Effect of some sulfonate analogues of ursodeoxycholic acid on biliary lipid secretion in the rat

Takahiro Mikami,[1,*] Kenji Kihira,[†] Seiichirou Ikawa,[*] Michiko Yoshii,[*] Erwin H. Mosbach,[§] and Takahiko Hoshita[*]

Institute of Pharmaceutical Sciences,[*] Hiroshima University School of Medicine, and Department of Pharmaceutical Services,[†] Hiroshima University Hospital, Kasumi 1-2-3 Minami-ku, Hiroshima 734, Japan, and Department of Surgery,[§] Beth Israel Medical Center, First Avenue at 16th Street, New York, NY 10003

Abstract The effect of the sulfonate analogues of ursodeoxycholic acid, namely sodium 3α,7β-dihydroxy-24-nor-5β-cholane-23-sulfonate (norUDC-SO_3Na) and sodium 3α,7β-dihydroxy-5β-cholane-24-sulfonate (UDC-SO_3Na), on biliary lipid secretion was studied in bile fistula rats. During intravenous infusion of the two sulfonate analogues, bile flow and biliary lipid secretion were stimulated in a dose-dependent manner. This suggests that the analogues exert an effect on biliary lipid secretion comparable to that of the naturally occurring bile acid, ursodeoxycholyltaurine (UDC-tau). The effects of norUDC-SO_3Na and UDC-SO_3Na on bile flow were similar but slightly smaller than that of UDC-tau. The output of bile salts was similar with both sulfonates but greater than that with UDC-tau. The infusion of norUDC-SO_3Na or UDC-SO_3Na induced cholesterol secretion and phospholipid secretion more significantly than UDC-tau infusion. The increase in phospholipid secretion was particularly pronounced during high-dose administration of norUDC-SO_3Na. Although norUDC-SO_3Na stimulated cholesterol secretion more intensely than the other two bile salts, it also facilitated phospholipid output, perhaps as a compensatory mechanism, and the biliary cholesterol/phospholipid ratio was decreased to a greater extent by the sulfonates than by UDC-tau. Consequently, the administration of norUDC-SO_3Na or UDC-SO_3Na produces a more "stable" bile than UDC-tau, suggesting that these sulfonates possess potential cholelitholytic activity.—**Mikami, T., K. Kihira, S. Ikawa, M. Yoshii, E. H. Mosbach, and T. Hoshita.** Effect of some sulfonate analogues of ursodeoxycholic acid on biliary lipid secretion in the rat. *J. Lipid Res.* 1996. **37:** 1181–1188.

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Biliary cholesterol is kept in solution as mixed micelles with bile acids and phospholipid or as cholesterol-phospholipid vesicles (1, 2). The mechanism whereby cholesterol gallstones form from these particles has not been fully elucidated, but an important aspect is the presence of excess cholesterol in a supersaturated bile (3). Chenodeoxycholic acid (CDCA) and ursodeoxycholic acid (UDCA) are still used in humans to desaturate the bile and dissolve cholesterol gallstones (4, 5). CDCA and UDCA dissolve gallstones by different mechanisms: CDCA acts by transferring cholesterol monohydrate from gallstones into micelles, while UDCA acts, in part, via the formation of liquid crystals (6, 7). However, both bile acids reduce the cholesterol saturation of bile, which appears to be one of the requirements for successful gallstone dissolution (8–10).

The relative concentration of biliary cholesterol is further influenced by the type and concentration of the biliary bile acids (11–14). While the oral administration of CDCA or UDCA decreases hepatic cholesterol output, cholic acid produces an increase and deoxycholic acid feeding does not change cholesterol output so that neither cholic acid nor deoxycholic acid is suitable for gallstone dissolution therapy (10, 15). Thus, the effect of bile acids on biliary lipid secretion is an important factor in the dissolution of cholesterol gallstones. We recently synthesized new bile acid analogues that are potentially useful as cholelitholytic agents (16, 17). These analogues possess a terminal sulfonic acid group in the side chain, like the taurine-conjugated bile acids,

Abbreviations: UDC-tau, ursodeoxycholyltaurine; UDC-SO_3Na, sodium 3α,7β-dihydroxy-5β-cholane-24-sulfonate; norUDC-SO_3Na, sodium 3α,7β-dihydroxy-24-nor-5β-cholane-23-sulfonate; CMC, critical micellar concantretion; CDCA, chenodeoxycholic acid; UDCA, ursodeoxycholic acid; HDC-tau, hyodeoxycholyltaurine.

[1]To whom correspondence should be addressed.

but possess no peptide bond and are therefore not hydrolyzable by the intestinal microorganisms. We subsequently demonstrated that sulfonate analogues of CDCA or UDCA resisted bacterial 7-dehydroxylation and did not form potentially hepatotoxic analogues of lithocholic acid (18, 19). In the present study we investigated the effect of norUDC-SO_3Na or UDC-SO_3Na on bile secretion and biliary lipid output in comparison with UDC-tau.

MATERIAL AND METHODS

Bile acids

UDC-tau was prepared by a method published previously (20). The syntheses of UDC-SO_3Na and norUDC-SO_3Na were described previously (18, 19).

Critical micellar concentration (CMC)

The CMC was measured by a method using the solubilization of Orange OT (Aldrich Chemical Co., Milwaukee, WI) (21).

Animal experiments

Male Wistar rats (Hiroshima Experimental Animal Center, Hiroshima, Japan), weighing 200–250 g were maintained with commercial rodent chow and water ad libitum, under a controlled 12-h light/dark cycle. The animals were anesthetized by intraabdominal injection of somnopentyl (Pitman-Moore, Inc., Mundelein, IL). After laparotomy, the common bile duct and the femoral vein were cannulated using polyethylene tubing (PE-10, 0.28 mm I.D., Becton, Dickinson & Company, Parisippany, NJ). Saline was infused intravenously, and bile was collected for 8 h to deplete the endogenous bile salts. At the end of this period a saline solution of the bile salt to be studied was infused at increasing rates (50, 100, 200, and 500 nmol/min per 100 g). Each dose was infused for consecutive 30-min periods; bile samples were collected every 10 min. After infusion of the bile acid analogues, bile was collected for an additional hour.

Analytical method

The bile flow rate was determined gravimetrically, assuming the specific gravity to be 1.0 g/ml. The total bile acid, phospholipid, and cholesterol concentrations of bile were determined with an enzymatic kit (bile acid and phospholipid were obtained from International Reagents Corp., Kobe, Japan, and the cholesterol kit, Monotest cholesterol, was from Boehringer-Mannheim GmbH, Germany).

Statistical analysis

The numerical data are expressed as mean ± SD. The statistical comparisons were made by ANOVA (Fisher PLSD) and P values < 0.05 were considered to be significant.

RESULTS

Table 1 lists the CMC values of the sulfonate analogues. The CMC values for norUDC-SO_3Na, UDC-SO_3Na, and UDC-tau in 0.15 mM Na^+ were 7.0, 3.0, and 2.0 mM, respectively. The CMC was related inversely to the length of the side chain.

Figure 1 (a–d) illustrates the time course of bile flow and biliary lipid output during intravenous infusion of the three compounds under study. The infusion of the bile acid analogues increased bile flow and biliary lipid secretion. The choleresis induced was similar for the sulfonates and UDC-tau (Fig. 1a). Bile acid output was significantly increased by norUDC-SO_3Na and UDC-SO_3Na in comparison with UDC-tau (Fig. 1b). NorUDC-SO_3Na infusion significantly stimulated cholesterol output and phospholipid output, particularly the latter during high dose infusion (Figs. 1c and 1d). The stimulation of phospholipid output by UDC-SO_3Na was significantly greater than that produced by UDC-tau.

Figure 2 (a–c) illustrates the relationships between bile flow and bile acid output; the linear regression equations are listed in **Table 2.** There was a linear correlation between bile acid output and bile flow; the sulfonates and UDC-tau gave similar effects. Table 2 shows that the rate of bile flow during the infusion of norUDC-SO_3Na, UDC-SO_3Na, and UDC-tau ranged from 1.10×10^{-2} to 1.17×10^{-2} µl/nmol.

The effects of the sulfonate analogues on biliary cholesterol output are shown in **Fig. 3 (a–c).** Cholesterol output was linearly related to bile acid output over the range from 0 to 150 nmol/min per 100 g, then reached a plateau. **Table 3** summarizes the linear regression

TABLE 1. Effect of side chain length on CMC values of sulfonate analogues

Bile Acids	norUDC-SO_3Na	UDC-SO_3Na	UDC-tau	UDCA[a]
CMC (mM)	7.0	3.0	2.0	7.0
Length of side chain[b]	5	6	9	5

[a]Experimental data from Roda, Hofmann, and Mysels (21).
[b]Number of carbon, nitrogen, and sulfur atoms.

equations during bile acid output over the range from 0 to 150 nmol/min per 100 g. During norUDC-SO_3Na infusion, the rate of cholesterol secretion (slope $4.80 \times 10^{-2} \pm 1.27 \times 10^{-2}$ nmol cholesterol/nmol bile acid) was significantly higher than that of UDC-SO_3Na (slope, $2.74 \times 10^{-2} \pm 0.55 \times 10^{-2}$ nmol cholesterol/nmol bile acid) $P < 0.05$. The difference between UDC-tau ($3.74 \times 10^{-2} \pm 0.68 \times 10^{-2}$ nmol cholesterol/nmol bile acid) and UDC-SO_3Na was not significant.

Figure 4 (a–c) illustrates the relationships between phospholipid output and bile acid output. The phospholipid output was linearly related to bile acid output up to a bile acid output of 150 nmol/min per 100 g, just like the cholesterol secretion. The linear regression equations are shown in **Table 4,** over the range from 0 to 150 nmol/min per 100 g. The slopes for norUDC-SO_3Na, UDC-SO_3Na, and UDC-tau were 3.46×10^{-1}, 1.79×10^{-1}, and 1.44×10^{-1} nmol phospholipid/nmol bile acid, respectively. The phospholipid output was stimulated significantly by norUDC-SO_3Na compared with UDC-SO_3Na ($P < 0.005$) and UDC-tau ($P < 0.001$). The difference between UDC-SO_3Na and UDC-tau was not significant.

Figure 5 (a–c) shows the relationships between cholesterol and phospholipid output. The cholesterol output was lineally related to the phospholipid output regardless of the bile acid output. The linear regression equations are shown in **Table 5.** During norUDC-SO_3Na

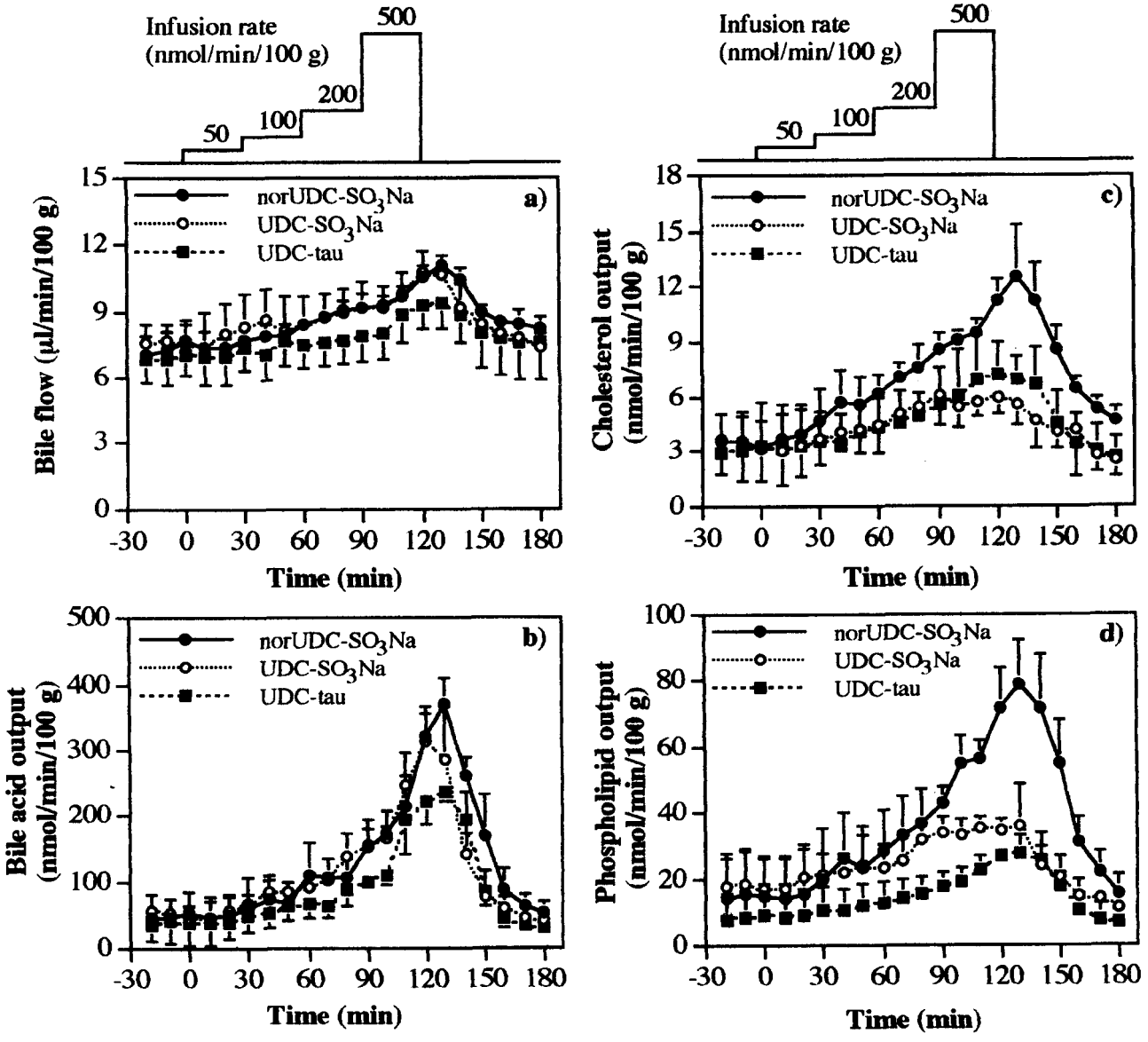

Fig. 1. Effect of norUDC-SO_3Na (●), UDC-SO_3Na (○), and UDC-tau (■) infusion on a) bile flow; b) bile acid output; c) cholesterol output; and d) phospholipid output in bile fistula rats.

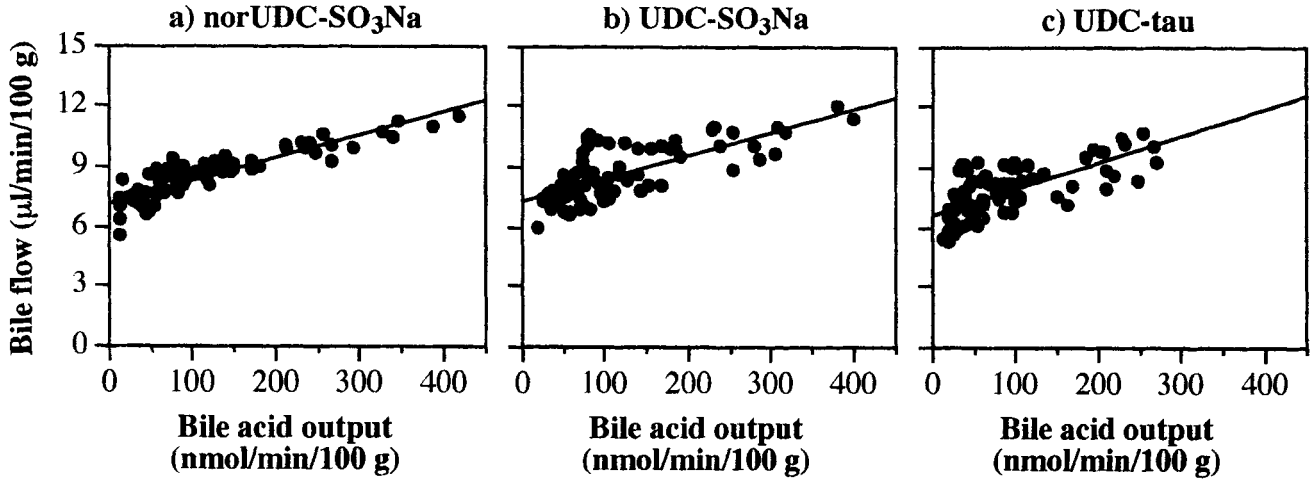

Fig. 2. Relationships between bile flow and bile acid output during intravenous infusion of a), norUDC-SO_3Na; b), UDC-SO_3Na; c), UDC-tau in bile fistula rats.

TABLE 2. Coefficients of linear regression lines for relationships between bile flow and bile acid output during intravenous infusion of norUDC-SO_3Na, UDC-SO_3Na, and UDC-tau in bile fistula rats

Bile Acid	Intercept[a]	Slope[b]	r
NorUDC-SO_3Na	7.24 ± 0.40	$1.10 \times 10^{-2} \pm 0.18 \times 10^{-2}$	0.90 ± 0.04
UDC-SO_3Na	7.34 ± 0.95	$1.09 \times 10^{-2} \pm 0.24 \times 10^{-2}$	0.87 ± 0.15
UDC-tau	6.65 ± 0.93	$1.17 \times 10^{-2} \pm 0.11 \times 10^{-2}$	0.94 ± 0.04

Values are mean ± SD for four rats.
[a]Expressed as μl/min per 100 g.
[b]Expressed as μl/nmol bile acid output.

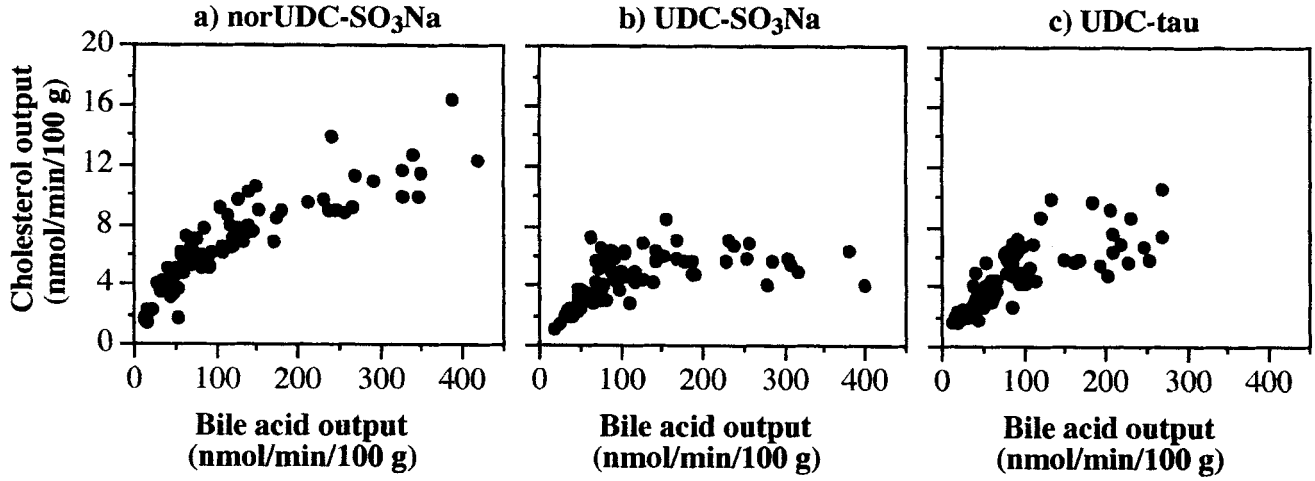

Fig. 3. Relationships between cholesterol and bile acid output during intravenous infusion of a), norUDC-SO_3Na; b), UDC-SO_3Na; c), UDC-tau in bile fistula rats.

TABLE 3. Coefficients of linear regression lines for relationships between cholesterol output and bile acid output (range 0 to 150 nmol/min per 100 g) during intravenous infusion of norUDC-SO_3Na, UDC-SO_3Na, and UDC-tau in bile fistula rats

Bile Acid	Intercept[a]	Slope[b]	r
NorUDC-SO_3Na	1.97 ± 0.63	$4.80 \times 10^{-2} \pm 1.27 \times 10^{-2}$[c]	0.90 ± 0.08
UDC-SO_3Na	1.89 ± 1.23	$2.74 \times 10^{-2} \pm 0.55 \times 10^{-2}$	0.83 ± 0.16
UDC-tau	1.61 ± 0.64	$3.74 \times 10^{-2} \pm 0.68 \times 10^{-2}$	0.93 ± 0.03

Values are mean ± SD for four rats.
[a]Expressed as nmol/min per 100 g.
[b]Expressed as nmol cholesterol output/nmol bile acid output.
[c]$P < 0.05$ versus UDC-SO_3Na.

and UDC-SO_3Na infusion, the rate of cholesterol secretion (slope, 1.29×10^{-1} and 1.33×10^{-1} cholesterol/nmol phospholipid, respectively) was significantly lower than that of UDC-tau (slope, 1.90×10^{-1} nmol cholesterol/nmol phospholipid) $P < 0.05$.

DISCUSSION

Biliary secretions of cholesterol and phospholipid are affected by the physicochemical properties of bile acids (22). For example, non-micelle-forming bile acids such as dehydrocholyltaurine do not stimulate the secretion of biliary cholesterol or phospholipid (23). NorUDC-SO_3Na and UDC-SO_3Na readily form micelles as shown in the present study in which the CMCs were determined (CMCs: norUDC-SO_3Na, 7.0; UDC-SO_3Na, 3.0; UDC-tau, 2.0 mM). The CMC values are related inversely to the length of the bile acid side chain (21). The number of atoms (C, N, and S) in the side chains of norUDC-SO_3Na, UDC-SO_3Na, and UDC-tau were 5, 6, and 9, respectively. The CMC of norUDC-SO_3Na was similar to that of free UDCA (7.0 mM), and both compounds have a 5-atom side chain (21). These results indicate that the higher CMC value of norUDC-SO_3Na compared to UDC-SO_3Na and UDC-tau is presumably a function of its shorter side chain.

In order to determine the effects of the sulfonate analogues on the bile flow and biliary cholesterol and phospholipid secretion, the compounds were infused intravenously into bile fistula rats. Both norUDC-SO_3Na and UDC-SO_3Na increased bile flow and biliary lipid output in a dose-dependent manner (Fig. 1 a–d). These results indicate that sulfonate analogues of the natural bile acids can exert biliary secretory effects resembling those of the natural bile acids. With all three compounds studied, bile secretion was linearly related to bile acid output throughout the experimental period (Fig. 2, Table 2). The effects of the three bile acids studied were very similar.

Biliary cholesterol and phospholipid secretion related nearly linearly to function of bile acid output up to a bile acid output of 150 nmol/min per 100 g. When the bile acid output exceeded 150 nmol/min per 100 g, the

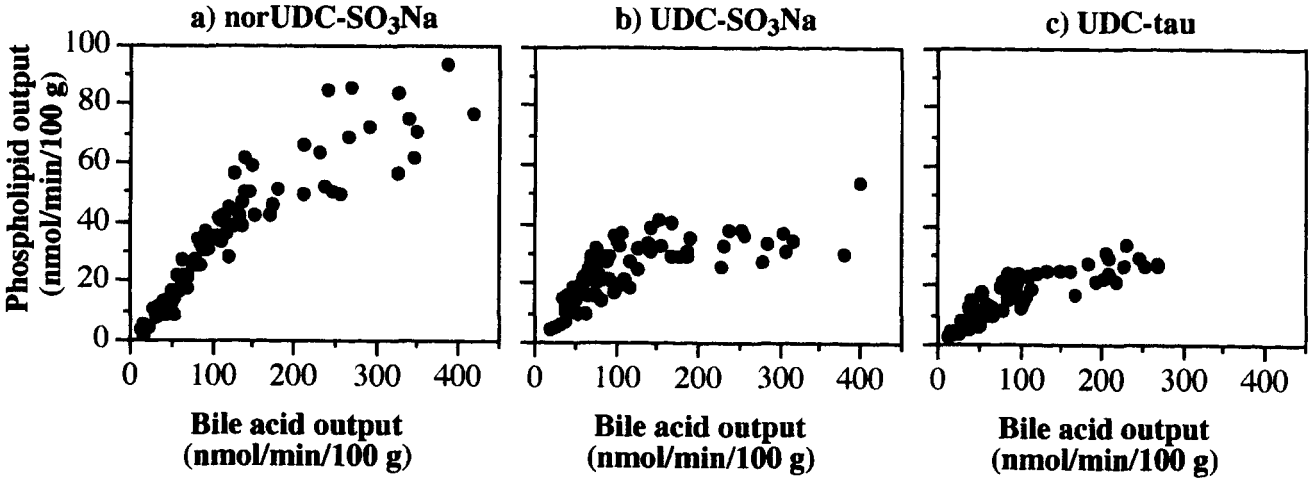

Fig. 4. Relationships between phospholipid and bile acid output during intravenous infusion of a), norUDC-SO_3Na; b), UDC-SO_3Na; c), UDC-tau in bile fistula rats.

TABLE 4. Coefficients of linear regression lines for relationships between phospholipid output and bile acid output (range 0 to 150 nmol/min per 100 g) during intravenous infusion of norUDC-SO_3Na, UDC-SO_3Na, and UDC-tau in bile fistula rats

Bile Acid	Intercept[a]	Slope[b]	r
NorUDC-SO_3Na	-0.62 ± 3.15[e]	$3.46 \times 10^{-1} \pm 0.59 \times 10^{-1}$[c,d]	0.96 ± 0.03
UDC-SO_3Na	6.92 ± 3.89	$1.79 \times 10^{-1} \pm 0.60 \times 10^{-1}$	0.81 ± 0.23
UDC-tau	3.45 ± 4.65	$1.44 \times 10^{-1} \pm 0.28 \times 10^{-1}$	0.95 ± 0.04

Values are mean ± SD for four rats.
[a]Expressed as nmol/min per 100 g.
[b]Expressed as nmol phospholipid output/nmol bile acid output.
[c]$P < 0.05$; [d]$P < 0.005$ versus UDC-SO_3Na.
[e]$P < 0.001$ versus UDC-tau.

biliary lipid output reached a plateau (Figs. 3 and 4). The effects of the bile acid sulfonates and UDC-tau on biliary lipid secretion are best compared in the lower range (bile acid output < 150 nmol/min per 100 g). The linear regression equations relating cholesterol-phospholipid output to bile acid output (range 0 to 150 nmol/min per 100 g), summarized in Tables 3 and 4, indicated that during UDC-SO_3Na infusion cholesterol secretion was lower and phospholipid secretion was higher than during infusion of UDC-tau. Cholesterol and phospholipid secretion were most strongly induced with norUDC-SO_3Na. The mechanisms of higher phospholipid secretion induced by norUDC-SO_3Na were not clearly explained by this study. The main physicochemical difference of the analogues examined, which may influence the phospholipid secretion, would be their hydrophilicity. NorUDC-SO_3Na would be considered more hydrophilic than UDC-SO_3Na and UDC-tau as indicated by its shorter side chain. It is known that biliary lipid secretion is stimulated by hydrophobic bile salts (12, 22). In contrast, recent study has demonstrated that the more hydrophilic bile acid is reported to be more effective in the biliary lipid secretion (24). The intravenous infusion of hyodeoxycholyltaurine (HDC-tau), one of the hydrophilic bile salts, in bile fistula rats produced higher phospholipid secretion compared to cholyltaurine and UDC-tau (24). The mechanism of this contrasting result will remain unexplained for the time being but in the present study, the higher phospholipid secretion with norUDC-SO_3Na seems to be attributable to its higher hydrophilicity. The mechanisms of bile acid-induced cholesterol and phospholipid secretion were not clear, but at the present time an intracanalicular hypothesis is advocated. In this hypothesis, the biliary secretion of cholesterol and phospholipid are mediated by bile acids in the bile canaliculus (25). These facts suggest that some hydrophilic bile salts may strongly affect secretion of phospholipid in the bile canalicular membrane. Further studies would be required for the definite mechanisms of simulated phospholipid secretion by hydrophilic bile salts to be elucidated.

Biliary phospholipid plays an important role in the solubilization of biliary cholesterol via mixed micelles and vesicles (1, 2). Cholesterol-phospholipid vesicles are much more efficient cholesterol carriers compared to mixed micelles. However, recent study indicated that

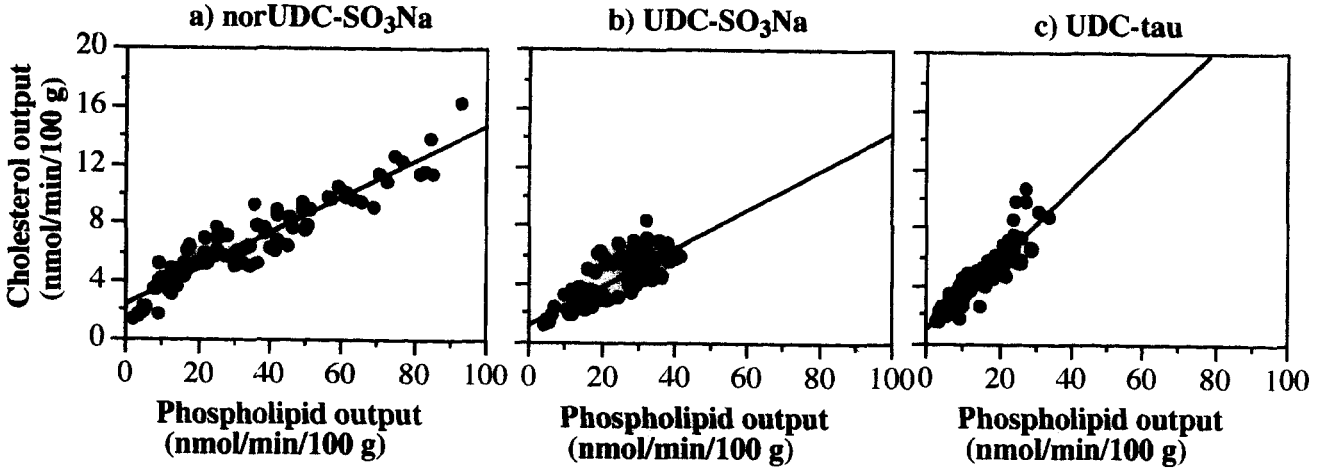

Fig. 5. Relationships between cholesterol and phospholipid output during intravenous infusion of a), norUDC-SO_3Na; b), UDC-SO_3Na; c), UDC-tau in bile fistula rats.

TABLE 5. Coefficients of linear regression lines for relationships between cholesterol output and phospholipid output during intravenous infusion of norUDC-SO_3Na, UDC-SO_3Na, and UDC-tau in bile fistula rats

Bile Acid	Intercept[a]	Slope[b]	r
NorUDC-SO_3Na	2.22 ± 0.91	$1.29 \times 10^{-1} \pm 0.12 \times 10^{-1}$[c]	0.96 ± 0.02
UDC-SO_3Na	1.29 ± 1.12	$1.33 \times 10^{-1} \pm 0.21 \times 10^{-1}$[c]	0.90 ± 0.08
UDC-tau	1.20 ± 0.20	$1.90 \times 10^{-1} \pm 0.48 \times 10^{-1}$	0.96 ± 0.02

Values are mean ± SD for four rats.
[a]Expressed as nmol/min per 100 g.
[b]Expressed as nmol cholesterol output/nmol phospholipid output.
[c]$P < 0.05$ versus UDC-tau.

vesicles play an important role in cholesterol gallstone formation (26–28). Stability of vesicles is affected by biliary cholesterol/phospholipid ratio and an elevated cholesterol/phospholipid ratio in vesicles is associated with more rapid cholesterol nucleation in both model and native biles (27, 28). During infusion of norUDC-SO_3Na and UDC-SO_3Na, cholesterol output was significantly lower ($P < 0.05$) than during infusion of UDC-tau (see slopes of regression lines in Table 5). The cholesterol output was linearly related to the phospholipid output regardless of the bile acid output without saturation. These results indicate that the biliary cholesterol/phospholipid ratio depends upon the structure of the infused bile salts and norUDC-SO_3Na and UDC-SO_3Na lower this ratio. In a previous study we demonstrated that UDC-SO_3Na was more effective than UDC-tau in the prevention of cholesterol gallstone formation (29). It is known that hydrophilic bile acids dissolve cholesterol gallstone by way of a formation of liquid crystalline vesicles (30). In the feeding experiment with UDC-SO_3Na, it induced a higher amount of liquid crystalline vesicles (83%) in the bile than did UDC-tau (13%) (29). In the present study, norUDC-SO_3Na and UDC-SO_3Na produced bile with a lower cholesterol/phospholipid ratio. This suggests that stability of biliary liquid crystalline vesicles may be increased by administration of norUDC-SO_3Na and UDC-SO_3Na. These results indicated that norUDC-SO_3Na and UDC-SO_3Na might become more effective cholelitholytic agents than UDC-tau.

In conclusion, norUDC-SO_3Na and UDC-SO_3Na produced an increased bile flow and an increased biliary lipid secretion similar to the natural bile acids. In this regard, norUDC-SO_3Na was the most effective of the three compounds studied. With UDC-SO_3Na the rate of cholesterol secretion was lower and that of phospholipid secretion was higher compared to UDC-tau. Cholesterol output/phospholipid output was lower with the sulfonates than with UDC-tau. These results suggest that the sulfonate analogues produce a more "stable" bile than UDC-tau. It seems possible, therefore, that the bile acid sulfonates can exert cholelitholytic effects, but further studies are needed to define efficacy and mechanisms.

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