Taurine Conjugate of Ursodeoxycholate Plays a Major Role in the Hepatoprotective Effect against Cholestasis Induced by Taurochenodeoxycholate in Rats

ウルソデオキシコール酸のタウリン抱合体はラットにおけるタウロケノデオキシコール酸起因性胆汁うっ滞に対し肝障害防止上の主役を演じる

Koko Tenkawa

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Abstract

Rats taurine-deprived through β-alanine administration and untreated rats were used to elucidate the mechanism of hepatoprotective effects of ursodeoxycholate (UDC). Animals were infused with taurochenodeoxy-cholate (TCDC, 0.4 μmol ·min^{-1}·100g^{-1}) alone or in combination with tauro-ursodeoxycholate (TUDC), or with UDC (both 0.6 μmol·min^{-1}·100g^{-1}) for 2 hours. Ursodeoxycholate as well as TUDC prevented severe cholestasis and liver damage induced by TCDC infusion in both untreated and taurine-deprived rat groups. In untreated rats, however, UDC was less effective in hepatoprotection than TUDC as indicated by sequential changes in biliary LDH output during the period of 30 to 120 min ($P<0.05$). In rats receiving UDC and TCDC, total biliary output of LDH for 2 hr was significantly higher in taurine-deprived rats than that in the control (73.40 ± 10.10 vs 41.14 ± 12.56: $P<0.05$), suggesting that the difference became greater upon taurine deprivation. In contrast, in rats receiving TUDC and TCDC, the protective effect was comparable for the taurine-deprived and untreated rats. When the animals were infused with UDC and TCDC, taurine-deprived rats exhibited a biliary excretion rate for TUDC half that of control rats ($P<0.05$). Furthermore, a highly significant correlation was observed be-tween the biliary excretion rate of TUDC and biliary output of LDH ($r=-0.886$, $P<0.0001$). These results suggest that UDC conjugates, especially TUDC, and not UDC may play a major role in the prevention of cholestasis and liver cell damage caused by TCDC infusion.

Introduction

Ursodeoxycholate (UDC) has recently been introduced to treat different acute or chronic cholestatic diseases, including primary biliary cirrhosis (1–5). However, the mechanism underlying the beneficial effect of this bile salt remains unresolved. On the other hand, it has been shown that taurochenodeoxycholate (TCDC), taurolithocholate (TLC), or even taurocholate (TC), the most common bile salt in human being, induces severe cholestasis and causes death in rats when infused with large dose (6–10). However, the coinfusion of tauroursodeoxycholate (TUDC) or UDC with TC (6–8), TCDC (7) or even with TLC(9,10), a more potent cholestatic bile salt, protects against the cholestasis induced by the infusion of these toxic bile salts. The mechanisms underlying this anticholestatic effect of TUDC (or UDC) have been controversial. The infusion of UDC was shown to be as effective as that of TUDC in an in vivo study (8). Moreover, a recent in vitro study using primary human hepatocytes has shown that UDC functions hepatoprotectively in itself without being biotransformed to its conjugates (11). However, the physiological property and intrahepatic biotransformation of UDC differ in many respects from those of TUDC (12–15). It remains still unclear whether UDC works directly in an unconjugated form or as its conjugates after amidation in the liver.

To clarify whether UDC in its unconjugated form is as protective against bile-salt-induced liver injury as is TUDC in vivo, we compared the hepatoprotective effects of UDC and TUDC in rats with intact and decreased

taurine pools. If UDC works after amidation, UDC infusion in taurine-deprived animals may be less efficient than TUDC infusion in preventing liver damage induced by toxic bile salts.

Materials and Methods

Materials.

TCDC was purchased from Sigma Chemical, St. Louis, MO. TUDC and ursodeoxycholic acid were generous gifts from Tokyo Tanabe (Tokyo, Japan). All of these bile salts were >99% pure. The major contaminants as examined by high-performance liquid chromatography (HPLC) were chenodeoxycholic acid and taurodeoxycholic acid for TCDC, ursodeoxycholic acid and chenodeoxycholic acid for TUDC and taurochenodeoxycholic acid for ursodeoxycholic acid preparation. None of these contaminants amounted to more than 0.6% of the total. All of these preparations were used without further purification. Ursodeoxycholic acid was converted to a sodium salt (UDC) in this laboratory as previously described (16). Three α-hydroxy steroid dehydrogenase was obtained from Worthington Biochemical Co. (Freehold, N.J., U.S.A.). β-Alanine was from Wako Junyaku (Tokyo, Japan). All of the chemical agents for measuring bile salt concentration and enzyme activities in the bile and plasma were of analytical grade.

In vivo studies.

Male Wistar rats (SLC, Shizuoka Jikken Doubutsu, Hamamatsu, Japan) were purchased at the age of 11 weeks and maintained for 2-3 weeks before use. Rats were given free access to food and water under controlled lighting (600-1800), humidity (55%), and temperature (23 ± 1 C°).

Taurine pool manipulation.

Taurine deprivation was induced according to the method reported by

Table 1. *Summary of experimental groups.*

Groups	Body wt, gm	ß-Alanine (3.6g/kg/day)	Infused bile salts, μmol·min^{-1}·100g^{-1} TCDC 0.4	TUDC 0.6	UDC 0.6
No treatment					
A (6)	316 ± 15.7	-	+		
B (3)	284 ± 5.8	-	+	+	
C (5)	317 ± 12.5	-	+		+
ß-Alanine treatment					
D (5)	319 ± 13.3	+	+		
E (4)	305 ± 8.5	+	+	+	
F (7)	311 ± 9.5	+	+		+

Numbers in parenthesis indicate the number of rats studied.

Shaffer and Kocsis (17). Beta-alanine (3.6g/kg body wt per day) in distilled water was administrated p.o. for two successive days before the bile salt infusion experiments. On the third day, the rats were used in an experiment. Under pentobarbital sodium anesthesia (4.5mg/100g body wt, Nembutal, Abbott, North Chicago, IL), the common bile duct was cannulated with PE-50 tubing (Clay-Adams, Parsippany, NJ) after an abdominal incision. The bile samples secreted during the first ten minutes shortly after the operation were discarded in order to minimize the influence on enzyme output caused by the operative manipulations. Then a 30-minute basal bile sample was collected in a tapered-bottom stock tube. Thereafter a bile salt solution was infused by a Harvard pump (model 9446, Harvard Apparatus, Mills, MA) through a jugular vein catheter. The infusate was made of bile salt(s) dissolved in 3% bovine serum albumin solution. The pH (7.4) and osmolality (300 mosm/kg) were adjusted to physiological levels, except for the pH of the UDC solution (8.3).

After a basal 30-min bile before infusion was obtained, TCDC at a rate of 0.4 μmol·min^{-1} ·100g^{-1} alone, or TCDC at the same rate with TUDC (or UDC) at a rate of 0.6 μmol·min^{-1}·100g^{-1} was infused iv. on the basis of the results of our previous studies. Six rat groups, that is, untreated groups A,B,C and taurine-pool-deprived groups D,E,F, received one of the three bile salt solutions (TCDC alone: A; D, TCDC+TUDC: B; E, and TCDC+UDC : C; F). The experimental groups are summarized in Table 1.

Bile salt infusions were continued for 2 hours, and four consecutive

30-min bile samples were collected during the infusion. Blood samples were also obtained at appropriate time intervals during the bile salt infusion and at the end of each experiment at 2 hr. The concentrations of bile salt and enzymes in bile and plasma samples were measured. The protocol of these experiments was approved by our institutional committee.

Analytical procedures.

The total bile salt concentration in the bile was determined by an enzymatic method. Individual bile salts in some bile samples were further analyzed by HPLC (Twincle, Nihonbunko) coupled with immobilized enzyme in a column (Bilepack II, Nihon-bunko) (7).

Taurine levels in the liver were measured in Mitsubishiyuka Bio-clinical Laboratories (Itabashi, Tokyo, Japan) with an amino-acid-auto-analyzer.

Enzyme assays.

The enzyme activities of lactate dehydrogenase (LDH) in bile and plasma, and alkaline phosphatase (ALP) in bile were determined by methods reported previously (7). The interactions of bile salts in the bile with the enzyme activities were appropriately corrected when necessary according to the bile salt concentration in the bile, as was previously reported (7).

Histological examination in the rat liver.

After a rat was infused with TCDC at a rate of 0.4 μmol $\cdot$min^{-1} $\cdot$100g^{-1}, the portal vein was cannulated and perfused with bufferred formaldehyde. Liver tissues were dehydrated with ethanol, embedded in JB-4 plastic

embedding medium, sectioned, subjected to hematoxylin–eosin stainning and observed with lightmicroscope.

Statistical analysis.

Effects of taurine deprivation and sequential changes of the data were analyzed by the repeated measurement that was one of analytical methods packed in a computer software SAS (18) through use of a personal computer (NEC9801–RA). The significances of p values for β–alanine treatment, time effects and interactive effects of the two components (β–Alanine x time) by Huynh and Feldt's method (19) are shown in another table. Scheffe's multiple comparison test was performed after ANOVA to compare any two sets of the values at corresponding time intervals. Wilcoxon's test was also used to compare values between only two groups. Values of p lower than 0.05 were considered to be significant.

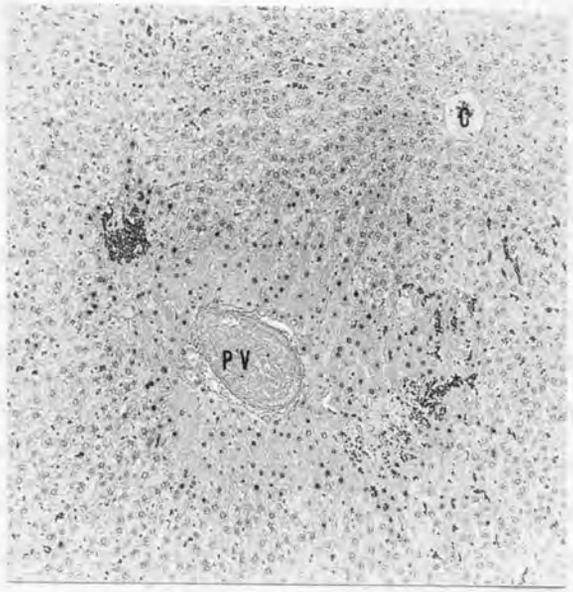

Fig.1. The light photomicrograph of rat liver tissue subjected to bile salt infusion (*i.e.*, TCDC at a rate of 0.4 μmol·min^{-1}·100g^{-1} for 120 min). Necrotic hepatocytes exhibiting pyknotic nuclei and karyorrhexis with no inflammation were observed in zone 1 tissue. The central zone is unaffected. **PV** = branch of portal vein. **C** = branch of central vein.(Original magnification x 950).

Results

TCDC alone infusion at a rate of 0.4 μmol·min^{-1} ·100g^{-1} for 120 min induced massive necrosis of hepatocytes around portal vein (PV) area (zone I) (Figure 1).

Taurine concentration in the liver in β-alanine-treated rats is one-fourth of that in untreated rats (0.9 ± 0.2 vs 3.6 ± 0.3 μmol/g liver, n=2). Basal bile flow rates in β-alanine-treated rats did not much differ from those in untreated rats when they received saline at a rate of 10 μl·min^{-1} ·100g^{-1}(Figure 2).

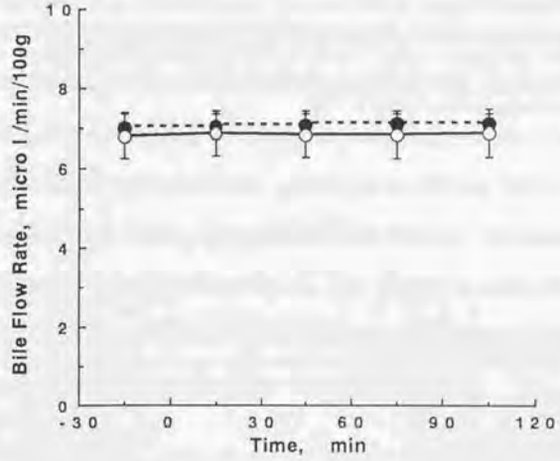

Fig. 2. Sequential changes in bile flow in rats infused with saline. Closed circles connected by broken line indicate the data in untreated rats (n=3). Open circles connected by solid line indicate the data in β-alanine-treated rats (n=3). All values are expressed as mean ± 1 SD. There is no significant difference between the untreated and β-alanine-treated rats.

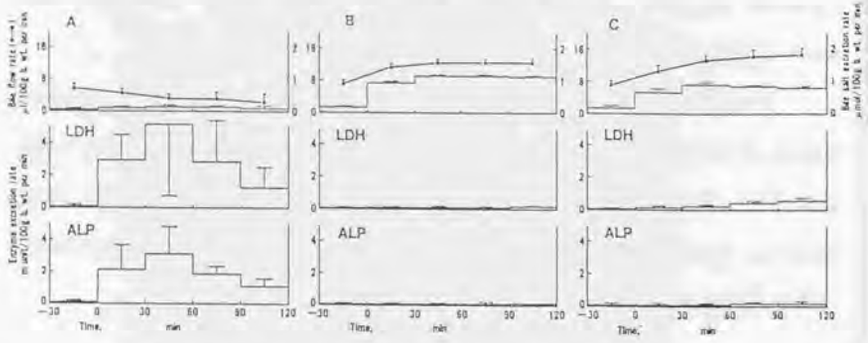

Fig. 3. Sequential changes in bile flow rates (closed circles) and excretion rates of bile salts (open columns, top panel) and enzyme activities in untreated rats infused with various bile salts. A:TCDC, 0.4 μmol·min^{-1}·100g^{-1} (n=6), B:TCDC, 0.4 μmol·min^{-1}·100g^{-1} + TUDC, 0.6 μmol·min^{-1} 100g^{-1} (n=3), C:TCDC, 0.4 μmol·min^{-1}·100g^{-1} + UDC, 0.6 μmol·min^{-1}· 100g^{-1} (n=5). All values are expressed as mean ± 1 SD.

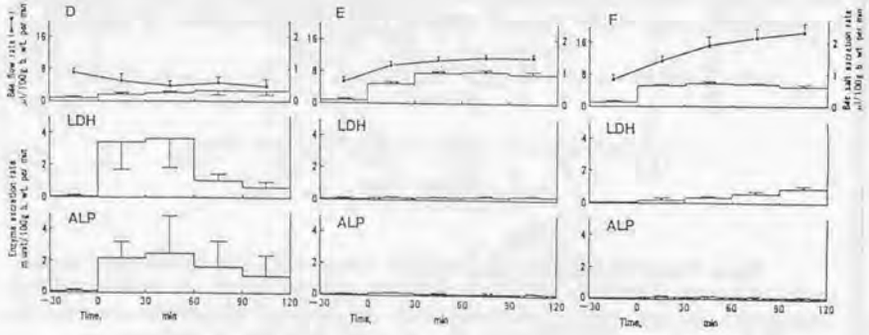

Fig. 4. Sequential changes in bile flow rates (closed circles) and biliary excretion rates of bile salts (open columns, top panel) and enzyme activities in β-alanine-treated rats infused with various bile salts. D:TCDC, 0.4 μmol·min^{-1}·100g^{-1} (n=5), E:TCDC, 0.4 μmol· min^{-1}·100g^{-1} + TUDC, 0.6 μmol·min^{-1}·100g^{-1} (n =3), F:TCDC, 0.4 μmol·min^{-1}·100g^{-1} + UDC, 0.6 μmol·min^{-1} ·100g^{-1} (n=7). All values are expressed as mean ± 1 SD.

Figures 3 and 4 show sequential changes in bile flow rate, bile salt excretion rate, and biliary outputs of LDH and ALP in untreated (Fig.3) and taurine pool-deprived rats (Fig.4). In untreated rats infused with TCDC alone (Fig.3A), the bile flow began to decrease immediately after the start of the infusion and continued to decline. However, in rats coinfused with TCDC and TUDC (Fig.3B) or UDC (Fig.3C), the bile flow began to increase after the infusion and remained at significantly higher rates compared with group A. The bile salt excretion rates in rats infused with TCDC alone (group A) increased only slightly compared with the basal levels before the infusion. In contrast, the excretion rates in the two coinfused groups (group B and C) increased rapidly after the start of the infusion and remained at a high plateau. In the rats infused with TCDC alone (group A), the LDH activity in the bile drastically increased, reaching a peak at 30 to 60 min which was 84 times higher than the basal level, and began to decline thereafter. In contrast, biliary LDH output during the period of 0 to 90 min was much lower in both groups B and C than that in group A. The excretion rates of ALP were also significantly lower in both group B and group C during the period of 0 to 90 min compared with corresponding values in group A.

P values in the "Time" component in Table 2 are all below 0.05 for all parameters. This indicates that any sequential pattern of these groups is significantly different from that of the other groups for all parameters. P values below 0.05 in the "Time x Treatment" component, which represents interactive effects of "Time" and "Treatment" components, indicate that the patterns of the sequential changes in untreated rat groups significantly differed from those in β-alanine-treated groups. The effects of β-alanine treatment were observed only in the TCDC+UDC coinfused group in three parameters, excluding the excretion rate of ALP.

Table 2. ***P values for analysis of variance in bile flow rate, bile salt excretion rate and biliary excretion rate of*** **LDH** ***and*** **ALP** ***in rat groups infused with various bile salts.***

Infused bile salts	Treatment	Time[a]	Time x Treatment[a]
Bile flow rate			
TCDC	0.1991	0.0155*	0.6359
TCDC + TUDC	0.0342*	0.0001**	0.0713
TCDC + UDC	0.0746	0.0001**	0.0025**
Bile Salt Excretion Rate			
TCDC	0.4287	0.0297*	0.6894
TCDC + TUDC	0.0015**	0.0001**	0.0703
TCDC + UDC	0.0001**	0.0001**	0.0090**
Excretion rate of LDH			
TCDC	0.0001**	0.3123	0.3126
TCDC + TUDC	0.6624	0.0007**	0.4775
TCDC + UDC	0.0373*	0.0001**	0.0005**
Excretion rate of ALP			
TCDC	0.2597	0.0001**	0.3377
TCDC + TUDC	0.5924	0.0001**	0.0728
TCDC + UDC	0.3066	0.0001**	0.0830

[a] P values for Time and Time x Treatment were calculated by Huynh and Feldt's method.
**P<0.01, *P<0.05

Table 3. *Comparison of bile flow rate, bile salt excretion rate, biliary excretion rate of LDH in the rats infused with TCDC + TUDC and TCDC + UDC.*

Group	Time interval, min				
	-30 to 0	0-30	30-60	60-90	90-120
Bile flow rates ($\mu l \cdot min^{-1} \cdot 100g^{-1}$)					
B(3)	7.63 ± 0.30	11.30 ± 0.43	12.02 ± 0.52	12.13 ± 0.78	11.82 ± 2.31
C(5)	7.57 ± 0.63	10.92 ± 1.13	13.27 ± 1.15	14.36 ± 2.16	14.45 ± 2.31
E(4)	5.87 ± 0.51	9.51 ± 0.63	10.65 ± 0.42	11.24 ± 0.44	11.45 ± 0.27
F(7)	6.74 ± 1.31	11.50 ± 1.45	15.12 ± 2.52[e]	17.21 ± 2.71[e]	18.27 ± 2.43[de]
Bile salt excretion rates ($nmol \cdot min^{-1} \cdot 100g^{-1}$)					
B(3)	199.70 ± 62.69	931.95 ± 79.49	1135.83 ± 42.12	1187.94 ± 42.12	1188.68 ± 32.84
C(5)	168.13 ± 36.10	675.42 ± 110.7[b]	907.15 ± 39.16[b]	869.09 ± 48.41[a]	833.09 ± 53.37[a]
E(4)	106.50 ± 14.48[b]	627.25 ± 142.8[a]	954.75 ± 43.87[a]	972.25 ± 36.41[a]	951.50 ± 49.71[a]
F(7)	133.86 ± 36.09	648.00 ± 43.94	735.43 ± 68.16[ce]	743.43 ± 35.79[ce]	682.14 ± 50.94[ce]
LDH excretion rates ($mIU \cdot min^{-1} \cdot 100g^{-1}$)					
B(3)	56.89 ± 21.75	87.15 ± 42.32	79.47 ± 27.07	129.35 ± 52.57	209.84 ± 105.0
C(5)	60.91 ± 44.11	162.58 ± 61.49	239.02 ± 80.88[b]	407.00 ± 138.8[b]	562.80 ± 171.1[b]
E(4)	29.00 ± 6.75	86.45 ± 31.62	118.18 ± 20.07	158.38 ± 45.91	222.55 ± 105.0
F(7)	27.34 ± 8.85	207.03 ± 86.89	360.54 ± 75.38[e]	546.19 ± 101.3[e]	829.97 ± 118.5[de]

Numbers in parenthesis indicate the number of rats studied.
[a]Significantly different from B(P<0.01), [b]Significantly different from B(P<0.05), [c]Significantly different from C(P<0.01), [d]Significantly different from C(P<0.05), [e]Significantly different from E(P<0.01).
f. B:untreated rats given the infusion of TCDC and TUDC, C:untreated rats given the infusion of TCDC and UDC
E:ß-alanine-treated rats given the infusion of TCDC and TUDC, F:ß-alanine-treated rats given the infusion of TCDC and UDC.

Table 3 shows the values of bile flow rate, bile salt excretion rate, and biliary output of LDH in each period in groups receiving TCDC+TUDC and TCDC+UDC with or without taurine pool manipulation in order to demonstrate more clearly the differences between the two groups receiving the mixed bile salts. The bile flow rates in group F were the highest of the 4 groups and were significantly higher than those in group E during the period of 30 to 120 min ($P<0.01$) and those in group C during the period of 90 to 120 min. The bile salt excretion rates at baseline period were significantly lower in group E than in group B ($P<0.05$). The bile salt excretion rates during the period of 30 to 120 min were significantly higher in the groups receiving TCDC+TUDC infusion than in those receiving TCDC+UDC infusion (group C vs group B, group E vs group F; $P<0.01$). Moreover, of the groups receiving the same infusion, the bile salt excretion rates were also higher in the untreated groups than in the β-alanine-treated groups during the same period (group B vs group E, group C vs group F; $P<0.01$). LDH excretion rates were significantly higher in group C than in group B ($P<0.01$), and also in group E than in group F, 30 min after infusion and thereafter. In addition, the values during the last 30-min period (90-120 min) were significantly higher in group F than in group C ($P<0.05$). In sum, these results showed a general tendency toward a greater protective effect in TUDC than in UDC against TCDC-induced liver damage. As for ALP excretion rates, there were no significant differences among the four groups (data not shown).

Figure 5 demonstrates the effect of β-alanine treatment on the 2-hr biliary output of LDH infused with TCDC (left panel), TCDC + TUDC (center panel) and TCDC + UDC (right panel). There were no significant differences between the intact and the β-alanine-treated groups infused with TCDC alone or with TCDC + TUDC, whereas the values were significantly higher in group F than in group C ($P<0.05$). The 2-hr biliary output of ALP showed no significant difference between the two treatments in all groups receiving the same bile salt(s) (data not shown).

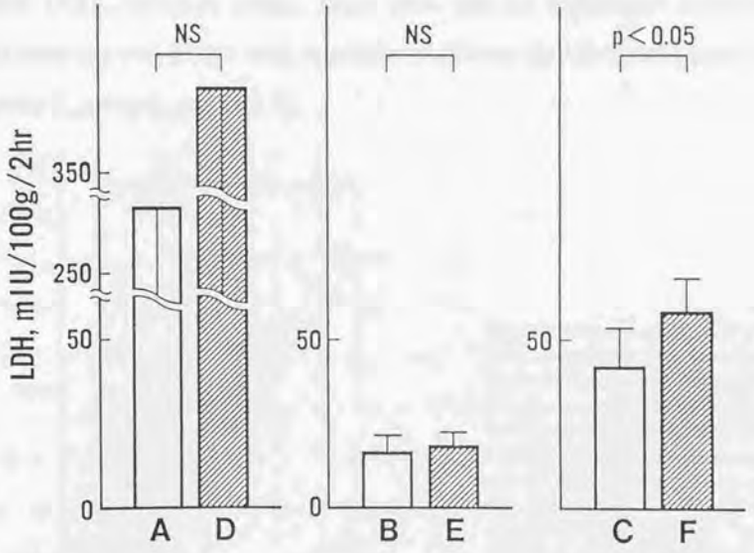

Fig. 5. Total biliary output of LDH for 2 hours in untreated rats (open columns) and in β-alanine-treated rats (hatched columns) infused with various bile salts. All values are expressed as mean ± 1 SD.

Figure 6 shows the sequential changes in LDH activities in plasma in different rat groups. Plasma LDH activities in groups infused with TCDC alone (A,D) increased sharply and became significantly higher than corresponding values in the other 4 groups with coinfusion at 45 min (and thereafter) ($P<0.01$). There were no significant differences between group A and group D. In contrast, in groups coinfused with TCDC and TUDC (or UDC), both with and without β-alanine pretreatment, LDH activity in plasma increased mildly (to about one-third to to one-half of the values in groups with TCDC infusion alone). There were also no significant differences between the two groups with or without β-alanine pretreatment (group B vs group E, group C vs group F).

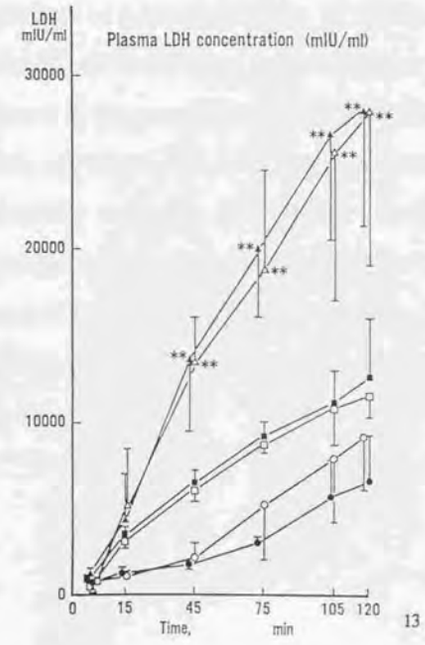

Fig.6. Sequential changes in plasma LDH activities in various rat groups. Symbols: ▲-▲,D; △-△,A; ■-■,F; □-□,C; ○-○, B; ●-●, E. Open symbols indicate the untreated groups. Closed symbols indicate the groups treated with β-alanine. Groups are expressed in Table 1. All values are expressed as mean ± 1SD. **p<0.01 compared with the groups receiving the same treatment and infused with the mixed infusate of TCDC and TUDC (or UDC).

Table 4. ***Biliary excretion of individual bile salts in the last 30 (90-120) min period.***

Infused bile salts, $\mu mol \cdot min^{-1} \cdot 100g^{-1}$	n	TUDC	GUDC	(TUDC+GUDC)	UDC	TC	TCDC
				$nmol \cdot min^{-1} \cdot 100g^{-1}$			
TCDC,0.4 + TUDC,0.6							
No treatment(B)	3	445 ± 68	ND	445 ± 68	ND	ND	352 ± 46
β-Alanine treatment(E)	4	590 ± 52[b]	ND	590 ± 52[a]	ND	ND	378 ± 22
TCDC,0.4 + UDC,0.6							
No treatment(C)	5	190 ± 57[a]	194 ± 31	384 ± 37	18 ± 9	ND	345 ± 17
β-Alanine treatment(F)	7	79 ± 22[cd]	238 ± 22[d]	306 ± 36[c]	25 ± 3[d]	ND	391 ± 63

n = numbers of rats studied
[a]Significantly different from B(P<0.01), [b]Significantly different from B(P<0.05), [c]Significantly different from E(P<0.01), [d]Significantly different from C(P<0.05). ND, not determined. Groups are expressed in Table 1.

Table 4 shows biliary excretion rates of TUDC, glycoursodeoxycholate (GUDC), UDC conjugates [TUDC+GUDC], UDC, TC, and TCDC in the period of 90–120 min in the 4 rat groups receiving TCDC+TUDC and TCDC+UDC, as examined by HPLC. In the groups receiving TCDC+TUDC infusion, major bile salts in this period consisted of infused TUDC and TCDC, and the excretion rates of TUDC were significantly higher in group E than in group B ($P<0.05$). In the groups receiving TCDC+UDC infusion, the major 3 bile salts consisted of TUDC and GUDC, which were converted from UDC, and infused TCDC. The rats infused with TCDC+UDC had a significantly lower excretion rate of TUDC than those infused with TCDC+TUDC regardless of taurine pool manipulation (group C vs group B, group F vs group E; $P<0.01$). In addition, the excretion rate of TUDC was almost 50 % lower in group F than in group C ($P<0.05$). GUDC was not found in the groups receiving TCDC+TUDC, and the excretion rate of this bile salt was significantly higher in group C than in group F ($P<0.05$). The summed excretion rate of UDC conjugates (TUDC+GUDC) was significantly higher in group F than in group C ($P<0.05$). In contrast, the excretion rate of UDC was significantly lower in group C than in group F. There was no significant difference regarding the excretion rate of TCDC among the 4 groups.

Figure 7 demonstrates highly significant negative correlations between the biliary output of LDH and the excretion rate of TUDC (upper panel) and UDC conjugates (TUDC and GUDC, lower panel). It is clear that LDH output became smaller as the excretion rate of UDC conjugates increased.

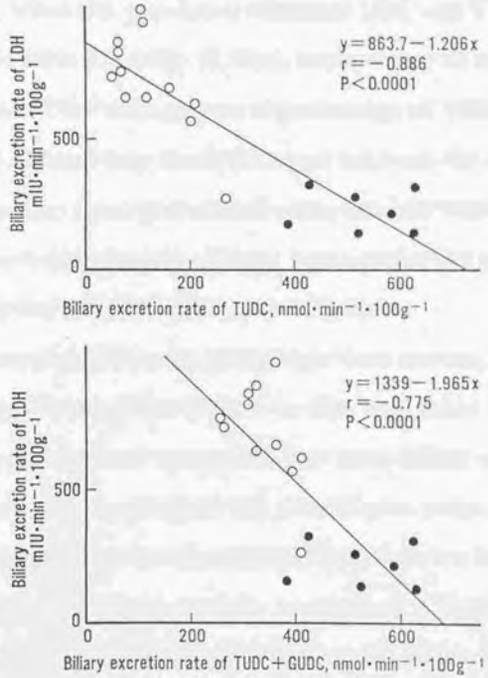

Fig. 7. Correlation between the biliary excretion rate of TUDC (upper panel) and conjugated UDCs (TUDC and GUDC) (lower panel) with the biliary excretion rate of LDH in the last 30 (90–120) min period. Open circles indicate the values receiving TCDC and UDC, closed circles indicate the values receiving TCDC and TUDC.

DISCUSSION

The present study has revealed that coinfused UDC prevented cholestasis and liver injury induced by TCDC infusion, both in untreated and β-alanine-pretreated rat groups (Figs.3, 4). As in the case of the coinfusion of TUDC, this observation is consistent with our preliminary observation (8). However, when the preventive effects of UDC and TUDC were carefully compared (Table 3 and Fig. 5), UDC was found to be significantly less efficient than TUDC with respect to prevention of biliary leakage of LDH (Table 3). Moreover, the differences between the effects of UDC and TUDC became more pronounced when animals were pretreated with β-alanine, revealing the less efficient hepatoprotective action of UDC compared with that of TUDC (Table 3 and Fig.5).

In our experiment the group sizes were uneven; that is, the sizes of group B (n=3) and E (n=4) were smaller than those of the other groups. However, the observed values (bile flow rates, biliary excretion rates of bile salts, LDH, ALP) in groups B and E were quite stable in comparison with respective values of the other groups. Therefore we think that the sizes of group B and E are large enough for comparisons with other groups.

Despite the significant differences in the biliary leakages of LDH, the values of plasma LDH activities in β-alanine-treated rats that received TCDC and TUDC (or UDC) were not significantly different from the values in their untreated counterparts (group E vs B, group F vs C). These results suggest that the mechanisms of release of LDH into bile and plasma

may differ in at least some respects. Our group has recently observed sporadic single, or groups of, necrotic hepatocytes in rats predominantly in zone 1 without obvious changes in tight junction when TCDC was infused at a rate of 0.6 μmol·min^{-1}·100g^{-1} for 60 min, and that there was a highly significant correlation between the number of necrotic hepatocytes and the biliary excretion rate of LDH (20). In this experiment, we also demonstrate massive necrotic hepatocytes around the portal vein when rats were infused with TCDC at a rate of 0.4 μmol·min^{-1}·100g^{-1} for 120 min. Therefore the biliary LDH output observed in our experiment may mainly reflect the necrosis of hepatocytes releasing LDH directly into the bile, even though some of the LDH molecules in bile may originate from plasma by means of the paracellular root.

Beta-alanine pretreatment has been shown to reduce the taurine pool in the liver of rats to about 20% of the control value (17). In our experiment, taurine concentration in the liver in β-alanine-treated rats was one fourth of that in untreated rats (See results). In addition, the manipulation of taurine deprivation itself did not affect basal bile flow rate (Figure 2). Among 4 groups receiving mixed bile salt infusions, bile salt excretion rates were significantly lower in the groups treated with β-alanine than in those receiving no treatment, 30 min after the start of the infusion (Table 3). Since the biliary LDH output is not greater in β-alanine-treated rats (E,F) than in untreated rats (B,C) during the preinfusion period, it is unlikely that β-alanine treatment directly caused liver damage, leading to the observed dif-

ferences between β-alanine-treated and untreated rat groups. Our preliminary study revealed no significant change, at least at the light microscopic level, caused by β-alanine treatment (data not shown), which supports this hypothesis. Furthermore, between the two groups receiving TCDC and TUDC infusion, the biliary output of LDH and ALP (data not shown) is comparable regardless of whether β-alanine treatment was administered. In addition, in TUDC coinfused groups, the biliary excretion of bile salts during the last 30-min period of the experiment was even greater in β-alanine-treated groups (Table 3). Although the higher values observed in this group are not explainable, these observations are compatible with the notion that β-alanine-pretreatment per se does not affect either hepatocyte damage or mechanisms affecting hepatic transport systems. Accordingly, it appears unlikely that a possible cytotoxicity of β-alanine or taurine depletion itself caused the difference in LDH outputs noted between the rats with and without the β-alanine-treatment. Rather, it is most likely that the observed differences in the outputs of biliary LDH between the two rat groups (with or without β-alanine-treatment) infused with TCDC and UDC (group C and group F) were due to the differences in the taurine pool immediately available for conjugation with UDC, leading to lesser formation of TUDC in taurine-deprived rats (F).

Biliary excretion rates of individual bile salts in the last one-quarter of 2 hr period in β-alanine-treated rats are also compatible with this hypothesis (Table 4). The differences in biliary LDH output between UDC and

TUDC became more pronounced under these conditions, strongly suggesting that UDC was less effective than TUDC before its amidation.

In in vitro studies, Miyazaki et al. have shown the cytotoxic effect of TCDC on isolated human hepatocytes (21). In addition, Koga reported that the damage to isolated hepatocytes induced by TCDC was prevented when these cells were incubated together with TUDC (22). In our experiment, the excretion rate of TCDC did not differ much between the groups with or without taurine depletion in both groups infused with the mixed bile salts (Table 4), whereas LDH output used as an index of hepatocytotoxicity differed considerably between these groups. These results suggest that a less toxic and probably hepatoprotective bile salt such as TUDC excreted into bile with TCDC may weaken the cytotoxic potency of TCDC at least at the level of the bile canaliculus. The results shown in Fig. 5 support this consideration. Since GUDC also protects against cholestasis and hepatocellular necrosis induced by more toxic bile salts in rats (23,24), it probably has hepatoprotective effects, as does TUDC.

A previous study has shown that UDC has a stronger choleretic effect under a state of taurine deprivation than in an intact state (13). Indeed, in the present study among the 4 groups that received the mixed bile salt, the highest bile flow rates and the lowest bile salts excretion rates observed in group F (Table 3) suggest that UDC may have a specific choleretic effect similar to that reported in the past study (13) even with the coinfusion of other bile salts in the rats treated with β-alanine. However, the high bile

flow rate per se did not lead to greater prevention of LDH output into the bile (group F) compared with rats infused with TCDC and TUDC (group E). Accordingly, we conclude that conjugated UDC molecules, especially TUDC, play a major role in hepatocyte protection, rather than the specific choleretic effect of UDC shown previously (12,13). In conclusion, our results suggest that UDC most probably exerts its cytoprotective effect after amidation, although the possibility that UDC in an unconjugated form has a cytoprotective property, albeit less efficient than that of its conjugates, can not be ruled out.

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