

Altered bile acid metabolism in liver disease: concurrent occurrence of C-1 and C-6 hydroxylated bile acid metabolites and their preferential excretion into urine

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Abstract C-1 and C-6 hydroxylated bile acid metabolites in various biological specimens from subjects with liver disease (cholestasis, liver cirrhosis, chronic hepatitis, acute hepatitis) were determined by gas-liquid chromatography-mass spectrometry. Five C-1 hydroxylated bile acids and nine C-6 hydroxylated bile acids were identified in the urine studied; 1 β ,3 α ,12 α -trihydroxy-, 1 β ,3 α ,7 α -trihydroxy-, 1 β ,3 α ,7 α ,12 α -tetrahydroxy-, 3 α ,6 α ,7 α -trihydroxy-, and 3 α ,6 α ,7 α ,12 α -tetrahydroxy-5 β -cholanoic acids were found as the major components. Most of the 1 β - and 6 α -hydroxylated bile acids were excreted into urine in the nonsulfate-nonglucuronide form. The amounts in the urine were greater than those found in the bile, portal and peripheral venous sera, and liver specimens. The biliary excretion and hepatic extraction of 1 β -hydroxylated metabolites were more impaired and less efficient than for cholic acid. ■ These findings suggested that hepatic 1 β - or 6 α -hydroxylation of bile acids occurred concurrently in the patients with liver disease and that the resulting hydroxylated metabolites were efficiently excreted in the nonsulfate-nonglucuronide form into urine rather than into bile. —Shoda, J., N. Tanaka, T. Osuga, K. Matsuura, and H. Miyazaki. Altered bile acid metabolism in liver disease: concurrent occurrence of C-1 and C-6 hydroxylated bile acid metabolites and their preferential excretion into urine. *J. Lipid Res.* 1990. 31: 249-259.

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A number of unusual bile acids have been found in the urine of patients with liver diseases (1-5) and their production has suggested activation of an altered bile acid metabolism associated with disturbances of bile acid synthesis (6). It has been reported that there are hydroxylated bile acid metabolites with a hydroxyl group at C-1, (1-3), C-2 (7), C-6 (1-3), and C-23 (8) in human biological specimens, and there is much interest in the formation of

these hydroxylated metabolites in connection with liver diseases (9-12). Tohma et al. (13, 14) have synthesized some 1 β -hydroxylated bile acids and identified them in biological fluids. We have also reported on three 1 β -hydroxylated bile acids in the biological specimens of newborns and patients with cholestatic liver diseases (15). However, little is known about these hydroxylated bile acids with respect to enterohepatic circulation and route of excretion and to their correlation with the severity of liver diseases.

This report deals with identification, quantitation, and metabolism of C-1 and C-6 hydroxylated bile acids in various liver diseases.

MATERIALS AND METHODS

Sample collections

The experiments were carried out using biological specimens from 111 male and female subjects studied in our

Abbreviations: LCA, lithocholic acid (3 α -hydroxy-5 β -cholanoic acid); DCA, deoxycholic acid (3 α ,12 α -dihydroxy-5 β -cholanoic acid); CDCA, chenodeoxycholic acid (3 α ,7 α -dihydroxy-5 β -cholanoic acid); UDCA, ursodeoxycholic acid (3 α ,7 β -dihydroxy-5 β -cholanoic acid); CA, cholic acid (3 α ,7 α ,12 α -trihydroxy-5 β -cholanoic acid); DCA-1 β -ol, 1 β ,3 α ,12 α -trihydroxy-5 β -cholanoic acid; CDCA-1 β -ol, 1 β ,3 α ,7 α -trihydroxy-5 β -cholanoic acid; CA-1 β -ol, 1 β ,3 α ,7 α ,12 α -tetrahydroxy-5 β -cholanoic acid; CDCA-6 α -ol (hyocholic acid) 3 α ,6 α ,7 α -trihydroxy-5 β -cholanoic acid; CA-6 α -ol, 3 α ,6 α ,7 α ,12 α -tetrahydroxy-5 β -cholanoic acid; DMES, dimethylethylsilyl; PHP-LH-20, piperidinohydroxypropyl Sephadex LH-20; GLC, gas-liquid chromatography; SIM, selected ion monitoring; MS, mass spectrometry.

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previous report (5): 17 control subjects without liver disease and 94 patients with liver diseases. There were 20 subjects with intra- and extrahepatic cholestasis, 21 with liver cirrhosis in compensated state, 19 with liver cirrhosis in decompensated state, 15 with chronic hepatitis, and 19 with acute hepatitis. The severity of the liver cirrhosis was judged using the criteria described previously (16). Patients with liver cirrhosis were divided into two groups: compensated and decompensated states. All medications such as antibiotics and drugs that might affect bile acid metabolism were discontinued for 2 weeks before initiation of the study. Pertinent laboratory data of the 111 subjects are summarized in **Table 1**.

Urine and peripheral venous blood. Urine was collected during a 24-h period and kept refrigerated at -20°C until analyzed. Blood was taken from an antecubital vein from fasting subjects; serum was obtained by centrifugation and frozen at -20°C until analyzed.

Bile and liver tissue. Paired bile and liver tissue samples were obtained simultaneously during laparotomy or autopsy from 8 control subjects and 14 cirrhotic subjects. Liver tissue was also obtained from 7 cholestatic patients. Bile samples were taken by puncture aspiration of gallbladder contents and kept refrigerated at -20°C until analyzed. The resected liver samples were immediately rinsed with chilled saline, sliced into small blocks, briefly dried on filter paper, weighed, and kept refrigerated at -80°C until analyzed.

Portal venous blood, peripheral venous blood, and bile. Portal and peripheral venous blood were obtained during the examination of percutaneous portography for the evalua-

tion of esophageal varices in 8 cirrhotic patients. Simultaneously, peripheral venous blood was taken from an antecubital vein. Bile was obtained as bile-rich duodenal fluid by aspirating duodenal juice after contraction of the gallbladder using magnesium sulfate.

Reference compounds

Authentic standards of bile acids were obtained from Steraloids, Inc. (Wilton, NH). 1β -Hydroxylated bile acids such as $1\beta,3\alpha,12\alpha$ -trihydroxy-, $1\beta,3\alpha,7\alpha$ -trihydroxy-, and $1\beta,3\alpha,7\alpha,12\alpha$ -tetrahydroxy- 5β -cholanoic acids (13), and 6-hydroxylated bile acids such as $3\alpha,6\alpha,7\alpha,12\alpha$ -tetrahydroxy-, $3\alpha,6\beta,7\alpha,12\alpha$ -tetrahydroxy-, and $3\alpha,6\beta,7\beta,12\alpha$ -tetrahydroxy- 5β -cholanoic acids (17) were kindly supplied by Prof. M. Tohma et al. (Faculty of Pharmaceutical Sciences, Higashi-Nippon-Gakuen University, Hokkaido, Japan). These three 6-tetrahydroxylated bile acids (paper submitted for publication) were kindly supplied also by Prof. T. Hoshita (Institute of Pharmaceutical Sciences, Hiroshima University School of Medicine, Hiroshima, Japan). 6-Hydroxylated bile acids such as $3\alpha,6\beta,7\alpha$ -trihydroxy- (α -muricholic acid), $3\alpha,6\beta,7\beta$ -trihydroxy- (β -muricholic acid), and $3\alpha,6\alpha,7\beta$ -trihydroxy- 5β -cholanoic acids (ω -muricholic acid) were kindly supplied by Prof. T. Nambara and Dr. J. Goto (Pharmaceutical Institute, Tohoku University, Miyagi, Japan). Authentic standards of bile acid-3-sulfates and bile acid-3-glucuronides were synthesized according to the method described by Goto et al. (18, 19). $[2,2,4,4\text{-}^2\text{H}_4]$ Lithocholic acid (LCA), $[2,2,4,4\text{-}^2\text{H}_4]$ deoxycholic acid (DCA), and $[2,2,4,4\text{-}^2\text{H}_4]$ cholic acid (CA)

TABLE 1. Biochemical data of the patients

	n (Sex)	Age yr	T-Bil	S-GOT	S-GPT	ALP
			mg/dl	U/l	U/l	K-AU
Controls	17 (M 9, F 8)	22-56	0.5 ± 0.2^a $0.2 - 1.1^b$	14.1 ± 9.4 5 - 35	10.1 ± 8.4 4 - 35	7.2 ± 1.4 3 - 8
CHOL	20 (M 15, F 5)	38-69	17.0 ± 8.4 5.0 - 34.6	137.2 ± 105.1 19 - 388	117.5 ± 113.6 14 - 273	42.9 ± 25.6 12 - 90
LC (com) ^c	21 (M 17, F 4)	32-60	0.8 ± 0.3 0.5 - 1.8	87.6 ± 67.7 25 - 263	64.8 ± 59.4 10 - 275	11.7 ± 5.3 6 - 27
LC (dec) ^c	19 (M 13, F 6)	45-63	5.3 ± 4.0 0.5 - 14.5	129.9 ± 100.7 31 - 348	68.4 ± 67.5 8 - 224	21.2 ± 13.0 7 - 55
CH	16 (M 12, F 4)	20-43	0.9 ± 0.5 0.3 - 1.8	76.0 ± 56.8 20 - 213	126.4 ± 109.5 28 - 394	7.9 ± 1.5 5 - 10
AH	19 (M 14, F 5)	24-48	8.4 ± 7.2 1.9 - 37.1	523.7 ± 478.9 82 - 1566	859.0 ± 636.2 186 - 2490	16.2 ± 7.2 7 - 24
Normal range			0.2 - 1.2	< 40	< 40	3 - 10

Abbreviations used: CHOL, cholestasis; LC (com), compensated liver cirrhosis; LC (dec), decompensated liver cirrhosis; CH, chronic hepatitis; AH, acute hepatitis; T-Bil, serum total bilirubin; S-GOT, serum glutamic oxaloacetic transaminase; S-GPT, serum glutamic pyruvic transaminase; ALP, alkaline phosphatase.

^aValues represent mean \pm SD.

^bRange of values.

^cPatients with liver cirrhosis were divided into two groups of LC (com) (total clinical score 2.7 ± 0.8 points (mean \pm SD) (1-4 points)) and LC (dec) (14.1 ± 2.7 points (10-17 points)) according to an index of the severity of liver disease described by McCormick et al. (ref. 16).

were obtained from MSD Isotopes (Montreal, Canada), and [6,6,7,7- $^2\text{H}_4$]chenodeoxycholic acid (CDCA) was prepared in the Research Laboratory of Nippon Kayaku, Co. (Tokyo, Japan). [2,2,4,4- $^2\text{H}_4$]LCA-3-sulfate was synthesized according to the method described by Goto et al. (18). [11,11,12,12- $^2\text{H}_4$]ursodeoxycholic acid (UDCA) and [2,2,4,4- $^2\text{H}_4$]DCA-3-sulfate were kindly supplied by Tokyo Tanabe Co. (Tokyo, Japan). [2,2,4,4- $^2\text{H}_4$]LCA-3-glucuronide and [11,11,12,12- $^2\text{H}_3$]DCA-3-glucuronide (20) were kindly supplied by Dr. H. Takikawa (The 1st Department of Internal Medicine, Faculty of Medicine, Teikyo University, Tokyo, Japan). Purity of bile acids was checked by thin-layer chromatography using the following solvent systems; isopropyl ether-isooctane-acetic acid 10:5:5 (v/v/v) (21), for unconjugated bile acids, and n-butanol-acetic acid-water 10:1:1 (v/v/v) (22) for conjugated bile acids. Each of their chromatograms showed only a single spot.

Chemicals

All solvents were of analytical grade. Bond Elut C_{18} cartridge (octadecylsilane-bonded silica) was obtained from Analytichem International Inc. (Harbor City, CA), cholyglycine hydrolase (3 α ,7 α ,12 α -trihydroxy-5 β -cholan-24-oylglycine amidohydrolase from *Clostridium perfringens*) (EC 3.5.1.24), and β -glucuronidase (β -D-glucuronide glucuronosohydrolase from *Helix pomatia*) (EC 3.2.1.31) were obtained from Sigma Chemical Co. (St. Louis, MO), Sephadex LH-20 was from Pharmacia Fine Chemicals

(Uppsala, Sweden) and dimethylethylsilyl imidazole (DMESI) was from Tokyo Kasei Kogyo Co. (Tokyo, Japan). Piperidinohydroxypropyl Sephadex LH-20 was kindly supplied by Prof. T. Nambra and Dr. J. Goto (Pharmaceutical Institute, Tohoku University, Miyagi, Japan).

Gas-liquid chromatography (GLC)

Gas-liquid chromatography was carried out according to the method previously described (15).

Gas-liquid chromatography-mass spectrometry (GLC-MS)

Gas-liquid chromatography-mass spectrometry was carried out as previously reported (4, 5).

Clean-up procedure

Urine, serum, and bile were stored at -20°C and liver specimens at -80°C until analyzed. These biological specimens were purified according to the method described by Yanagisawa et al. (23, 24) with minor modifications. A detailed description of the clean-up procedure is described in recent reports (5, 15).

Urine serum and bile. To 1–2 ml of serum, 5–10 ml of urine, and 5–10 μl of bile or bile-rich duodenal juice, adequate amounts of internal standards were added as a mixture of [$^2\text{H}_4$]LCA, [$^2\text{H}_4$]DCA, [$^2\text{H}_4$]CDCA, [$^2\text{H}_4$]UDCA

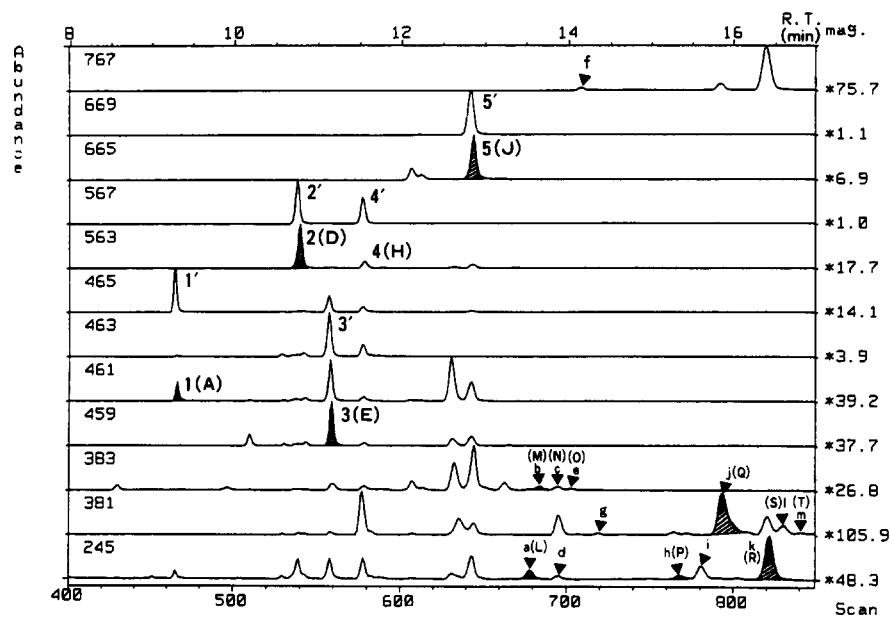


Fig. 1. A representative selected ion recording of urinary bile acids of a patient with extrahepatic cholestasis; 1, LCA; 1', [$^2\text{H}_4$]LCA; 2, DCA; 2', [$^2\text{H}_4$]DCA; 3, CDCA; 3', [$^2\text{H}_4$]CDCA; 4, UDCA; 4', [$^2\text{H}_4$]UDCA; 5, CA; 5', [$^2\text{H}_4$]CA; a, 1 β ,3 α ,12 α -ol (DCA-1 β -ol); b, 3 α ,6 α ,7 α -ol (CDCA-6 α -ol); c, 3,6,7-ol; d, 1 β ,3,12-ol; e, 3,6,7-ol; f, 3 α ,6 β ,7 α ,12 α -ol; g, 3 α ,6 β ,7 β ,12 α -ol; h, 1 β ,3 α ,7 α -ol (CDCA-1 β -ol); i, 1 β ,3 α ,7 β -ol (UDCA-1 β -ol); j, 3 α ,6 α ,7 α ,12 α -ol (CA-6 α -ol); k, 1 β ,3 α ,7 α ,12 α -ol (CA-1 β -ol); l, 3,6,7,12-ol; m, 3,6,7,12-ol. Upper case letters in parentheses correspond to the peaks of a reconstructed ion profile in our previous report (Fig. 2 and Table 1, ref. 15). Lower case letters correspond to those in Table 2. The peaks with cross-hatched areas represent those for quantitation by GLC-SIM.

TABLE 2. Completely and partially identified C-1 or C-6 hydroxylated

Bile Acids Identified ¹	MUv ²	Mr	[M] ³	[M-C ₂ H ₅] ³	[M-DMESOH] ³	Fragment Ions ³ (m/z)
						[M-2 × DMESOH] ³ or [M-2 × DMESOH + H] ³
a ⁴ (L) ⁵ 5βB-1β,3α,12α-ol	36.52	694	694 (-)	665 (33)	590 (14)	486 (5)
b (M) 5βB-3α,6α,7α-ol	36.62	694	694 (-)	665 (4)	590 (1)	487 (2)
c (N) B-3,6,7-ol ⁶	36.79	694	694 (-)	665 (2)	590 (-)	486 (2)
d 5βB-1β,3,12-ol ⁶	36.99	694	694 (-)	665 (29)	590 (21)	486 (11)
e (O) B-3,6,7-ol ⁶	37.19	694	694 (-)	665 (8)	590 (34)	486 (39)
f 5βB-3α,6β,7α,12α-ol	37.24	796	796 (-)	767 (100)	692 (2)	588 (17)
g 5βB-3α,6β,7α,12α-ol	37.34	796	796 (-)	767 (38)	692 (3)	588 (11)
h (P) 5βB-1β,3α,7α-ol	37.58	694	694 (-)	665 (4)	590 (2)	486 (4)
i 5βB-1β,3α,7β-ol ⁶	37.68	694	694 (-)	665 (15)	590 (17)	486 (28)
j (Q) 5βB-3α,6α,7α,12α-ol	37.76	796	796 (-)	767 (9)	692 (-)	588 (4)
k (R) 5βB-1β,3α,7α,12α-ol	37.97	796	796 (-)	767 (32)	692 (4)	588 (4)
l (S) B-3,6,7,12-ol ⁶	38.45	796	796 (-)	767 (19)	692 (46)	588 (32)
m (T) B-3,6,7,12-ol ⁶	38.94	796	796 (-)	767 (8)	692 (1)	588 (3)

¹B, cholanoic acid; configurations at C-5 and of hydroxyl groups are indicated by Greek letters.

²Methylene unit values of ethyl ester DMES ether derivatives.

³Chemical formulas after M indicate mass fragments lost. DMESOH represents dimethylethylsilanol.

⁴Lower case letters correspond to the peaks of the selected ion recording in Fig. 1.

and [²H₄]CA in 0.5 M potassium phosphate buffer (pH 7.0); the samples were then applied to a Bond Elut C₁₈ cartridge. The eluted bile acids were subjected to solvolysis according to the method described by Kornel (25), followed by enzymatic hydrolysis using cholyglycine hydrolase (26) and derivatization into the Et-DMES ether derivatives.

Conjugate forms of bile acids (nonglucuronide-nonsulfate, glucuronides and sulfates). In some cirrhotic patients, the forms of conjugation such as nonglucuronide-nonsulfate, glucuronide, and sulfate in the urine were studied. To 5–10 ml of urine, adequate amounts of internal standards were added as a mixture of [²H₄]LCA, [²H₄]DCA, [²H₄]CDCA, [²H₄]UDCA, [²H₄]CA, [²H₄]LCA-3-sulfate, [²H₄]DCA-3-sulfate, [²H₄]LCA-3-glucuronide, and [²H₃]DCA-3-glucuronide. After enzymatic cleavage of amino acid conjugates, the resulting bile acids were separated into these three fractions using a column of piperidinoxypropyl Sephadex LH-20 (PHP-LH-20) (20, 27). Nonglucuronidated-nonsulfated bile acids, deglucuronidated bile acids after glucuronic acid cleavage by treatment with β-glucuronidase (28), and desulfated bile acids after solvolysis (25) were converted into the ethyl ester dimehtylethylsilyl (Et-DMES) ether derivatives.

Liver. Liver tissue (about 300 mg wet weight) was homogenized in 2 ml of ice-cold 95% aqueous ethanol containing 0.1% ammonium hydroxide using a Teflon pestle homogenizer at about 700 rpm for 5 min. The homogenate was transferred into a centrifuge tube and washed with three 3-ml portions of 95% aqueous ethanol-0.1% ammonium hydroxide with the aid of ultrasoni-

cation. The combined washings were heated in a water bath at 80°C for 10 min under continuous stirring, and then centrifuged at 0°C for 10 min at 5000 g. The supernatant was stored at -20°C. To an aliquot of the pooled supernatants, adequate amounts of a mixture of internal standards were added and then the supernatants were purified.

Identification and quantitation of individual bile acids. The identification of individual bile acid derivatives was based on the comparison of the methylene unit values (MUv) (29) of the peaks on reconstructed ion profiles and their mass spectra with those of authentic standards. Quantitation of individual bile acids was carried out by GLC-selected ion monitoring-MS, using the ions as the Et-DMES ethers at m/z 461/465 for LCA/[²H₄]LCA; m/z 563/567 for DCA/[²H₄]DCA; m/z 459/463 for CDCA/[²H₄]CDCA; m/z 563/567 for UDCA/[²H₄]UDCA; m/z 665/669 for CA/[²H₄]CA; m/z 245 for 1β,3α,12α-trihydroxy-, 1β,3α,7α-trihydroxy-, and 1β,3α,7α,12α-tetrahydroxy-5β-cholanoic acid; m/z 383 for 3α,6α,7α-trihydroxy-5β-cholanoic acid; and m/z 381 for 3α,6α,7α,12α-tetrahydroxy-5β-cholanoic acid. 1β- and 6α-hydroxylated bile acids were quantitated using [²H₄]CA as a convenient internal standard. All the monitoring ions except those of CDCA/[²H₄]CDCA, 1β- and 6α-hydroxylated bile acids were the characteristic ions of [M-C₂H₅]⁺, whereas the following ions were selected for the monitoring ions: m/z 459/463 [M-dimethylethylsilanol (DMESOH)-C₂H₅]⁺ for CDCA/[²H₄]CDCA; m/z 245 (m/z 143) with an additional DMES group for 1β-hydroxylated bile acids; and m/z 383 (m/z 381) [M-3(4) × DMESOH + H]⁺ for 6α-hydroxylated bile acids.

(Relative Intensities)

[M-3 × DMESOH] [‡] or [M-3 × DMESOH + H] [‡]	[M-4 × DMESOH] [‡] or [M-4 × DMESOH + H] [‡]	Other Ions					
382 (4)	-	330 (8)	253 (10)	<u>245</u> ⁷ (100)			
<u>383</u> (100)	-	337 (10)	319 (2)	275 (3)	161 (25)	159 (24)	
383 (100)	-	337 (6)	319 (2)	253 (6)	161 (24)	159 (12)	
382 (12)	-	330 (5)	253 (13)	245 (100)			
383 (100)	-	337 (2)	319 (6)	275 (10)	161 (32)	159 (24)	
484 (19)	381 (17)	355 (17)	251 (10)	161 (3)	159 (5)		
485 (54)	381 (100)	313 (48)	251 (8)	206 (40)	196 (8)		
382 (5)	-	330 (4)	253 (-)	<u>245</u> (100)	209 (18)	196 (14)	
382 (4)	-	330 (5)	253 (4)	245 (100)			
485 (81)	<u>381</u> (100)	335 (19)	251 (5)	161 (13)	159 (7)		
484 (16)	380 (18)	355 (6)	251 (11)	<u>245</u> (100)	209 (18)	196 (14)	
485 (21)	381 (100)	335 (13)	251 (22)	161 (53)	159 (42)		
485 (4)	381 (100)	335 (16)	251 (12)	161 (36)	159 (16)		

[‡]Upper case letters in parentheses correspond to the peaks of the reconstructed ion profile in our previous report (Fig. 2 and Table 1, ref. 15).

[‡]Positions of hydroxyl groups and stereochemistry are tentative.

⁷Fragment ions underlined were used for quantitation of the individual bile acids.

Good recoveries of unconjugated bile acids (83–91%), glucuronidated bile acids (93–100%), and sulfated bile acids (92–98%) were obtained by the present procedure using deuterated bile acids as internal bile acids, as observed in previous reports (30, 31).

RESULTS

Qualitative composition of C-1 and C-6 hydroxylated bile acid metabolites in liver diseases

Fig. 1 shows a typical selected ion recording obtained by analysis of urinary bile acids of cirrhotic patients. Table 2 lists the C-1 and C-6 hydroxylated bile acid metabolites identified in this study. Five 1 β -hydroxylated bile acids and nine 6 α - or 6 β -hydroxylated bile acids were completely or partially identified in the urine in comparison with reference compounds (13, 14). Of the 1 β -hydroxylated metabolites, 1 β ,3 α ,12 α -trihydroxy- (DCA-1 β -ol), 1 β ,3 α ,7 α -trihydroxy- (CDCA-1 β -ol), and 1 β ,3 α ,7 α ,12 α -tetrahydroxy-5 β -cholanoic acids (CA-1 β -ol) were always detected in the urine. Of the 6 α - or 6 β -hydroxylated metabolites, 3 α ,6 α ,7 α -trihydroxy- (CDCA-6 α -ol, so-called hyocholic acid) and 3 α ,6 α ,7 α ,12 α -tetrahydroxy-5 β -cholanoic acids (CA-6 α -ol) were always detected in the urine.

Quantitative composition of C-1 and C-6 hydroxylated bile acid metabolites in liver diseases

Urine. Table 3 shows the urinary excretion amounts of 1 β - and 6 α -hydroxylated bile acid metabolites and their proportions in total bile acids.

Of the three 1 β -hydroxylated bile acid metabolites, DCA-1 β -ol was found to be a major component in control subjects and patients with compensated liver cirrhosis and chronic hepatitis. A significant increase in compensated liver cirrhosis (9.2% of total bile acids) and significant decreases in cholestasis (0.2%) and acute hepatitis (0.2%) of the proportion in total bile acids were observed in comparison with control subjects (4.3%). CA-1 β -ol was a major component in cholestasis. Significant increases of this component in cholestasis (2.4%), compensated (1.5%) and decompensated liver cirrhosis (3.2%) were observed in comparison with control subjects (<0.1%).

The proportions of 1 β -hydroxylated metabolites to total bile acids and the ratio of [(CA-1 β -ol + CDCA-1 β -ol)/DCA-1 β -ol] were calculated from the data of Table 3. There were significant increases of the proportion (9.4%) and the ratio (6.2) in liver cirrhosis in comparison with control subjects (5.2% and 0.04, respectively). When the compensated liver cirrhosis was compared with the decompensated cirrhosis, the proportion of the former group (11.3%) was found to be higher than that of the latter group (6.8%), whereas the ratio of the latter (12.4) was found to be significantly higher than that of the former (0.7). As for the groups of patients with cholestasis and acute hepatitis, there were significant decreases in their proportions (3.2% and 0.6%, respectively) and significant increases in their ratios (31.6 and 8.1, respectively) in comparison with those of control subjects.

CDCA-6 α -ol (hyocholic acid) and CA-6 α -ol were always present as major components of 6 α - or 6 β -hydroxylated bile acid metabolites. There were significant increases

TABLE 3. Total bile acid levels and proportions of 1 β - and 6 α -hydroxylated bile acids to total bile acids in urine and serum

n	Total Bile Acids						1 β -Hydroxylated Bile Acids						6 α -Hydroxylated Bile Acids								
	Urine		Serum		Urine		Serum		Urine		Serum		Urine		Serum		Urine		Serum		
	mg/day	μ g/ml	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	
Controls	6.3 \pm 1.6 ^a	2.1 \pm 0.1	5.2 \pm 1.3	1.1 \pm 0.3	4.3 \pm 1.2	1.1 \pm 0.2	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	
CHOL	94.7 \pm 14.9 [†]	64.2 \pm 8.6 [†]	3.2 \pm 1.0	1.0 \pm 0.8	0.2 \pm 0.03 [‡]	0.1 \pm 0.05 [‡]	0.6 \pm 0.2	0.6 \pm 0.2	0.6 \pm 0.2	0.6 \pm 0.2	0.6 \pm 0.2	0.6 \pm 0.2	0.6 \pm 0.2	0.6 \pm 0.2	0.6 \pm 0.2	0.6 \pm 0.2	0.6 \pm 0.2	0.6 \pm 0.2	0.6 \pm 0.2	0.6 \pm 0.2	0.6 \pm 0.2
LC (com)	25.1 \pm 4.6 [†]	21.8 \pm 7.1 [†]	11.3 \pm 2.3 [†]	2.9 \pm 0.7 [†]	9.2 \pm 2.2 [†]	2.6 \pm 0.7 [†]	0.6 \pm 0.1 [†]	0.6 \pm 0.1 [†]	0.6 \pm 0.1 [†]	0.6 \pm 0.1 [†]	0.6 \pm 0.1 [†]	0.6 \pm 0.1 [†]	0.6 \pm 0.1 [†]	0.6 \pm 0.1 [†]	0.6 \pm 0.1 [†]	0.6 \pm 0.1 [†]	0.6 \pm 0.1 [†]	0.6 \pm 0.1 [†]	0.6 \pm 0.1 [†]	0.6 \pm 0.1 [†]	0.6 \pm 0.1 [†]
LC (dec)	32.2 \pm 7.3 [†]	37.2 \pm 10.7 [†]	6.8 \pm 2.0	1.7 \pm 0.7	2.5 \pm 0.8	0.5 \pm 0.1 [†]	1.2 \pm 0.3 [†]	1.2 \pm 0.3 [†]	1.2 \pm 0.3 [†]	1.2 \pm 0.3 [†]	1.2 \pm 0.3 [†]	1.2 \pm 0.3 [†]	1.2 \pm 0.3 [†]	1.2 \pm 0.3 [†]	1.2 \pm 0.3 [†]	1.2 \pm 0.3 [†]	1.2 \pm 0.3 [†]	1.2 \pm 0.3 [†]	1.2 \pm 0.3 [†]	1.2 \pm 0.3 [†]	1.2 \pm 0.3 [†]
CH	14.1 \pm 6.5 [†]	7.0 \pm 1.7 [†]	5.7 \pm 2.1	0.5 \pm 0.1	5.2 \pm 2.0	0.4 \pm 0.1 [†]	0.1 \pm 0.06	0.1 \pm 0.06	0.1 \pm 0.06	0.1 \pm 0.06	0.1 \pm 0.06	0.1 \pm 0.06	0.1 \pm 0.06	0.1 \pm 0.06	0.1 \pm 0.06	0.1 \pm 0.06	0.1 \pm 0.06	0.1 \pm 0.06	0.1 \pm 0.06	0.1 \pm 0.06	0.1 \pm 0.06
AH	219.3 \pm 53.8 [†]	78.3 \pm 14.0 [†]	0.6 \pm 0.1 [†]	0.1 \pm 0.03 [‡]	0.2 \pm 0.03 [‡]	0.1 \pm 0.02 [‡]	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1

n	Total				CDCA-1 β -ol				CDCA-6 α -ol								
	Urine		Serum		Urine		Serum		Urine		Serum		Urine		Serum		
	[(CDCA-1 β -ol + CA-1 β -ol)/DCA-1 β -ol]	%	[(CDCA-1 β -ol + CA-1 β -ol)/DCA-1 β -ol]	%	[(CDCA-1 β -ol + CA-1 β -ol)/DCA-1 β -ol]	%	[(CDCA-1 β -ol + CA-1 β -ol)/DCA-1 β -ol]	%	[(CDCA-1 β -ol + CA-1 β -ol)/DCA-1 β -ol]	%	[(CDCA-1 β -ol + CA-1 β -ol)/DCA-1 β -ol]	%	[(CDCA-1 β -ol + CA-1 β -ol)/DCA-1 β -ol]	%	[(CDCA-1 β -ol + CA-1 β -ol)/DCA-1 β -ol]	%	
Controls	0.04 \pm 0.01	2.9 \pm 1.3	5.5 \pm 2.0	3.4 \pm 1.4	5.4 \pm 2.0	0.6 \pm 0.1	ND	0.6 \pm 0.1	ND	0.6 \pm 0.1	ND	0.6 \pm 0.1	ND	0.6 \pm 0.1	ND	0.6 \pm 0.1	ND
CHOL	31.6 \pm 14.3 [†]	14.5 \pm 2.5 [†]	3.3 \pm 1.0	8.9 \pm 1.6 [†]	3.8 \pm 1.1	2.4 \pm 0.5 [†]	0.2 \pm 0.01	2.4 \pm 0.5 [†]	0.2 \pm 0.01	2.4 \pm 0.5 [†]	0.2 \pm 0.01	2.4 \pm 0.5 [†]	0.2 \pm 0.01	2.4 \pm 0.5 [†]	0.2 \pm 0.01	2.4 \pm 0.5 [†]	0.2 \pm 0.01
LC (com)	0.7 \pm 0.3 [†]	5.7 \pm 1.3	2.7 \pm 0.5	3.9 \pm 1.1	2.6 \pm 0.5	2.4 \pm 0.4 [†]	0.8 \pm 0.4	2.4 \pm 0.4 [†]	0.8 \pm 0.4	2.4 \pm 0.4 [†]	0.8 \pm 0.4	2.4 \pm 0.4 [†]	0.8 \pm 0.4	2.4 \pm 0.4 [†]	0.8 \pm 0.4	2.4 \pm 0.4 [†]	0.8 \pm 0.4
LC (dec)	12.4 \pm 4.5 [†]	8.6 \pm 1.5 [†]	2.4 \pm 0.4	7.8 \pm 1.5 [†]	2.3 \pm 0.4	2.1 \pm 0.2 [†]	0.2 \pm 0.04	2.1 \pm 0.2 [†]	0.2 \pm 0.04	2.1 \pm 0.2 [†]	0.2 \pm 0.04	2.1 \pm 0.2 [†]	0.2 \pm 0.04	2.1 \pm 0.2 [†]	0.2 \pm 0.04	2.1 \pm 0.2 [†]	0.2 \pm 0.04
CH	0.1 \pm 0.02	3.3 \pm 0.6	1.7 \pm 0.3	2.2 \pm 0.5	1.8 \pm 0.3	0.5 \pm 0.1	ND	0.5 \pm 0.1	ND	0.5 \pm 0.1	ND	0.5 \pm 0.1	ND	0.5 \pm 0.1	ND	0.5 \pm 0.1	ND
AH	8.1 \pm 2.3 [†]	5.1 \pm 1.2	2.2 \pm 0.5	3.2 \pm 0.8	2.1 \pm 0.4	1.5 \pm 0.3 [†]	0.3 \pm 0.1	1.5 \pm 0.3 [†]	0.3 \pm 0.1	1.5 \pm 0.3 [†]	0.3 \pm 0.1	1.5 \pm 0.3 [†]	0.3 \pm 0.1	1.5 \pm 0.3 [†]	0.3 \pm 0.1	1.5 \pm 0.3 [†]	0.3 \pm 0.1

Abbreviations used: DCA-1 β -ol, 1 β , 3 α , 12 α -trihydroxy-5 β -cholanolic acid; CDCA-1 β -ol, 1 β , 3 α , 7 α , 12 α -trihydroxy-5 β -cholanolic acid; CA-1 β -ol, 1 β , 3 α , 7 α , 12 α -trihydroxy-5 β -cholanolic acid; CDCA-6 α -ol, 3 α , 6 α , 7 α , 12 α -trihydroxy-5 β -cholanolic acid; [(CDCA-1 β -ol + CA-1 β -ol)/DCA-1 β -ol] and [CDCA-6 α -ol/CDCA-6 α -ol] represent the ratios calculated from the data of urine; CHOL, cholestasis; LC (com), compensated liver cirrhosis; LC (dec), decompensated liver cirrhosis; CH, chronic hepatitis; AH, acute hepatitis.

^aValues represent mean \pm SEM; [†]P < 0.05; [‡]P < 0.025; [§]P < 0.01; [¶]P < 0.005; significant as compared with those of control subjects (\uparrow , increase; \downarrow , decrease); ND, nondetectable.

of the proportion of CDCA-6 α -ol to total bile acids in cholestasis (8.9%) and decompensated liver cirrhosis (7.8%) in comparison with that of control subjects (3.4%). There were also significant increases of the CA-6 α -ol proportion in cholestasis (2.4%), compensated (2.4%) and decompensated liver cirrhosis (2.1%), and acute hepatitis (1.5%) in comparison with that of control subjects (0.6%).

The proportions of CDCA-6 α -ol and CA-6 α -ol to total bile acids and the ratio of [CA-6 α -ol/CDCA-6 α -ol] were calculated from the data of Table 3. There were significant increases of both the proportions and the ratios in cholestasis (14.5% and 0.8), liver cirrhosis (7.0% and 2.1), and acute hepatitis (5.1% and 0.6) in comparison with those of control subjects (2.9% and <0.1). When compensated liver cirrhosis was compared with decompensated cirrhosis, the proportion was found to be higher in the latter (8.6%) than the former (5.7%), whereas the ratio of the former (2.6%) was found to be significantly higher than that of the latter (0.9%).

Fig. 2 shows the ratio of [1 β -/6 α -hydroxylated bile acids] calculated from Tables 3 and 4. There were significant decreases of these ratios in cholestasis (0.3), decompensated liver cirrhosis (0.9), and acute hepatitis (0.3) in comparison with that of control subjects (3.8). The ratios in compensated liver cirrhosis (3.1) and chronic hepatitis (1.8) were similar to that of control subjects (3.8). 6 α -Hydroxylation of bile acids might take precedence over 1 β -hydroxylation in the subjects with severe liver diseases.

The conjugate forms of 1 β - and 6 α -hydroxylated bile acid metabolites in the urine were studied and each fraction (nonglucuronide-nonsulfate, glucuronide, and sulfate) was determined; LCA nonglucuronide-nonsulfate (N) 5.6 \pm 2.0% (mean \pm SEM), glucuronide (G) 4.7 \pm 1.9, sulfate

(S) 89.3 \pm 3.3, DCA N 17.1 \pm 3.8, G 13.6 \pm 1.9, S 69.2 \pm 5.1, CDCA N 17.6 \pm 3.4, G 3.3 \pm 1.0, S 76.5 \pm 2.5, UDCA N 22.5 \pm 7.2, G 7.4 \pm 3.0, S 70.0 \pm 8.1, CA N 49.5 \pm 4.0, G 14.4 \pm 2.5, S 35.9 \pm 2.4, DCA-1 β -ol N 88.2 \pm 3.2, G 6.2 \pm 3.0, S 5.4 \pm 2.1, CDCA-1 β -ol N 90.9 \pm 3.3, G 4.3 \pm 2.4, S 4.7 \pm 1.9, CA-1 β -ol N 87.4 \pm 5.8, G 9.2 \pm 6.4, S 3.4 \pm 0.8, CDCA-6 α -ol N 85.8 \pm 3.0, G 11.2 \pm 3.1, S 2.9 \pm 1.0, CA-6 α -ol N 90.5 \pm 1.8, G 4.9 \pm 1.4, S 4.4 \pm 0.8. It is worthy of notice that major portions of these 1 β - and 6 α -hydroxylated bile acid metabolites were found as the non-glucuronide-nonsulfate conjugates.

Serum. Table 3 shows the serum concentrations of 1 β - and 6 α -hydroxylated bile acid metabolites and their proportions to total bile acids in liver diseases, respectively. A few differences between the control subjects and those with liver disease were found in the serum concentration and proportion of these metabolites. The urine/serum (U/S) ratio of each bile acid proportion to total bile acids was estimated from the data of Table 3. The U/S ratios were found to be 44.5 \pm 4.2 (mean \pm SEM) for the total 1 β -hydroxylated bile acids and 10.5 \pm 1.0 for the total 6 α -hydroxylated bile acids. The high U/S ratio indicates the efficient excretion of these 1 β - and 6 α -hydroxylated bile acid metabolites into urine.

Bile and liver. Table 4 shows the level and composition of individual bile acids in the paired bile and liver samples. Only DCA-1 β -ol and CDCA-6 α -ol, which were always found in these specimens, were determined. In some bile and liver samples, CA-1 β -ol and CDCA-1 β -ol could be detected in small amounts; they constituted up to 1.0% and 0.5% of total bile acids, respectively, indicating the much lower proportion of 1 β -hydroxylated bile acid metabolites in the bile and liver specimens in comparison with that in urine.

The paired bile and liver specimens obtained from the cirrhotic patients were analyzed according to the report of Akashi et al. (32). The results indicated that the ratio of [CA/CDCA] in bile to [CA/CDCA] in liver (1.71), and that of [CA/CDCA-1 β -ol] in bile to [CA/DCA-1 β -ol] in liver of the cirrhotic patients (1.31) were significantly higher than of [CA/CDCA] ratio (0.82) and [CA/DCA-1 β -ol] ratio (0.53) of control subjects. The ratio of [CDCA/DCA-1 β -ol] was also significantly higher in liver cirrhosis (1.07) than in control subjects (0.61). However, in the case of the ratio of [CA/CDCA-6 α -ol] and [CDCA/CDCA-6 α -ol], there was no significant difference between the control subjects and cirrhotic patients. This can be explained by postulating the impaired biliary excretion of CDCA and DCA-1 β -ol in comparison with that of CA in the cirrhotic state. When CDCA-6 α -ol is compared with DCA-1 β -ol, the biliary excretion of the former was more preserved than that of the latter in the cirrhotic state.

Portal and peripheral venous blood. The urine, bile, and blood specimens from portal and peripheral veins of eight cirrhotic patients during the examination of percutaneous

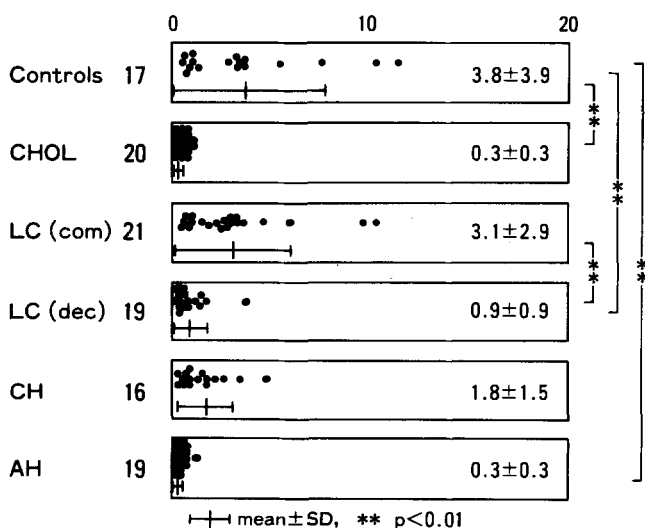


Fig. 2. The ratios of [1 β -/6 α -hydroxylated bile acids] in the urine. Controls, control subjects; CHOL, cholestasis; LC (com), compensated liver cirrhosis; LC (dec), decompensated liver cirrhosis; CH, chronic hepatitis; AH, acute hepatitis.

TABLE 4. Bile acids in bile and supernatant of liver tissue

Compound	Controls		Liver Cirrhosis		Cholestasis Liver (n = 7)
	Bile (n = 8)	Liver (n = 8)	Bile (n = 14)	Liver (n = 14)	
Total	10.1 ± 3.4 ^a	36.6 ± 8.6 ^b	6.7 ± 1.6 ^a	65.9 ± 9.4 ^b	112.8 ± 33.8 ^b
% Distribution of Bile Acids					
LCA	1.9 ± 0.4	3.0 ± 1.0	2.5 ± 1.1	1.2 ± 0.2	2.2 ± 1.0
DCA	11.6 ± 2.5	28.2 ± 5.0	6.8 ± 1.0	4.5 ± 1.1	1.9 ± 0.4
CDCA	51.7 ± 3.3	37.1 ± 5.1	55.5 ± 5.9	62.2 ± 3.9	61.1 ± 6.2
UDCA	5.2 ± 1.3	3.4 ± 1.5	1.1 ± 0.5	0.9 ± 0.1	1.2 ± 0.4
CA	28.1 ± 2.2	23.4 ± 3.4	28.6 ± 4.5	25.2 ± 3.2	22.6 ± 5.1
DCA-1β-ol	0.4 ± 0.08	0.1 ± 0.02	0.5 ± 0.1	0.6 ± 0.1	0.2 ± 0.2
CDCA-6α-ol	0.3 ± 0.05	0.9 ± 0.7	8.4 ± 1.1	1.4 ± 0.4	0.7 ± 0.9
CA/CDCA (Bile/liver)	0.54 ± 0.06 (0.82 ± 0.09)	0.87 ± 0.27	0.85 ± 0.26 (1.71 ± 0.16)*	0.38 ± 0.08	
CA/DCA-1β-ol (Bile/liver)	82.6 ± 11.8 (0.53 ± 0.06)	158.3 ± 24.9	101.9 ± 20.6 (1.31 ± 0.13)**	89.7 ± 19.7	
CDCA/DCA-1β-ol (Bile/liver)	150.2 ± 41.9 (0.61 ± 0.05)	235.0 ± 44.7	312.2 ± 64.0 (1.07 ± 0.16)*	299.1 ± 81.1	
CA/CDCA-6α-ol (Bile/liver)	79.2 ± 10.0 (1.38 ± 0.35)	78.0 ± 36.1	11.1 ± 3.4 (1.14 ± 0.18)	9.4 ± 1.1	
CDCA/CDCA-6α-ol (Bile/liver)	107.9 ± 15.8 (1.87 ± 0.81)	78.4 ± 26.3	60.9 ± 26.6 (0.89 ± 0.24)	46.2 ± 10.3	

Values represent mean ± SEM. **P* < 0.05; ***P* < 0.01: significant as compared to those of control subjects. Abbreviations used: total, total bile acids; LCA, lithocholic acid; DCA, deoxycholic acid; CDCA, chenodeoxycholic acid; UDCA, ursodeoxycholic acid; CA, cholic acid; DCA-1β-ol, 1β,3α,12α-trihydroxy-5β-cholanoic acid; CDCA-6α-ol, 3α,6α,7α-trihydroxy-5β-cholanoic acid.

^aGiven as mg/ml.

^bGiven as μg per g liver.

portography were analyzed in order to elucidate a possibly different enterohepatic circulation of 1β- and 6α-hydroxylated bile acid metabolites compared to other normal bile acids. Table 5 shows the results.

The mean ratio between portal and peripheral venous serum concentrations (P/V ratio) of CA (1.37) was significantly higher than those of CDCA (0.90, *P* < 0.05), DCA-1β-ol (0.60, *P* < 0.01), and the 1β-ol-fraction (0.56, *P* < 0.01). This suggested that CA might be subjected to more efficient extraction by liver from portal venous flow than CDCA and 1β-hydroxylated bile acid metabolites in the cirrhotic patients. In contrast to the hepatic extraction of 1β-hydroxylated bile acid metabolites, that of CDCA-6α-ol (hypoCA) was speculated to be similar to that of CA.

DISCUSSION

Recent reports (1-3, 7, 8) have shown the occurrence in human subjects of hydroxylated bile acid metabolites substituted at other than normally hydroxylated positions such as C-3, C-7, or C-12. Bremmelgaard and Sjövall (9) reported the hydroxylated of exogenously injected [¹⁴C]-labeled bile acids at C-1 or C-6 under cholestatic conditions.

In this study, five 1β-hydroxylated bile acid metabolites were found in the urine of the patients with liver disease. 1β-Hydroxylation of bile acids has been speculated to be a major metabolic pathway during the neonatal period (13-15). It is of interest that 1β-hydroxylation is also accelerated in liver disease.

Urinary excretion of 1β-hydroxylated bile acid metabolites in subjects with liver disease increased in comparison to that of control subjects. With respect to the more severe liver diseases such as cholestasis, decompensated liver cirrhosis, and acute hepatitis, the increased urinary excretion of 1β-hydroxylated bile acids was due to CA-1β-ol and CDCA-1β-ol. On the other hand, the increases in the more mild liver diseases such as compensated liver cirrhosis and chronic hepatitis were attributed to DCA-1β-ol. The ratio of [(CA-1β-ol + CDCA-1β-ol)/DCA-1β-ol] showed the prominent difference between mild and severe liver disease, indicating that this index might be useful for evaluation of severity of liver disease.

In this study, nine 6α- or 6β-hydroxylated bile acid metabolites were found in the urine of patients with liver disease. These unusual hydroxylated bile acids have been reported in several papers (33-35). The two hydroxylated metabolites identified as having a hydroxyl group at 6β-configuration, 3α,6β,7α,12α- and 3α,6β,7β,12α-tetrahy-

TABLE 5. Portal/peripheral venous serum ratios of total bile acids, cholic acid, chenodeoxycholic acid, 1 β -hydroxylated, and 6 α -hydroxylated bile acids of eight cirrhotic patients

Total Bile Acids (n = 8)	Cholic Acid (n = 8)	Chenodeoxycholic Acid (n = 8)	DCA-1 β -ol ^d (n = 8)	1 β -ol-Fraction ^b (n = 8)	CDCA-6 α -ol ^c (n = 8)
1.44 \pm 0.13 ^d 1.04 - 2.35 ^f	1.37 \pm 0.17 0.85 - 2.26	0.90 \pm 0.07 ^{*↓} 0.58 - 1.26	0.60 \pm 0.14 ^{**↓} 0.12 - 1.00	0.56 \pm 0.13 ^{**↓} 0.12 - 1.00	1.20 \pm 0.70 0.10 - 6.33

^a1 β ,3 α ,12 α -Trihydroxy-5 β -cholanoic acid.

^bTotal of 1 β -hydroxylated bile acids.

^c3 α ,6 α ,7 α -Trihydroxy-5 β -cholanoic acid.

^dValues represent mean \pm SEM.

^e*↓, Significant decrease ($P < 0.05$) in comparison with the ratio of CA; **↓, significant decrease ($P < 0.01$) in comparison with the ratio of CA.

^fValues represent the range.

droxy-5 β -cholanoic acids, were identified as minor components in comparison with CA-6 α -ol. 6 α -Hydroxylation of bile acids might take precedence over 6 β -hydroxylation and be accelerated in liver disease, especially in the more severe forms. The ratio of [CA-6 α -ol/CDCA-6 α -ol] showed no remarkable difference mild and severe disease.

Conjugate forms of 1 β - and 6 α -hydroxylated bile acid metabolites in the urine were studied. Most of them were found as the nonglucuronide-nonsulfated form, and their U/S ratios were very high. This suggests that 1 β - and 6 α -hydroxylated bile acid metabolites may be two major metabolic transformations for the elimination of bile acids into urine in addition to sulfate and glucuronide bile acids.

Little is known about the synthesis of 1 β - or 6 α -hydroxylated bile acid metabolites. Fetal liver microsomes have been shown to be capable of 1 β -hydroxylation of steroids (36, 37) and bile acids (38). Trülch et al. (39) reported the existence of microsomal 6 α -hydroxylase activity toward tauroolithocholic acids in liver biopsies. Some 1 β - and 6 α -hydroxylated bile acid metabolites identified in the urine were also found in liver and bile samples in this study. Since bile acid composition in liver reflects both bile acid synthesis and the state of enterohepatic circulation of bile acids (40), possible hepatic 1 β - and 6 α -hydroxylation of bile acids is suggested. The low levels of 1 β - and 6 α -hydroxylated bile acid metabolites in the portal venous blood support a hypothesis that these metabolites are formed in the liver rather than the intestine.

The paired bile and liver samples were analyzed with special attention to biliary excretion of bile acids. When the biliary excretions of DCA-1 β -ol, CDCA-6 α -ol, CA, and CDCA were compared, the degree of the impairment was in the order DCA-1 β -ol > CDCA > CA in the cirrhotic patients. On the other hand, the biliary excretion of CDCA-6 α -ol, which was quite similar to that of CA, was preserved in comparison with that of DCA-1 β -ol.

There are a few reports (41, 42) about the enterohepatic circulation of normal bile acids suggesting the efficient hepatic extraction of cholic acid compared with chenode-

oxycholic and deoxycholic acids in control subjects; however, there are only a few reports about the possible enterohepatic circulation of 1 β - and 6 α -hydroxylated bile acid metabolites. A significant difference of P/V ratio was observed between CA and other bile acids such as CDCA, DCA-1 β -ol, and the other 1 β -ol-metabolites. However, the P/V ratio of CDCA-6 α -ol was found to be almost equal to that of CA. These findings suggested that there is preferential hepatic extraction of CA compared to CDCA and DCA-1 β -ol, although it may be necessary to consider the existence of porto-systemic shunting. Urinary abundance of 1 β - and 6 α -hydroxylated bile acid metabolites in liver disease may be attributed to their increased hepatic biosynthesis, impaired biliary excretion, or inefficient extraction which results in efficient urinary excretion.

Excessive bile acids in the body appears to be toxic in species that are unable to convert them into more polar compounds (43). Conjugation with glycine or taurine does little to decrease the toxic potentiality of excessive bile acids (44), but sulfation (45, 46) glucuronidation, and hydroxylation may provide detoxication of bile acids.

In conclusion, transformations such as 1 β - or 6 α -hydroxylation are activated in liver disease for increasing the water solubility of bile acids and their excretion into urine. ■

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