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The choleretic effects of *N*-acetylglucosaminides, major urinary metabolites of ursodeoxycholic acid, in bile fistula rats

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Abstract

We investigated the effects of three bile acids conjugated with *N*-acetylglucosamine, ursodeoxycholate *N*-acetylglucosaminide, tauroursodeoxycholate *N*-acetylglucosaminide and glycoursodeoxycholate *N*-acetylglucosaminide, on bile flow and biliary excretion of various markers in comparison with ursodeoxycholic acid, tauroursodeoxycholic acid and glycoursodeoxycholic acid in bile fistula rats. These bile acids were infused intravenously at a constant rate of 0.3 or 0.6 μmol/min/100 g b.w. for 2 h. All bile acids examined increased bile flow in a dose-dependent manner. In particular, ursodeoxycholate *N*-acetylglucosaminide has a longer-lasting effect after its infusion on bile flow than the other bile acids. Furthermore, these bile acids markedly increased biliary total bile acid excretion. At a higher dose level, the coefficient of determination (r^2) between the biliary total bile acid excretion and bile flow for ursodeoxycholate *N*-acetylglucosaminide ($r^2 = 0.39$) was lower than that for the other bile acids ($r^2 = 0.75$–0.92). The ursodeoxycholate *N*-acetylglucosaminide, as well as tauroursodeoxycholic acid, glycoursodeoxycholic acid, tauroursodeoxycholate *N*-acetylglucosaminide and glycoursodeoxycholate *N*-acetylglucosaminide, was mostly excreted in an unchanged form in bile, whereas ursodeoxycholic acid was excreted as a conjugate with taurine. The three *N*-acetylglucosaminides as well as ursodeoxycholic acid, tauroursodeoxycholic acid and glycoursodeoxycholic acid significantly increased the biliary excretion of cholesterol, phospholipid, bilirubin and total Ca^{2+}. In contrast, the *N*-acetylglucosaminides significantly decreased in biliary bicarbonate concentration, whereas ursodeoxycholic acid significantly increased biliary bicarbonate concentration. However, tauroursodeoxycholic acid and glycoursodeoxycholic acid did not significantly change the biliary bicarbonate concentration. The results indicate that *N*-acetylglucosaminides have a choleretic effect in bile fistula rats. Our present study also demonstrates that *N*-acetylglucosaminides, but not ursodeoxycholic acid, tauroursodeoxycholic acid or glycoursodeoxycholic acid, can significantly reduce the biliary bicarbonate concentration. Furthermore, our findings suggest that ursodeoxycholate *N*-acetylglucosaminide may partly exert a choleretic effect via mechanisms different from those of the other bile acids.

Keywords: *N*-acetylglucosamine; Ursodeoxycholic acid; Glycoursodeoxycholic acid; Tauroursodeoxycholic acid; Bile flow; Biliary excretion; (Rat)

1. Introduction

It is generally established that ursodeoxycholic acid can improve clinical and biochemical indices in patients with primary biliary cirrhosis (Poupon et al., 1987, 1991; Leuschner et al., 1989; Oka et al., 1990) and some other cholestatic liver diseases (James, 1990).

Interestingly, a previous study showed that *N*-acetylglucosaminides, bile acids which are conjugated with *N*-acetylglucosamine, could be detected in urine at a very low concentration in healthy humans by gas chromatography–mass spectrometry (Marschall et al., 1988). The *N*-acetylglucosaminides are excreted in the urine of patients with cholestasis in large amounts as metabolites of ursodeoxycholic acid after oral administration of ursodeoxycholic acid. Therefore, it is suggested that *N*-acetylglucosaminidation is a metabolic pathway for 7β-hydroxylated bile acids such as ursodeoxycholic acid (Marschall et al., 1989, 1992, 1994a,b; Matern et al., 1990). Furthermore, a recent study suggested that *N*-acetylglucosaminides, which constitute 50% of the metabolites of ursodeoxycholic acid, are the major metabolites in the

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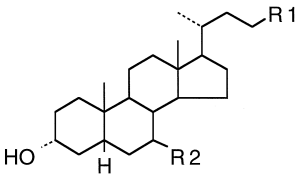
urine of healthy subjects given ursodeoxycholic acid (Marschall et al., 1994a). Kimura et al. (1994) and Kimura et al. (1995) measured the concentrations of *N*-acetylglucosaminides in the serum and urine of patients with various liver diseases during ursodeoxycholic acid treatment by high-performance liquid chromatography, and they suggested that *N*-acetylglucosaminidation was one of the major pathways of ursodeoxycholic acid metabolism in patients with primary biliary cirrhosis. These observations seem to indicate that *N*-acetylglucosaminides are major urinary metabolites of ursodeoxycholic acid in patients with cholestasis such as primary biliary cirrhosis. However, little is still known about the effects of *N*-acetylglucosaminide on bile flow and the biliary excretion of various markers. In the present study, therefore, we examined the effects of ursodeoxycholate *N*-acetylglucosaminide, tauroursodeoxycholate *N*-acetylglucosaminide and glycoursodeoxycholate *N*-acetylglucosaminide on the bile flow and biliary excretion of total bile acid, cholesterol, phospholipid, bilirubin and total Ca^{2+}, and biliary bicarbonate concentration and biliary bile acid composition in bile fistula rats in comparison with ursodeoxycholic acid, tauroursodeoxycholic acid and glycoursodeoxycholic acid.



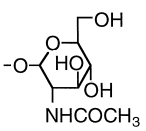
Bile acid	R1	R2
UDCA	-COOH	-OH
TUDCA	$-CONHCH_2CH_2SO_3H$	
GUDCA	$-CONHCH_2COOH$	
GlcNAc-UDCA	-COOH	
GlcNAc-TUDCA	$-CONHCH_2CH_2SO_3H$	
GlcNAc-GUDCA	$-CONHCH_2COOH$	

Fig. 1. Chemical structures of ursodeoxycholic acid (UDCA), tauroursodeoxycholic acid (TUDCA), glycoursodeoxycholic acid (GUDCA), ursodeoxycholate *N*-acetylglucosaminide (GlcNAc-UDCA), tauroursodeoxycholate *N*-acetylglucosaminide (GlcNAc-TUDCA) and glycoursodeoxycholate *N*-acetylglucosaminide (GlcNAc-GUDCA).

2. Materials and methods

2.1. Materials

Sodium ursodeoxycholate *N*-acetylglucosaminide, sodium tauroursodeoxycholate *N*-acetylglucosaminide, sodium glycoursodeoxycholate *N*-acetylglucosaminide, ursodeoxycholic acid, sodium tauroursodeoxycholate and sodium glycoursodeoxycholate were prepared by our laboratory. These compounds were more than 98% pure on high-performance liquid chromatography.

2.2. Experimental protocol

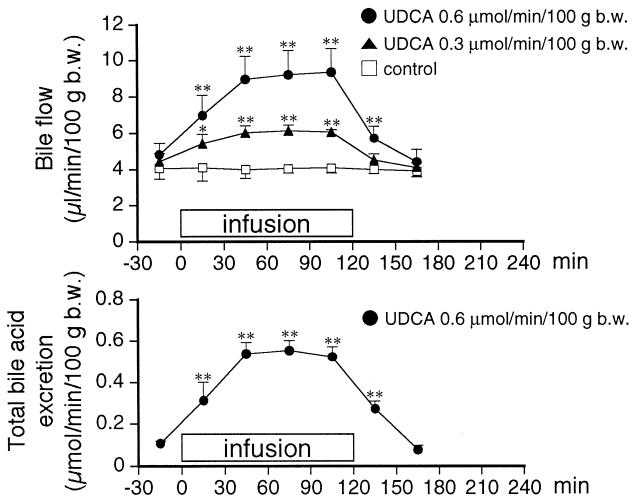
Male Wistar rats (Japan SLC, Hamamatsu, Japan) weighing between 260 and 320 g were used. Sodium ursodeoxycholate *N*-acetylglucosaminide, sodium tauroursodeoxycholate *N*-acetylglucosaminide, sodium glycoursodeoxycholate *N*-acetylglucosaminide, sodium tauroursodeoxycholate and sodium glycoursodeoxycholate were dissolved in saline. Ursodeoxycholic acid was dissolved in 0.5 N NaOH and adjusted to pH 8.3 with 0.5 N HCl. Rats were anesthetized by intraperitoneal injection of urethane (1 g/kg), and the common bile duct and the femoral vein were cannulated with polyethylene SP 28 tubes (Natume, Tokyo, Japan) after laparotomy. Thirty minutes after bile duct cannulation, bile was collected in preweighed tubes every 30 min to examine the change in bile flow, biliary excretion of total bile acid, cholesterol, phospholipid, bilirubin and total Ca^{2+}, biliary bicarbonate concentration and biliary bile acid composition. Thirty minutes after the start of bile collection, ursodeoxycholate *N*-acetylglucosaminide, tauroursodeoxycholate *N*-acetylglucosaminide, glycoursodeoxycholate *N*-acetylglucosaminide, ursodeoxycholic acid, tauroursodeoxycholic acid, glycoursodeoxycholic acid or saline (control) was infused in the femoral vein for 2 h at a rate of 2 ml/h. Each bile acid was infused at a constant rate of 0.3 or 0.6 μmol/min/100 g b.w. The chemical structures of ursodeoxycholic acid, tauroursodeoxycholic acid, glycoursodeoxycholic acid, ursodeoxycholate *N*-acetylglucosaminide, tauroursodeoxycholate *N*-acetylglucosaminide and glycoursodeoxycholate *N*-ace-tylglucosaminide are shown in Fig. 1.

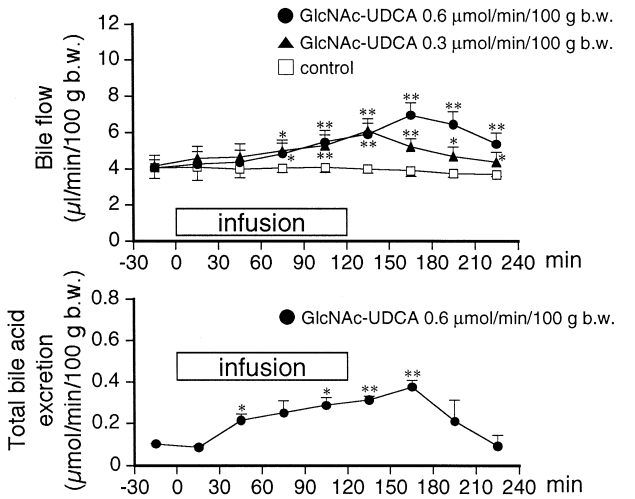
2.3. Analytical methods

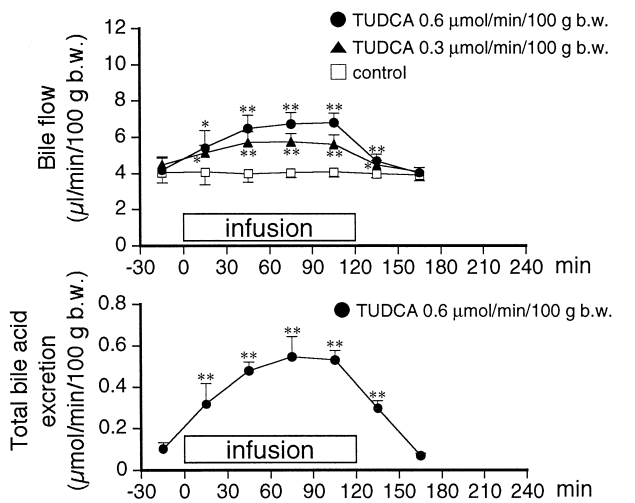
Biliary concentrations of cholesterol, phospholipid, bilirubin, total Ca^{2+} and total bile acid, except for

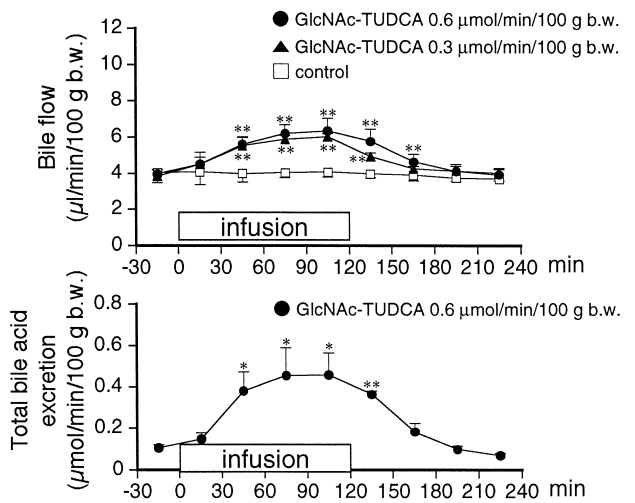
Fig. 2. Effects of ursodeoxycholic acid (UDCA), tauroursodeoxycholic acid (TUDCA), glycoursodeoxycholic acid (GUDCA), ursodeoxycholate *N*-acetylglucosaminide (GlcNAc-UDCA), tauroursodeoxycholate *N*-acetylglucosaminide (GlcNAc-TUDCA) and glycoursodeoxycholate *N*-acetylglucosaminide (GlcNAc-GUDCA) infusion on bile flow and biliary total bile acid excretion in bile fistula rats. Bile acids were infused at a constant rate of 0.3 or 0.6 μmol/min/100 g b.w. for 2 h. Values are means ± S.D. from three to seven rats. $*\ P < 0.05$, $**\ P < 0.01$ vs. control in bile flow (Dunnett's multiple comparison test). $*\ P < 0.05$, $**\ P < 0.01$ vs. −30–0 min in bile acid (paired *t*-test).

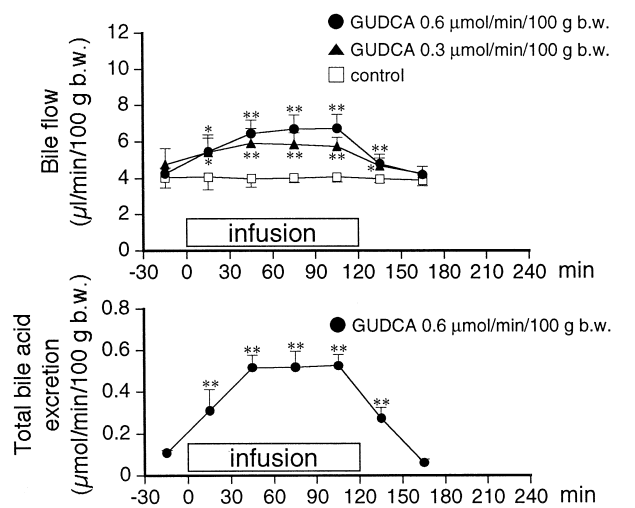
N-acetylglucosaminide, were all measured with a Hitachi automatic analyzer (Hitachi, Tokyo, Japan). The biliary concentration of *N*-acetylglucosaminide was measured by high-performance liquid chromatography (Niwa et al., 1992). The biliary bicarbonate concentration was measured by Acid Base Laboratory ABL300 (Radiometer, Tokyo,

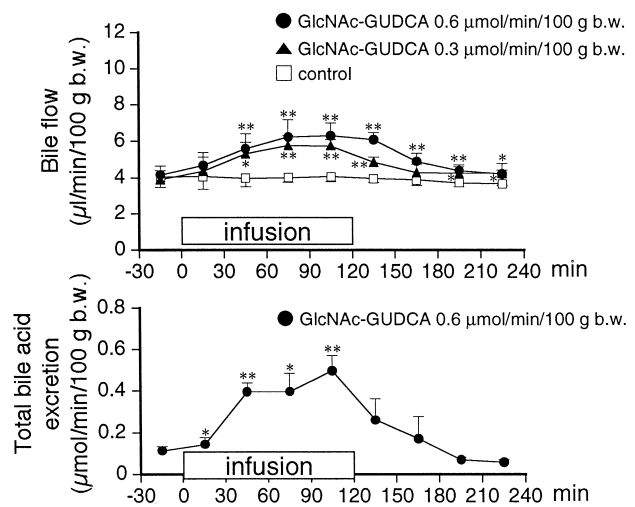












Japan). The biliary bile acid composition was analyzed by high-performance liquid chromatography (Sakakura et al., 1993).

2.4. Statistical analysis

Data are expressed as means ± S.D. The effects of drugs on bile flow and biliary excretion of total bile acid, cholesterol, phospholipid, bilirubin and total Ca^{2+}, and biliary bicarbonate concentration were analyzed by a repeated measures analysis of variance (ANOVA) for statistical comparison. When ANOVA showed a statistical difference, the effects of each drug on bile flow and biliary bicarbonate concentration were compared with the effects of control, using a general linear models procedure for ANOVA or Dunnett's multiple comparison test, and other parameters were assessed using a paired *t*-test for comparison with pre-value. Regression analysis was performed to reveal any correlations between bile acid excretion rate and bile flow. Data were regarded as significant at a *P*-value of < 0.05.

3. Results

3.1. Bile flow and total bile acid excretion

As shown in Fig. 2, all bile acids examined increased bile flow in a dose-dependent manner in bile fistula rats. Furthermore, these bile acids at a higher dose markedly increased biliary bile acid excretion. The maximal increase in bile flow at a higher dose was greatest with ursodeoxycholic acid (9.38 ± 1.31 μl/min/100 g b.w., $P < 0.01$), followed by ursodeoxycholate *N*-acetylglucosaminide (6.96 ± 0.68 μl/min/100 g b.w., $P < 0.01$), tauroursodeoxycholic acid (6.81 ± 0.54 μl/min/100 g b.w., $P < 0.01$), glycoursodeoxycholic acid (6.74 ± 0.79 μl/min/100 g b.w., $P < 0.01$), tauroursodeoxycholate *N*-acetylglucosaminide (6.36 ± 0.69 μl/min/100 g b.w., $P < 0.01$) and glycoursodeoxycholate *N*-acetylglucosaminide (6.32 ± 0.71 μl/min/100 g b.w., $P < 0.01$). The increase in bile flow after treatment with tauroursodeoxycholate *N*-acetylglucosaminide or glycoursodeoxycholate *N*-acetylglucosaminide was slower than that after treatment with ursodeoxycholic acid, tauroursodeoxycholic acid or glycoursodeoxycholic acid. The increase in bile flow after ursodeoxycholate *N*-acetylglucosaminide treatment was much slower than that after treatment with tauroursodeoxycholate *N*-acetylglucosaminide and glycoursodeoxycholate *N*-acetylglucosaminide. Also, ursodeoxycholate *N*-acetylglucosaminide increased bile flow maximally at 30–60 min after the end of infusion. In contrast, the other five bile acids had a maximal effect during the final 30 min of infusion.

The maximal rate of total bile acid excretion was greatest with ursodeoxycholic acid (0.554 ± 0.050 μmol/min/100 g b.w., $P < 0.01$), followed by tauroursodeoxycholic acid (0.548 ± 0.098 μmol/min/100 g b.w., $P < 0.01$), glycoursodeoxycholic acid (0.529 ± 0.053 μmol/min/100 g b.w., $P < 0.01$), glycoursodeoxycholate *N*-acetylglucosaminide (0.499 ± 0.074 μmol/min/100 g b.w., $P < 0.01$), tauroursodeoxycholate *N*-acetylglucosaminide (0.459 ± 0.106 μmol/min/100 g b.w., $P < 0.05$) and ursodeoxycholate *N*-acetylglucosaminide (0.377 ± 0.033 μmol/min/100 g b.w., $P < 0.01$).

The relationships between total bile acid excretion and bile flow are shown in Fig. 3. The slope of the regression line, indicating apparent choleretic activity (Takikawa et al., 1992), was highest for ursodeoxycholic acid, followed by tauroursodeoxycholate *N*-acetylglucosaminide, ursodeoxycholate *N*-acetylglucosaminide, tauroursodeoxycholic acid, glycoursodeoxycholic acid and glycoursodeoxycholate *N*-acetylglucosaminide. The r^2 was highest for tauroursodeoxycholate *N*-acetylglucosaminide (0.92), followed by ursodeoxycholic acid (0.84), glycoursodeoxycholic acid (0.78), glycoursodeoxycholate *N*-acetylglucosaminide (0.78) and tauroursodeoxycholic acid (0.75). However, the r^2 (0.39) for ursodeoxycholate *N*-ace-

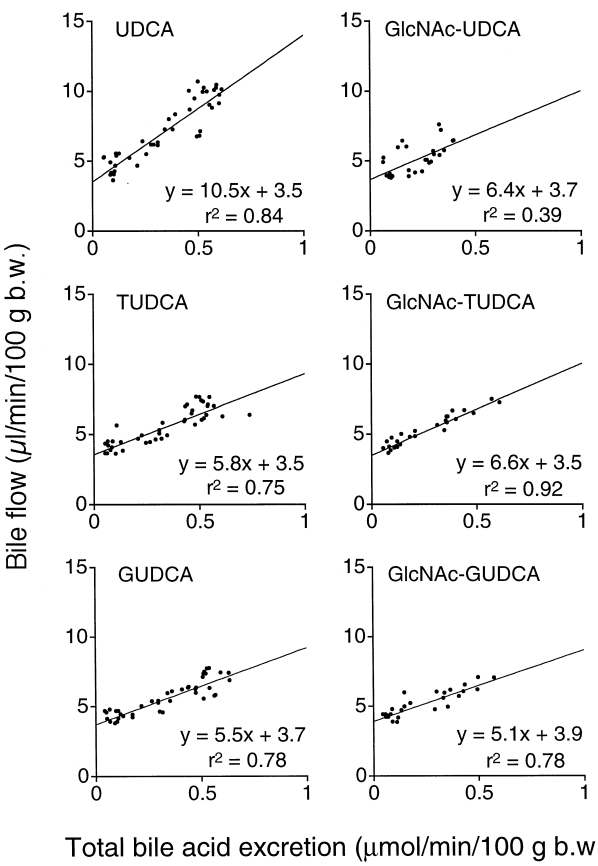


Fig. 3. Relationship between total bile acid excretion and bile flow after infusion of ursodeoxycholic acid (UDCA), tauroursodeoxycholic acid (TUDCA), glycoursodeoxycholic acid (GUDCA), ursodeoxycholate *N*-acetylglucosaminide (GlcNAc-UDCA), tauroursodeoxycholate *N*-acetylglucosaminide (GlcNAc-TUDCA) and glycoursodeoxycholate *N*-acetylglucosaminide (GlcNAc-GUDCA) in three to six rats.

tylglucosaminide was lower than that for the other five bile acids.

3.2. Biliary bile acid composition

Ursodeoxycholic acid was mostly excreted into bile in bile fistula rats as a conjugate with taurine. In contrast, most of the ursodeoxycholate *N*-acetylglucosaminide was excreted in an unchanged form. Tauroursodeoxycholate *N*-acetylglucosaminide, glycoursodeoxycholate *N*-acetylglucosaminide, tauroursodeoxycholic acid and glycoursodeoxycholic acid were excreted in an unchanged form (data not shown). In addition, the rats treated with saline showed no changes in biliary bile acid composition throughout the experiments. After treatment of normal rats with ursodeoxycholic acid, tauroursodeoxycholic acid, glycoursodeoxycholic acid, ursodeoxycholate *N*-acetylglucosaminide, tauroursodeoxycholate *N*-acetylglucosaminide and glycoursodeoxycholate *N*-acetylglucosaminide the bile content of these bile acids was 64.4%, 72.5%, 73.2%, 64.8%, 78.5% and 72.0%, respectively. The percentages of ursodeoxycholic acid, tauroursodeoxycholic acid and glycoursodeoxycholic acid were similar to those of their respective *N*-acetylglucosaminides (data not shown).

3.3. Biliary excretion of cholesterol, phospholipid, bilirubin and total Ca^{2+}

As shown in Table 1, all six bile acids significantly increased the biliary excretion of cholesterol and phospholipid in bile fistula rats. The maximal rate of biliary cholesterol excretion was greatest with ursodeoxycholate *N*-acetylglucosaminide (1.29 ± 0.19 μg/min/100 g b.w., $P < 0.01$), followed by ursodeoxycholic acid (1.23 ± 0.45 μg/min/100 g b.w., $P < 0.01$), glycoursodeoxycholic acid (1.20 ± 0.26 μg/min/100 g b.w., $P < 0.01$), tauroursodeoxycholic acid (1.05 ± 0.20 μg/min/100 g b.w., $P < 0.01$), glycoursodeoxycholate *N*-acetylglucosaminide

Table 1
Effects of ursodeoxycholic acid (UDCA), tauroursodeoxycholic acid (TUDCA), glycoursodeoxycholic acid (GUDCA), ursodeoxycholate *N*-acetylglucosaminide (GlcNAc-UDCA), tauroursodeoxycholate *N*-acetylglucosaminide (GlcNAc-TUDCA) and glycoursodeoxycholate *N*-acetylglucosaminide (GlcNAc-GUDCA) on biliary excretion of cholesterol, phospholipid, bilirubin and total Ca^{2+} in bile fistula rats

	Min								
	−30–0	0–30	30–60	60–90	90–120	120–150	150–180	180–210	210–240
Cholesterol excretion (μg / min / 100 g b.w.)									
UDCA	0.49 ± 0.13	0.86 ± 0.27^{b}	1.21 ± 0.45^{b}	1.21 ± 0.42^{b}	1.23 ± 0.45^{b}	0.94 ± 0.26^{b}	0.70 ± 0.14^{b}		
TUDCA	0.47 ± 0.10	0.68 ± 0.20^{b}	0.95 ± 0.20^{b}	1.00 ± 0.20^{b}	1.05 ± 0.20^{b}	0.79 ± 0.18^{b}	0.57 ± 0.14^{a}		
GUDCA	0.49 ± 0.16	0.71 ± 0.17^{b}	1.11 ± 0.23^{b}	1.18 ± 0.24^{b}	1.20 ± 0.26^{b}	0.88 ± 0.24^{b}	0.61 ± 0.21^{b}		
GlcNAc-UDCA	0.55 ± 0.06	0.56 ± 0.08	0.84 ± 0.10^{b}	1.01 ± 0.12^{b}	1.11 ± 0.18^{b}	1.17 ± 0.17^{b}	1.29 ± 0.19^{b}	1.04 ± 0.26^{b}	0.72 ± 0.16
GlcNAc-TUDCA	0.52 ± 0.05	0.59 ± 0.06^{b}	0.79 ± 0.07^{b}	0.86 ± 0.05^{b}	0.87 ± 0.07^{b}	0.79 ± 0.09^{b}	0.70 ± 0.08^{b}	0.64 ± 0.07^{a}	0.53 ± 0.05
GlcNAc-GUDCA	0.58 ± 0.10	0.67 ± 0.12^{b}	0.89 ± 0.17^{b}	0.96 ± 0.16^{b}	0.93 ± 0.15^{b}	0.95 ± 0.13^{b}	0.81 ± 0.10^{b}	0.64 ± 0.13	0.52 ± 0.11
Phospholipid excretion (μg / min / 100 g b.w.)									
UDCA	11.8 ± 2.2	21.7 ± 5.2^{b}	32.1 ± 7.6^{b}	32.4 ± 7.0^{b}	32.9 ± 7.4^{b}	24.9 ± 3.5^{b}	16.3 ± 1.8^{a}		
TUDCA	12.1 ± 1.7	17.5 ± 3.9^{b}	24.9 ± 4.1^{b}	26.3 ± 3.4^{b}	27.2 ± 4.0^{b}	19.9 ± 2.8^{b}	12.6 ± 2.1		
GUDCA	11.4 ± 2.3	17.0 ± 1.9^{b}	27.6 ± 1.7^{b}	29.9 ± 2.3^{b}	30.5 ± 2.9^{b}	21.1 ± 2.7^{b}	11.8 ± 2.6		
GlcNAc-UDCA	13.0 ± 1.5	11.6 ± 1.4^{a}	15.2 ± 1.6^{a}	17.9 ± 1.7^{b}	19.4 ± 2.8^{b}	19.6 ± 2.5^{b}	22.0 ± 2.9^{b}	17.4 ± 4.6^{a}	10.9 ± 2.3
GlcNAc-TUDCA	12.0 ± 0.9	11.7 ± 1.0	16.2 ± 1.4^{b}	17.3 ± 1.5^{b}	16.9 ± 1.6^{b}	15.2 ± 2.0^{a}	12.6 ± 2.2	10.1 ± 1.6^{a}	8.2 ± 0.9^{b}
GlcNAc-GUDCA	13.6 ± 2.7	13.2 ± 2.2	16.6 ± 2.6^{b}	17.8 ± 2.6^{b}	17.3 ± 2.5^{b}	18.6 ± 2.1^{b}	14.0 ± 1.8	10.2 ± 2.0^{a}	8.0 ± 1.5^{b}
Bilirubin excretion (μg / min / 100 g b.w.)									
UDCA	0.25 ± 0.03	0.31 ± 0.04^{b}	0.32 ± 0.07^{a}	0.43 ± 0.19^{a}	0.56 ± 0.33	0.56 ± 0.36	0.70 ± 0.50		
TUDCA	0.20 ± 0.01	0.27 ± 0.04^{b}	0.31 ± 0.09^{a}	0.32 ± 0.09^{a}	0.32 ± 0.09^{a}	0.27 ± 0.08	0.27 ± 0.08		
GUDCA	0.23 ± 0.03	0.27 ± 0.04^{b}	0.29 ± 0.03^{b}	0.30 ± 0.04^{b}	0.30 ± 0.04^{b}	0.28 ± 0.05^{a}	0.29 ± 0.06^{a}		
GlcNAc-UDCA	0.21 ± 0.03	0.22 ± 0.04	0.22 ± 0.03	0.30 ± 0.06^{b}	0.35 ± 0.07^{b}	0.39 ± 0.07^{b}	0.49 ± 0.11^{b}	0.40 ± 0.11^{b}	0.29 ± 0.07^{b}
GlcNAc-TUDCA	0.24 ± 0.04	0.27 ± 0.05^{a}	0.30 ± 0.05^{a}	0.33 ± 0.06^{a}	0.33 ± 0.06^{a}	0.36 ± 0.07^{a}	0.36 ± 0.06^{b}	0.39 ± 0.05^{b}	0.42 ± 0.06^{b}
GlcNAc-GUDCA	0.23 ± 0.04	0.26 ± 0.05^{b}	0.28 ± 0.05^{b}	0.31 ± 0.05^{b}	0.31 ± 0.05^{b}	0.32 ± 0.04^{b}	0.28 ± 0.04^{b}	0.26 ± 0.04	0.25 ± 0.04
Total Ca^{2+} excretion (μg / min / 100 g b.w.)									
UDCA	0.38 ± 0.04	0.68 ± 0.12^{b}	1.08 ± 0.13^{b}	1.11 ± 0.15^{b}	1.09 ± 0.16^{b}	0.60 ± 0.12^{b}	0.32 ± 0.05		
TUDCA	0.35 ± 0.05	0.56 ± 0.09^{b}	0.90 ± 0.14^{b}	0.93 ± 0.14^{b}	0.94 ± 0.17^{b}	0.48 ± 0.05^{a}	0.29 ± 0.03^{a}		
GUDCA	0.36 ± 0.02	0.67 ± 0.09^{b}	1.16 ± 0.08^{b}	1.19 ± 0.11^{b}	1.17 ± 0.06^{b}	0.55 ± 0.03^{b}	0.30 ± 0.01^{b}		
GlcNAc-UDCA	0.36 ± 0.03	0.37 ± 0.03	0.46 ± 0.03^{b}	0.54 ± 0.04^{b}	0.59 ± 0.05^{b}	0.62 ± 0.04^{b}	0.70 ± 0.04^{b}	0.53 ± 0.15^{a}	0.37 ± 0.07
GlcNAc-TUDCA	0.35 ± 0.03	0.38 ± 0.03^{a}	0.54 ± 0.04^{b}	0.58 ± 0.05^{b}	0.56 ± 0.06^{b}	0.50 ± 0.06^{b}	0.36 ± 0.05	0.29 ± 0.03^{b}	0.25 ± 0.02^{b}
GlcNAc-GUDCA	0.38 ± 0.03	0.46 ± 0.06^{b}	0.70 ± 0.06^{b}	0.75 ± 0.06^{b}	0.73 ± 0.05^{b}	0.66 ± 0.10^{b}	0.39 ± 0.07	0.30 ± 0.04^{a}	0.27 ± 0.03^{b}

Values are means ± S.D. from six to seven rats.
[a] $P < 0.05$ and [b] $P < 0.01$ vs. −30–0 min (paired *t*–test).

(0.96 ± 0.16 μg/min/100 g b.w., $P < 0.01$) and tauroursodeoxycholate *N*-acetylglucosaminide (0.87 ± 0.07 μg/min/100 g b.w., $P < 0.01$). In contrast, the maximal rate of biliary phospholipid excretion was greatest with ursodeoxycholic acid (32.9 ± 7.4 μg/min/100 g b.w., $P < 0.01$), followed by glycoursodeoxycholic acid (30.5 ± 2.9 μg/min/100 g b.w., $P < 0.01$), tauroursodeoxycholic acid (27.2 ± 4.0 μg/min/100 g b.w., $P < 0.01$), ursodeoxycholate *N*-acetylglucosaminide (22.0 ± 2.9 μg/min/100 g b.w., $P < 0.01$), glycoursodeoxycholate *N*-acetylglucosaminide (18.6 ± 2.1 μg/min/100 g b.w., $P < 0.01$) and tauroursodeoxycholate *N*-acetylglucosaminide (17.3 ± 1.5 μg/min/100 g b.w., $P < 0.01$). Biliary bilirubin and total Ca^{2+} excretion were significantly increased by all six bile acids. The maximal rate of biliary Ca^{2+} excretion was greatest with glycoursodeoxycholic acid (1.19 ± 0.11 μg/min/100 g b.w., $P < 0.01$), followed by ursodeoxycholic acid (1.11 ± 0.15 μg/min/100 g b.w., $P < 0.01$), tauroursodeoxycholic acid (0.94 ± 0.17 μg/min/100 g b.w., $P < 0.01$), glycoursodeoxycholate *N*-acetylglucosaminide (0.75 ± 0.06 μg/min/100 g b.w., $P < 0.01$), ursodeoxycholate *N*-acetylglucosaminide (0.70 ± 0.04 μg/min/100 g b.w., $P < 0.01$) and tauroursodeoxycholate *N*-acetylglucosaminide (0.58 ± 0.05 μg/min/100 g b.w., $P < 0.01$).

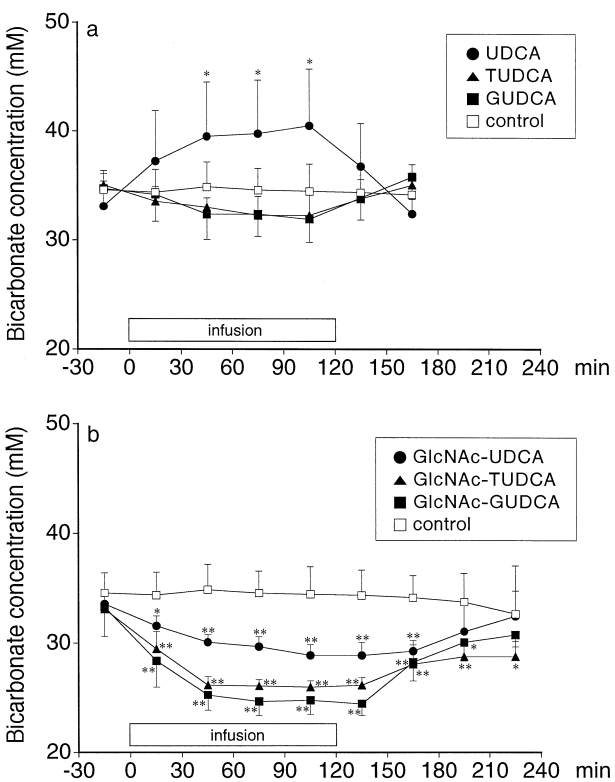


Fig. 4. Effects of ursodeoxycholic acid (UDCA), tauroursodeoxycholic acid (TUDCA), glycoursodeoxycholic acid (GUDCA), ursodeoxycholate *N*-acetylglucosaminide (GlcNAc-UDCA), tauroursodeoxycholate *N*-acetylglucosaminide (GlcNAc-TUDCA) and glycoursodeoxycholate *N*-acetylglucosaminide (GlcNAc-GUDCA) on biliary bicarbonate concentration in bile fistula rats. (a) UDCA (closed circle), TUDCA (closed triangle), GUDCA (closed square) and control (open square), (b) GlcNAc-UDCA (closed circle), GlcNAc-TUDCA (closed triangle), GlcNAc-GUDCA (closed square) and control (open square). Bile acids were infused at a constant rate of 0.6 μmol/min/100 g b.w. for 2 h. Values are means ± S.D. from six to seven rats. * $P < 0.05$, ** $P < 0.01$ vs. control (Dunnett's multiple comparison test).

3.4. Biliary bicarbonate concentration

As shown in Fig. 4, ursodeoxycholic acid markedly increased the biliary bicarbonate concentration in bile fistula rats, whereas tauroursodeoxycholic acid and glycoursodeoxycholic acid did not significantly change the biliary bicarbonate concentration throughout the experiment. In contrast, the three *N*-acetylglucosaminides significantly reduced the biliary bicarbonate concentration in bile fistula rats.

4. Discussion

Ursodeoxycholic acid is widely used for the treatment of liver dysfunction in patients with primary biliary cirrhosis (Leuschner et al., 1989; Poupon et al., 1991) and acute and chronic intrahepatic cholestatic disorders (Ullrich et al., 1987; Ctting et al., 1990; Nakagawa et al., 1990; Lindor and Burnes, 1991; O'Brien et al., 1991). The beneficial effect of ursodeoxycholic acid is attributed to its powerful choleretic action. In recent years, considerable attention has been focused on the metabolism of bile acids in connection with hepatobiliary diseases, because *N*-acetylglucosaminide, a novel conjugate, has been detected in human urine (Kimura et al., 1994, 1995). To our knowledge, however, there is no report on the biochemical and physiological importance of *N*-acetylglucosaminide. Therefore, the main aim of this study was to evaluate the biochemical effect of three bile acids conjugated with *N*-acetylglucosamine (ursodeoxycholate *N*-acetylglucosaminide, tauroursodeoxycholate *N*-acetylglucosaminide and glycoursodeoxycholate *N*-acetylglucosaminide) in comparison with that of ursodeoxycholic acid, tauroursodeoxycholic acid and glycoursodeoxycholic acid.

The present study showed that ursodeoxycholate *N*-acetylglucosaminide, tauroursodeoxycholate *N*-acetylglucosaminide and glycoursodeoxycholate *N*-acetylglucosaminide significantly increased bile flow and biliary total bile acid excretion in bile fistula rats. The effect of *N*-acetylglucosaminides on bile flow was less pronounced than that of ursodeoxycholic acid, tauroursodeoxycholic acid and glycoursodeoxycholic acid. However, ursodeoxycholate *N*-acetylglucosaminide showed a long-lasting effect after its infusion on bile flow in bile fistula rats, as compared with that of the other five bile acids. Furthermore, the r^2 between bile flow and biliary total bile acid excretion for ursodeoxycholate *N*-acetylglucosaminide (0.39) was lower than that for the other bile acids (ursodeoxycholic acid; 0.84, tauroursodeoxycholic acid; 0.75,

glycoursodeoxycholic acid; 0.78, tauroursodeoxycholate *N*-acetylglucosaminide; 0.92, glycoursodeoxycholate *N*-acetylglucosaminide; 0.78). These results suggest that the increase in bile flow seen with ursodeoxycholate *N*-acetylglucosaminide dose not directly depend on the increase in biliary total bile acid excretion, whereas the increase in bile flow elicited by the other five bile acids is closely related to the increase in biliary total bile acid excretion. The findings are, at least in part, consistent with previous reports, indicating a positive correlation between bile flow and bile acid excretion with ursodeoxycholic acid, glycoursodeoxycholic acid and tauroursodeoxycholic acid (Wheeler, 1975; Norman and Javitt, 1976; Forker, 1977). Therefore, our findings demonstrate that the choleretic effect of ursodeoxycholate *N*-acetylglucosaminide is partly exerted via mechanisms different from those of the other bile acids.

In the analysis of biliary bile acid composition, infused *N*-acetylglucosaminides were mostly excreted in an unchanged form into bile in bile fistula rats, as were tauroursodeoxycholic acid and glycoursodeoxycholic acid. In contrast, ursodeoxycholic acid was mostly excreted as a conjugate with taurine into bile in bile fistula rats. Furthermore, we observed that *N*-acetylglucosaminides were excreted gradually into bile in bile fistula rats, as compared with ursodeoxycholic acid, tauroursodeoxycholic acid and glycoursodeoxycholic acid (data not shown). The precise reason for this phenomenon is presently unclear. However, we speculate that bile acids conjugated with *N*-acetylglucosamine, which binds to R2 (7β-position), do not easily pass through the cell membrane in liver as compared to ursodeoxycholic acid, tauroursodeoxycholic acid and glycoursodeoxycholic acid, which are not conjugated with *N*-acetylglucosamine. In the present study, we did not measure the concentration of *N*-acetylglucosaminides in liver in bile fistula rats. Therefore, further studies should be performed to investigate the detailed mechanisms underlying our findings.

In agreement with previous studies (Hoffman et al., 1975; Poupon et al., 1976; Sama et al., 1982; Gurantz and Hofmann, 1984), ursodeoxycholic acid, tauroursodeoxycholic acid and glycoursodeoxycholic acid significantly increased biliary cholesterol, phospholipid, bilirubin and total Ca^{2+} excretion during each infusion. Also, *N*-acetylglucosaminides significantly increased biliary cholesterol and phospholipid excretion in bile fistula rats. Biliary bilirubin and total Ca^{2+} excretion were also increased in bile fistula rats by *N*-acetylglucosaminides. Of the three *N*-acetylglucosaminides, ursodeoxycholate *N*-acetylglucosaminide showed a long-lasting effect after its infusion on biliary cholesterol, phospholipid, bilirubin and total Ca^{2+} excretion in bile fistula rats. Tauroursodeoxycholate *N*-acetylglucosaminide also exhibited a long-lasting effect after its infusion on biliary bilirubin excretion in bile fistula rats, as compared with glycoursodeoxycholate *N*-acetylglucosaminide. In contrast, tauroursodeoxycholate *N*-acetylglucosaminide and glycoursodeoxycholate *N*-acetylglucosaminide mildly decreased biliary phospholipid and total Ca^{2+} excretion in bile fistula rats at a later stage after the infusion of each compound. The reason for this is difficult to explain. Therefore, further studies are required to clarify this phenomenon. However, the present study demonstrates that *N*-acetylglucosaminides can increase the biliary excretion of cholesterol, phospholipid, bilirubin and total Ca^{2+} in bile fistula rats, as can ursodeoxycholic acid, tauroursodeoxycholic acid and glycoursodeoxycholic acid. Furthermore, our findings suggest that ursodeoxycholate *N*-acetylglucosaminide has a longer-lasting effect after its infusion on biliary cholesterol, phospholipid and total Ca^{2+} excretion in bile fistula rats than do tauroursodeoxycholate *N*-acetylglucosaminide and glycoursodeoxycholate *N*-acetylglucosaminide.

We also observed that ursodeoxycholic acid significantly increased the biliary bicarbonate concentration in bile fistula rats during its infusion. This finding is consistent with previous reports (Kitani and Kanai, 1982). However, tauroursodeoxycholic acid and glycoursodeoxycholic acid did not significantly change the biliary bicarbonate concentration in bile fistula rats. In contrast, *N*-acetylglucosaminides significantly reduced the biliary bicarbonate concentration in bile fistula rats. Kitani and Kanai (1982) previously reported that the increase in bile flow produced by ursodeoxycholic acid is closely related to the increase in biliary bicarbonate concentration. Therefore, it is conceivable that bicarbonate may play a role in the regulation of bile flow. In the present study, however, tauroursodeoxycholic acid and glycoursodeoxycholic acid did not significantly change the biliary bicarbonate concentration in bile fistula rats, although both compounds significantly increased bile flow. These findings suggest that the increase in bile flow induced by tauroursodeoxycholic acid or glycoursodeoxycholic acid is mainly related to the increase in bile acid excretion. The precise mechanism for the decrease in biliary bicarbonate concentration induced by *N*-acetylglucosaminides is presently unclear. However, this finding seems to provide valuable information concerning the possible role of bicarbonate in the effect of *N*-acetylglucosaminides and bile acids that are not conjugated with *N*-acetylglucosamine.

In conclusion, the present study provides evidence that *N*-acetylglucosaminides as well as ursodeoxycholic acid, tauroursodeoxycholic acid and glycoursodeoxycholic acid have a choleretic effect. Our study also indicates that *N*-acetylglucosaminides, but not ursodeoxycholic acid, tauroursodeoxycholic acid and glycoursodeoxycholic acid, can cause a conspicuous reduction in biliary bicarbonate concentration. Furthermore, our findings suggest that ursodeoxycholate *N*-acetylglucosaminide exerts a choleretic effect via mechanisms different from those of the other five bile acids. Thus, the present study demonstrates for the first time the choleretic effect of *N*-acetylgluco-

saminides, although further studies are needed to investigate the clinical and physiological significance of these compounds.

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