_3 (○), [^{3}H]NUDC-SO_3 (△), and [^{14}C]CDC (●).

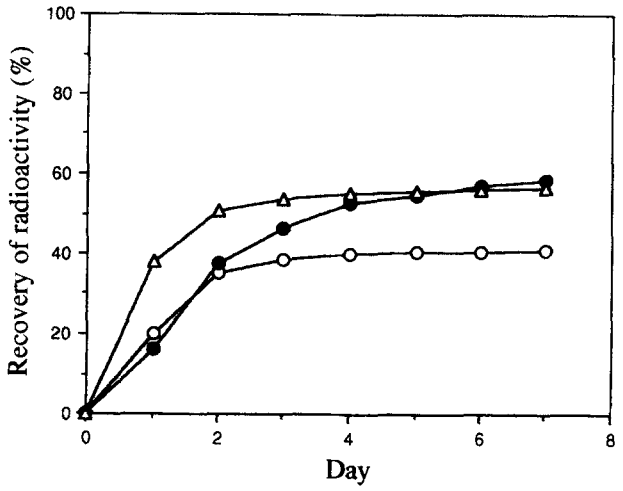

Fig. 4. Cumulative fecal recovery of radioactivity in hamsters after intragastric administration of [^{3}H]UDC-SO_3 (○), [^{3}H]NUDC-SO_3 (△), and [^{14}C]CDC (●).

The effect of UDC-SO_3 or NUDC-SO_3 on biliary bile acid composition was studied by feeding these sulfonate analogs to hamsters for 2 weeks. UDC-tau and UDCA were also fed as reference bile acids. As shown in **Table 1**, UDC-tau and UDCA gave essentially the same results, as UDC-tau would be readily hydrolyzed by intestinal bacteria to yield UDCA. As reported previously, UDCA feeding to hamsters resulted in a predominance of CDCA

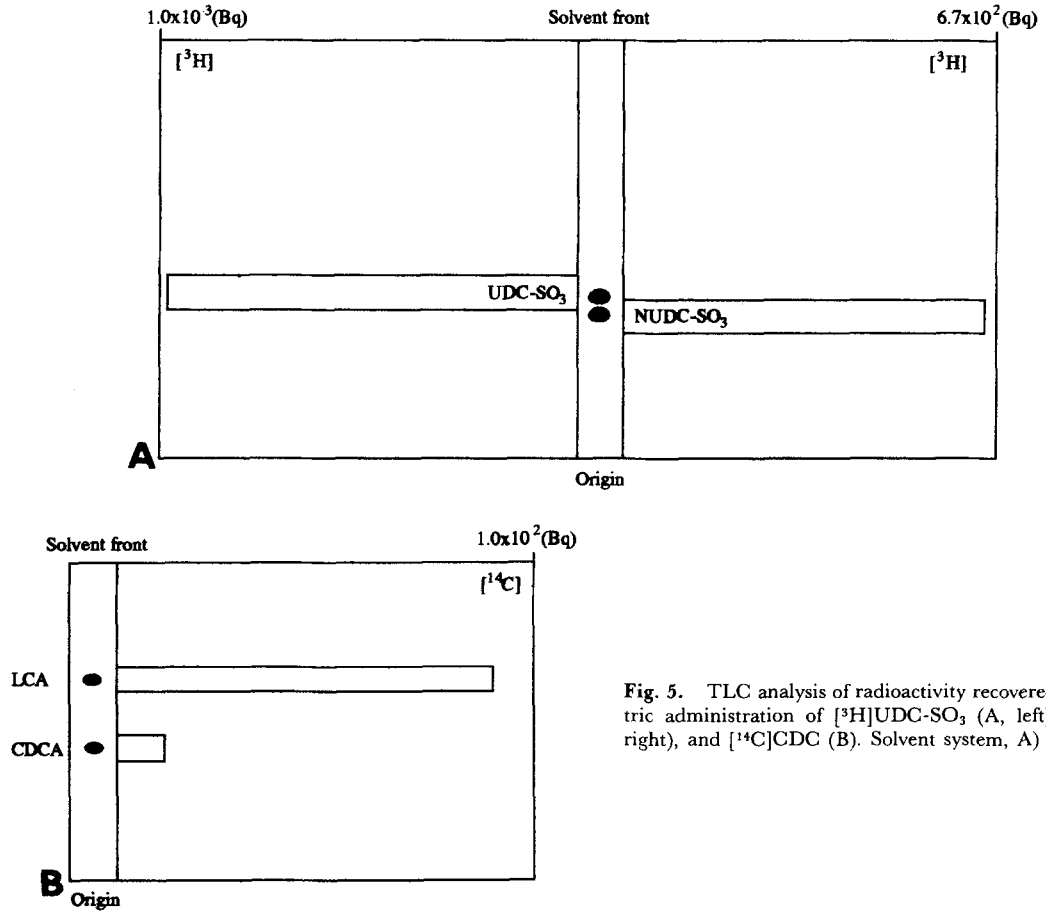

Fig. 5. TLC analysis of radioactivity recovered in feces after intragastric administration of [^{3}H]UDC-SO_3 (A, left), [^{3}H]NUDC-SO_3 (A, right), and [^{14}C]CDC (B). Solvent system, A) CMAW, B) S-9.

TABLE 1. Effects of dietary bile acids on biliary bile acid composition

Bile Acid Composition	Diet				
	Control	UDC-SO_3	NUDC-SO_3	TUDC	UDCA
			%		
TCA	46.1 ± 5.3	30.1 ± 6.7 (39.4)[a]	36.7 ± 3.9 (44.2)	12.0 ± 3.9	11.1 ± 3.1
GCA	21.6 ± 4.0	15.3 ± 3.5 (20.1)	16.0 ± 3.0 (19.3)	18.4 ± 1.3	14.9 ± 3.2
TCDC	18.6 ± 5.3	15.7 ± 1.7 (20.6)	17.8 ± 1.3 (21.4)	25.3 ± 4.4	27.5 ± 3.2
GCDC	7.0 ± 3.5	7.7 ± 1.9 (10.1)	6.2 ± 1.4 (7.5)	33.7 ± 9.3	35.8 ± 3.5
TDCA	4.7 ± 2.4	5.6 ± 1.5 (7.3)	5.0 ± 1.5 (6.0)	2.3 ± 1.5	2.1 ± 1.4
GDCA	1.1 ± 0.2	1.9 ± 0.7 (2.5)	1.4 ± 0.7 (1.7)	2.5 ± 1.0	2.0 ± 1.6
TUDC	n.d.	n.d.	n.d.	2.7 ± 1.6	2.5 ± 1.2
GUDC	n.d.	n.d.	n.d.	3.3 ± 1.0	4.1 ± 1.2
UDC-SO_3	n.d.	24.0 ± 8.0	n.d.	n.d.	n.d.
NUDC-SO_3	n.d.	n.d.	16.9 ± 4.0	n.d.	n.d.
CA/CDC ratio	2.88 ± 1.02	2.00 ± 0.61	2.22 ± 0.38	0.52 ± 0.10[b]	0.41 ± 0.09[b]
G/T ratio	0.42 ± 0.10	0.48 ± 0.07	0.40 ± 0.09	1.47 ± 0.58[c]	1.34 ± 0.27[c]

Values are mean ± SD for three animals; n.d., not detected.
[a]Figures in parentheses indicate proportion (%) of natural bile salts without including the administered sulfonate analogs.
[b]$P < 0.05$; [c], $P < 0.001$ versus control group.

(about 60%) in the bile. In addition to the change of the bile acid composition, the ratio of glycine-conjugated bile acids to taurine-conjugated bile acids (G/T ratio) was significantly elevated to 1.47 and 1.34 by feeding UDC-tau and UDCA, respectively, whereas it was 0.42 in the control group. UDC-SO_3 and NUDC-SO_3 accounted for 24.0% and 16.9% of total biliary bile acids, respectively. The biliary percent composition of the natural bile acids and the G/T ratio were not affected by UDC-SO_3 and NUDC-SO_3.

The effects of UDC-SO_3, NUDC-SO_3, UDC-tau, and UDCA on serum cholesterol concentration and cholesterol absorption are shown in **Table 2** and **Table 3**. As shown in Table 2, serum cholesterol levels were significantly elevated by feeding a high cholesterol diet ($P < 0.01$). No significant changes of serum cholesterol concentration were seen with UDC-SO_3 and NUDC-SO_3, or with UDC-tau and UDCA. Intestinal absorption of cholesterol was 66% on the control diet and 57.8% on the high cholesterol diet (Table 3). On the high cholesterol diet containing 0.1% UDC-tau and UDCA, corresponding values were 61.0% and 59.8%, respectively. Thus there were no significant differences in intestinal cholesterol absorption between controls, UDC-tau, and UDCA groups in the present study. Intestinal cholesterol absorption was 60.2% and 65.3% on the high cholesterol diet with either 0.1% UDC-SO_3 or NUDC-SO_3, respectively. Therefore UDC-SO_3 and NUDC-SO_3 had no significant effect on intestinal cholesterol absorption.

TABLE 2. Effect of dietary bile acids on serum cholesterol

Diet	Free	Total
	mg/dl	
Control diet	38.8 ± 3.4	142.2 ± 15.6
+ UDC-SO_3	34.9 ± 7.3	125.5 ± 8.7
+ NUDC-SO_3	35.0 ± 4.4	127.4 ± 9.9
+ TUDC	35.9 ± 3.3	139.1 ± 7.5
+ UDCA	36.4 ± 5.9	129.1 ± 8.5
High cholesterol diet	73.4 ± 5.9[a]	188.9 ± 13.5[a]
+ UDC-SO_3	66.1 ± 2.7[a]	182.1 ± 8.8[a]
+ NUDC-SO_3	72.8 ± 6.5[a]	199.7 ± 18.2[a]
+ TUDC	77.8 ± 6.4[a]	206.7 ± 11.7[a]
+ UDCA	71.6 ± 6.9[a]	195.3 ± 13.6[a]

Values are mean ± SD for four animals.
[a]$P < 0.01$ versus corresponding control diet group.

DISCUSSION

UDC-SO_3 and NUDC-SO_3 were absorbed from the ileum and participated in the enterohepatic circulation as efficiently as endogenous bile acids; they did not exhibit any hepatic biotransformation during enterohepatic circulation. Bile acids are absorbed from the ileum by active

TABLE 3. Effects of dietary bile acids on cholesterol absorption

Diet	Cholesterol Absorption
	%
Control	66.7 ± 8.2
High cholesterol diet	57.8 ± 9.3
+ UDC-SO_3	60.2 ± 1.2
+ NUDC-SO_3	65.3 ± 2.5
+ TUDC	61.0 ± 7.4
+ UDCA	59.8 ± 8.0

Values are mean ± SD for four animals.

transport and more polar bile acids (i.e., taurine-conjugated bile acids) are absorbed more efficiently (20). On the other hand, bile acids are absorbed from the jejunum by passive diffusion and nonpolar bile acids (i.e., unconjugated bile acids) are efficiently absorbed. On TLC and PHP-LH 20 analysis, UDC-SO_3 and NUDC-SO_3 have been shown to exhibit a polarity similar to that of UDC-tau (11, 12). These facts suggest that UDC-SO_3 and NUDC-SO_3 should be absorbed from the ileum by active transport just like taurine-conjugated bile acids. The absorbed sulfonate analogs should then participate in the enterohepatic circulation just like the endogenous bile acids.

UDC-SO_3 and NUDC-SO_3 resisted biotransformation by intestinal microorganisms. The resistance of UDC-SO_3 and NUDC-SO_3 to bacterial 7-dehydroxylation is not due to an alteration of the intestinal flora, because CDCA administered simultaneously was converted to LCA. It has been shown that bacterial 7-dehydroxylation takes place mainly after deconjugation. This supposes that 7-dehydroxylation is possibly a function of the polarity of the bile salt molecule. The sulfonate analogs have a polarity similar to that of taurine conjugate bile acids (11, 12). Recently it has been demonstrated that 7-dehydroxylation of cholic acid requires the presence of a free carboxyl group and its linkage to an adenosine nucleotide (7). Apparently, the sulfonic acid moiety on the side chain of the sulfonate derivatives cannot form a bond with the nucleotide.

Intestinal cholesterol absorption affects serum and liver cholesterol levels, and perhaps, biliary cholesterol secretion. There have been reports that UDCA either inhibited intestinal cholesterol absorption (21, 22) or had no effect (23), and UDCA reduced serum cholesterol concentration (24, 25) and had no effect (26). In the present study, feeding of UDCA had no significant effect on intestinal cholesterol absorption or serum cholesterol concentration. Similarly, no significant changes of cholesterol absorption or serum cholesterol concentration were seen with UDC-SO_3 and NUDC-SO_3. These results indicate that UDC-SO_3 and NUDC-SO_3 have no profound effects on cholesterol metabolism.

Feeding of UDCA or UDC-tau to hamsters resulted in a predominance of CDCA in bile. The same results have been reported previously (27). CDCA was presumably formed from UDCA via the 7-oxo compound (bacterial) followed by reduction to the 7α-hydroxyl compound (hepatic) (28) and/or 7α-hydroxylation of lithocholic acid (29). The elevated G/T ratio reflects the depletion of the hepatic taurine pool caused by the conjugation of UDCA and its metabolites during enterohepatic cycling. Orally administered UDC-SO_3 and NUDC-SO_3 accounted for 24.0% and 16.9% of biliary total bile acids, but had no effect on the relative proportions of the naturally occurring bile acids or the G/T ratio. However, the proportion of the sulfonate analogs was relatively low in comparison with the results reported for UDCA and its metabolite CDCA (27). The unchanged proportion of the natural bile acids, the unchanged G/T ratio, and the relatively low proportion of sulfonate analogs in bile suggest that UDC-SO_3 and NUDC-SO_3 do not inhibit cholesterol 7α-hydroxylase. It has been shown that UDCA and its conjugates were poor inhibitors of cholesterol 7α-hydroxylation (22, 30). It is of interest whether this apparent lack of inhibition of cholesterol 7α-hydroxylase by the sulfonate analogs is a function of the structure of the steroid nuclear possessing the 3α,7β-dihydroxy moiety or the lack of a peptide bond in the side chain.

These results suggest that UDC-SO_3 and NUDC-SO_3 have little effect on endogenous bile acid metabolism and may be suitable as potential gallstone-dissoluting agents. Whether they are safer and more effective than UDCA requires further studies.

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REFERENCES

1. Danzinger, R. G., A. F. Hofmann, L. J. Schoenfield, and J. L. Thistle. 1972. Dissolution of cholesterol gallstones by chenodeoxycholic acid. *N. Engl. J. Med.* **286:** 1-8.
2. Makino, I., K. Shinozaki, K. Yoshino, and S. Nagasawa. 1975. Dissolution of cholesterol gallstones by ursodeoxycholic acid. *Jpn. J. Gastroenterol.* **72:** 690-702.
3. Hunt, R. D., G. A. Leveille, and H. E. Sauberlich. 1964. Dietary bile acid and lipid metabolism. III. Effect of lithocholic acid in mammalian species. *Proc. Soc. Exp. Biol. Med.* **115:** 227-280.
4. Palmer, R. H. 1982. Bile salts and the liver. *Prog. Liver Dis.* **7:** 221-242.
5. Reddy, B. S., and K. Watanabe. 1979. Effect of cholesterol metabolites and promoting effect of lithocholic acid in colon carcinogenesis in germ-free and conventional F334 rats. *Cancer Res.* **39:** 1521-1524.
6. Batta, A. K., G. Salen, R. Arora, S. Shefer, M. Batta, and A. Person. 1990. Side chain conjugation prevents bacterial 7-dehydroxylation of bile acids. *J. Biol. Chem.* **265:** 10925-10928.
7. Björkhem, I., K. Einarsson, P. Melone, and P. Hylemon. 1989. Mechanism of intestinal formation of deoxycholic acid from cholic acid in humans: evidence for a 3-oxo-Δ^4-steroid intermediate. *J. Lipid Res.* **30:** 1033-1039.
8. Kimura, M., S. Hatono, M. Une, T. Fukuoka, and T. Hoshita. 1984. Synthesis, intestinal absorption and metabolism of sarcosine-conjugated ursodeoxycholic acid. *Steroids.* **43:** 677-687.
9. Hatono, S., Y. Hironaka, T. Kuramoto, and T. Hoshita. 1984. Metabolism of bile acids. XIV. Sarcosine-conjugated bile acids. *Yakugaku Zasshi.* **104:** 466-471.
10. Schmassmann, A., A. Angellotti, H. T. Ton-Nu, C. D. Schteingart, S. N. Marcus, S. S. Rossi, and A. F. Hofmann.

1990. Transport, metabolism, and effect of chronic feeding of cholylsarcosine, a conjugated bile acid resistant to deconjugation and dehydroxylation. *Gastroenterology.* **98:** 163–174.
11. Kihira, K., M. Yoshii, A. Okamoto, S. Ikawa, H. Ishii, and T. Hoshita. 1990. Synthesis of new bile salt analogues, sodium 3α,7α-dihydroxy-5β-cholane-24-sulfonate and sodium 3α,7β-dihydroxy-5β-cholane-24-sulfonate. *J. Lipid Res.* **31:** 1323–1326.
12. Kihira, K., T. Mikami, S. Ikawa, A. Okamoto, M. Yoshii, S. Miki, E. H. Mosbach, and T. Hoshita. 1992. Synthesis of sulfonate analogs of bile acids. *Steroids.* **57:** 193–198
13. Kihira, K., A. Okamoto, S. Ikawa, T. Mikami, M. Yoshii, E. H. Mosbach, and T. Hoshita. 1991. Metabolism of sodium 3α,7α-dihydroxy-5β-cholane-24-sulfonate in hamsters. *J. Biochem.* **109:** 879–881.
14. Miyazaki, K., F. Nakayama, and A. Koga. 1984. Effect of chenodeoxycholic and ursodeoxycholic acids on isolated adult human hepatocytes. *Dig. Dis. Sci.* **29:** 1123–1130.
15. Galle, P. R., L. Theilmann, R. Raedsch, G. Otto, and A. Stiehl. 1990. Ursodeoxycholate reduces hepatotoxicity of bile salts in primary human hepatocytes. *Hepatology.* **12:** 486–491.
16. Lack, L., F. O. Dorrity, Jr., T. Walker, and G. D. Singletary. 1973. Synthesis of conjugated bile acids by means of a peptide coupling reagent. *J. Lipid Res.* **14:** 367–370.
17. Goto, J., M. Saito, T. Chikai, N. Goto, and T. Nambara. 1983. Studies on steroids. CLXXXVI. Determination of serum bile acids by high-performance liquid chromatography with fluorescence labeling. *J. Chromatogr.* **276:** 286–300.
18. Quintao, E., S. M. Grundy, and E. H. Ahrens, Jr. 1971. An evaluation of four methods for measuring cholesterol absorption by the intestine in man. *J. Lipid Res.* **12:** 221–232.
19. Overturf, M. L., S. A. Smith, A. M. Gotto, Jr., J. D. Morrisett, T. Tewson, J. Poorman, and D. S. Loose-Mitchell. 1990. Dietary cholesterol absorption, and sterol and bile acid excretion in hypercholesterolemia-resistant white rabbits. *J. Lipid Res.* **31:** 2019–2027.
20. Schiff, E. R., N. C. Small, and J. M. Dietschy. 1972. Characterization of the kinetics of the passive and active transport mechanisms for bile acid absorption in the small intestine and colon of the rat. *J. Clin. Invest.* **51:** 1351–1362.
21. Lanzini, A., and T. C. Northfield. 1988. Effect of ursodeoxycholic acid on biliary lipid coupling and on cholesterol absorption during fasting and eating in subjects with cholesterol gallstones. *Gastroenterology.* **95:** 408–416.
22. Hardison, W. G., and S. M. Grundy. 1984. Effect of ursodeoxycholic and its taurine conjugate on bile acid synthesis and cholesterol absorption. *Gastroenterology.* **87:** 130–135.
23. LaRusso, N. F., and J. L. Thistle. 1983. Effect of litholytic bile acids on cholesterol absorption in gallstone patients. *Gastroenterology.* **84:** 265–271.
24. Eusufzai, S., S. Ericsson, T. Cederlund, K. Einarsson, and B. Angelin. 1991. Effect of ursodeoxycholic acid treatment on ileal absorption of bile acids in man as determined by the SeHCAT test. *Gut.* **32:** 1044–1048.
25. Angelin, B., S. Ewerth, and K. Einarsson. 1983. Ursodeoxycholic acid treatment in cholesterol gallstone disease: effects on hepatic 3-hydroxy-3-methylglutaryl coenzyme A reductase activity, biliary lipid composition, and plasma lipid levels. *J. Lipid Res.* **24:** 461–468.
26. Bachrach, W. H., and A. F. Hofmann. 1982. Ursodeoxycholic acid in the treatment of cholesterol cholelithiasis. *Dig. Dis. Sci.* **27:** 737–856.
27. Matejka, M., C. Vescina, C. N. Carducci, A. Alayon, A. Dios, E. Scarlatto, and A. Mamianetti. 1990. Effect of ursodeoxycholic acid administration on bile acid composition in hamster bile. *Pharmacol. Res.* **22:** 297–305.
28. Fromm, H., G. L. Carlson, A. F. Hofmann, S. Farivar, and P. Amin. 1980. Metabolism in man of 7-ketolithocholic: precursor of cheno- and ursodeoxycholic acids. *Am. J. Physiol.* **239:** G161–166.
29. Emerman, S., and N. B. Javitt. 1967. Metabolism of taurolithocholic acid in the hamster. *J. Biol. Chem.* **242:** 661–664.
30. Bertolotti, M., N. Carulli, D. Menozzi, F. Zironi, A. Digrisolo, A. Pinetti, and M. G. Baldini. 1986. In vivo evaluation of cholesterol 7α-hydroxylation in humans: effect of disease and drug treatment. *J. Lipid Res.* **27:** 1278–1286.