Hydrophilic 7β-Hydroxy Bile Acids, Lovastatin, and Cholestyramine Are Ineffective in the Treatment of Cerebrotendinous Xanthomatosis

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We compared the effect of treatments with hydrophilic bile acids (ursodeoxycholic and ursocholic acids), cholestyramine, and lovastatin versus chenodeoxycholic acid in 4 patients with cerebrotendinous xanthomatosis (CTX). Bile acids and bile alcohols in plasma, bile, and urine before and after treatment were quantitated by gas-liquid chromatography. Untreated, all patients showed abnormal biliary bile acid composition: cholic acid (72.7%) and chenodeoxycholic acid (6.2%), and polyhydroxylated C_{27}-bile alcohols (10.0%), and elevated plasma cholestanol levels. Treatment with hydrophobic chenodeoxycholic acid inhibited abnormal bile acid synthesis (virtual disappearance of C_{27}-bile alcohols from plasma, bile, and urine and marked reduction of plasma cholestanol levels). Hydrophilic ursodeoxycholic and ursocholic acids did not inhibit abnormal bile acid synthesis, while cholestyramine increased abnormal bile acid synthesis (continued increased formation of polyhydroxylated C_{27}-bile alcohols and further elevation of plasma cholestanol levels). Lovastatin did not affect abnormal bile acid synthesis or reduce plasma cholestanol levels. The results demonstrate that impaired side-chain oxidation in bile acid synthesis due to mutations of *Cyp27* results in increased formation of polyhydroxylated C_{27}-bile alcohols and cholestanol in CTX. Hydrophobic chenodeoxycholic acid, but not cholestyramine, lovastatin, or hydrophilic 7β-hydroxy acids, inhibited the abnormal synthetic pathway. The role of chenodeoxycholic acid in downregulating abnormal bile acid synthesis in CTX is emphasized.

CEREBROTENDINOUS xanthomatosis (CTX) is a rare, recessively inherited lipid-storage disease. Clinically, affected subjects present with progressive neurologic disease, tendon and tuberous xanthomas, cataracts, premature atherosclerosis, and endocrine abnormalities. Chemically, large amounts of cholestanol, the 5α-dihydro derivative of cholesterol, and C_{27} polyhydroxylated bile alcohols accumulate and are responsible for the development of progressive neurologic disease, atherosclerosis, and xanthomas.[1] Setoguchi et al first reported that the oxidation and shortening of the 8-carbon cholesterol side-chain in bile acid synthesis was impaired in CTX such that formation of primary bile acids, particularly chenodeoxycholic acid, is diminished, and as a consequence, C_{27} polyhydroxylated bile alcohols (Fig 1) are formed in abundance.[2] Previous studies have demonstrated that replenishing exogenous chenodeoxycholic acid in the enterohepatic pool inhibits abnormal bile acid synthesis, reduces the formation of cholestanol and bile alcohols, and stabilizes the neurologic disease.[3-8] However, ursodeoxycholic acid, the 7β-hydroxy epimer of chenodeoxycholic acid, was shown to be ineffective and abnormal bile acid synthesis continued.[9-11] Bile acid sequestrant, cholestyramine, was shown to exacerbate the condition, apparently due to increased cholesterol synthesis.[12] On the other hand, statins, which suppress cholesterol synthesis, were shown to be ineffective when given alone to a patient with CTX,[13] while they were beneficial when administered in combination with chenodeoxycholic acid.[14]

To gain further insight into the regulation of abnormal bile acid synthesis and effect of therapy in CTX, we compared treatments with chenodeoxycholic acid with its 7β-hydroxy epimer, ursodeoxycholic acid, and even more hydrophilic, ursocholic acid, on plasma and biliary concentrations and urinary outputs of the polyhydroxylated C_{27}-bile alcohols and the levels of cholestanol in plasma. In addition, the effect of intestinal bile acid malabsorption produced by feeding cholestyramine and the inhibition of hydroxymethylglutaryl coenzyme A (HMG-CoA) reductase by treatment with lovastatin was considered in the same patients with CTX.

The studies were conducted in 4 untreated CTX subjects and showed that chenodeoxycholic acid treatment was the most effective and powerful inhibitor of abnormal bile acid synthesis that eliminated polyhydroxylated C_{27}-bile alcohols from plasma, bile, and urine and reduced plasma cholestanol levels. Neither more hydrophilic ursodeoxycholic acid nor ursocholic acid was effective and both failed to inhibit abnormal bile acid synthesis. Similarly, lovastatin did not affect abnormal bile acid synthesis, while cholestyramine, which further depleted the endogenous bile acid pool, increased abnormal bile acid synthesis and the concentrations of the polyhydroxylated C_{27}-bile alcohols and cholestanol in plasma and urine.

MATERIALS AND METHODS

Materials

Cholic acid and nor-cholic acid were purchased from Steraloids Inc (Wilton, NH). Chenodeoxycholic acid was obtained from H. Falk, Freiburg, Germany. Ursocholic acid and ursodeoxycholic acid were gifts from Gipharmax, Milan, Italy. Lovastatin (Mevanolin, E-Merck, Rahway, NJ) was obtained from the hospital pharmacy. Sep-pak C_{18} cartridges were purchased from Waters Associates (Milford, MA). The acetone powder of cholylglycine hydrolase [from *Clostridium perfringens* (welchii)], β-glucuronidase (from *Helix pomatia*), and Sil-Prep (for preparation of the trimethylsilyl ether derivatives) were purchased from Sigma Chemical Co (St Louis, MO). Methanolic hydrochloric acid was obtained from Supelco (Bellefonte, PA). The bile alcohols, 5β-cholestane-3α,7α,12α,25-tetrol and 5β-cholestane-3α,7α,12α,24(R

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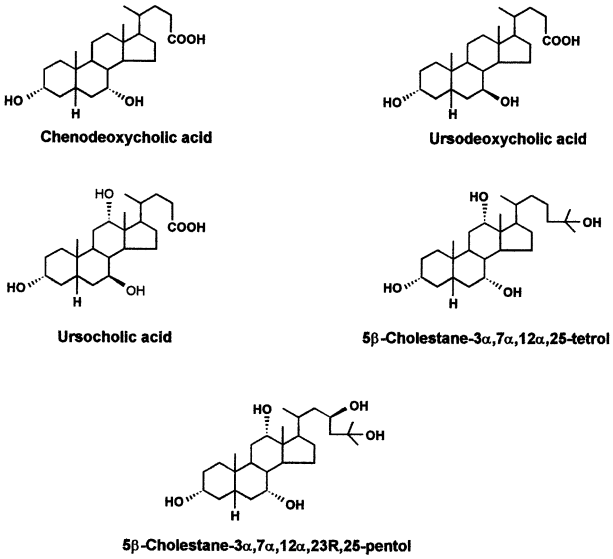

Fig 1. Structures of the bile acids and bile alcohols.

and S),25-pentols were synthesized from cholic acid, and 5β-cholestane-3α,7α,12α,23R,25-pentol was isolated from the bile of a patient with CTX as described previously.[6,15,16]

Clinical

Studies were performed in 4 unrelated male CTX homozygotes. The clinical histories of the patients are described in Table 1.

Experimental Protocol

Patients 1 and 2 were treated for 4 weeks with ursodeoxycholic acid (1 g/d), followed by a 4-week wash-out period without treatment. Ursocholic acid (1 g/d) was then administered for 4 weeks, followed by another 4-week of wash-out period when no drug was given. Both subjects were then treated with 0.75 g/d of chenodeoxycholic acid for 4 weeks. Patients 3 and 4 were treated with 18 g/day of cholestyramine for 4 weeks, followed by a 4-week wash-out period of no treatment. They were then treated with lovastatin (40 mg/d) for 15 weeks, followed by a 4-week wash-out period. They were then treated with chenodeoxycholic acid (0.75 g/d) for 4 weeks. The protocol was approved by the human studies committees at the Veterans Affairs Medical Center, East Orange, NJ, and University of Medicine and Dentistry–New Jersey Medical School, Newark, NJ.

Sample Collection

Blood samples were obtained from each patient before treatment and on the last day of each treatment regimen after an overnight fast of 12 hours and collected with anticoagulant, EDTA. After centrifugation (5,000 $\times$ *g* for 10 minutes) to remove the red blood cells, plasma was collected and stored at -20°C for sterol and bile alcohol analyses. Bile specimens were obtained pretreatment and on the last day of each treatment period after an overnight fast through an endoscope passed into the duodenum. The bile samples were stored at -20°C until used for analysis. Twenty-four–hour urine specimens were collected before treatment and on the last day of each treatment period and aliquots were stored at -20°C until used.

Thin-Layer Chromatography

Thin-layer chromatography of bile acid conjugates was carried out on silica gel O plates (Analabs, New Haven, CT) in a solvent system of chloform:methanol:acetic acid:water, 13:4:2:1 (vol/vol/vol/vol) and the spots were visualized by spraying the plate with phosphomolybdic acid (3.5% in isopropanol) and sulfuric acid (20%) and heating at 110°C for 2 minutes. R_f values of representative bile acid conjugates were as follows: taurocholate, 0.35; taurochenodeoxycholate, 0.45; tauroursodeoxycholate, 0.48; glycocholate, 0.63; glycochenodeoxycholate, 0.72; glycoursodeoxycholate, 0.76.

Gas-Liquid Chromatography

Sterols and bile alcohols in aliquots of plasma, bile, and urine, together with 10 μg coprostanol (for sterol measurement) or 10 μg 24-nor-5β-cholestane-3α,7α,12α,25-tetrol (for bile alcohol measurement) as internal recovery standard, were silylated with 100 μl of Sil-Prep (Supelco, Bellefonte, PA), for 30 minutes at 55°C. After evaporation of solvents under N_2, the trimethylsilyl (TMS) ethers were dissolved in 100 μL hexane and 1 to 5 μL was injected into the gas chromatograph (Hewlett-Packard, Palo Alto, CA, model 5980A, equipped with a split-splitless device for capillary columns). A fused silica CP-Sil-5 CP capillary column (25 m; inner diameter, 0.20 to 0.22 mm) was employed and helium was used as the carrier gas at a flow rate of 1.2 mL/min. The injector and detector temperatures were kept at 260°C. Oven temperature was initially 100°C for 2 minutes, and then programmed to increase at a rate of 35°C/min to a final temperature of 262°C for analysis of sterols and 278°C for analysis of bile alcohols.[17] The response factors of cholesterol and cholestanol were similar and the correction factor was found to be 1.05 for both sterols. The response factors of various bile alcohols were found to be similar and the correction factor corresponding to the peak area of the internal standard was found to be of the order of 1.05 to 1.15 and this correction factor was used for their quantitation. Retention times of various bile alcohols relative to 5α-cholestane (retention time, 13.20 minutes): 5β-cholestane-3α,7α,12α,25-tetrol, 2.01; 5β-cholestane-3α,7α,12α,24R,25-pentol, 2.63; 5β-cholestane-3α,7α,12α,24S,25-pentol, 2.66; 5β-cholestane-

Table 1. Clinical Findings in CTX Patients

Patient No.	Age (yr)	Sex	Clinical Findings
1	35	M	Dementia, spinal cord paresis, Achilles tendon xanthomas, xanthelasma. Symptoms began at age 18.
2	33	M	Achilles tendon xanthomas, tuberous xanthomas of forearm. Symptoms began at age 15.
3[15,35]	47	M	Spastic parplegia, cerebellar ataxia, Achilles tendon xanthomas, right inferior quadrant anopsia in both eyes. Symptoms began at age 12.
4[36]	38	M	Spinal cord paresis, Achilles tendon xanthomas. Symptoms began at age 22.

Table 2. Effect of Treatments on Plasma Sterols and Bile Alcohols in CTX Patients

Treatment	Patient No.	Cholesterol (mg/dL)	Cholestanol (mg/dL)	25-Tetrol (mg/dL)	Pentols (mg/dL)	Total Bile Alcohols (mg/dL)
Patients 1 and 2						
Untreated	1	191	2.30	0.80	0.84	1.64
	2	173	2.42	0.92	0.82	1.74
UDCA	1	180	2.22	1.10	0.90	2.00
	2	196	2.34	0.94	0.98	1.92
UCA	1	202	2.80	1.26	1.00	2.26
	2	182	2.56	1.30	1.11	2.41
CDCA	1	190	0.36	0.04	0.05	0.09
	2	202	0.39	0.06	0.06	0.12
Patients 3 and 4						
Untreated	3	180	2.36	0.73	0.44	1.17
	4	162	2.16	0.85	0.50	1.35
Cholestyramine	3	151	2.86	1.20	0.88	2.08
	4	172	2.90	1.27	0.89	2.16
Lovastatin	3	144	2.40	0.67	0.44	1.11
	4	152	2.48	0.60	0.40	1.00
CDCA	3	188	0.35	0.02	0.05	0.07
	4	205	0.32	0.02	0.04	0.06
Control (n = 6)						
Untreated		185 ± 5	0.2 ± 0.19	tr	tr	tr

NOTE. Values represent the mean values from 2 measurements.

Abbreviations: 25-Tetrol, 5β-cholestane-3α,7α,12α,25-tetrol; Pentols, 5β-cholestane-3α,7α,12α,23R,25-pentol + 5β-cholestane-3α,7α,12α,24S, 25-pentol + 5β-cholestane-3α,7α,12α,24R,25-pentol; tr, trace.

3α,7α,12α,23R,25-pentol, 2.59; 5β-cholestane-3α,7α,12α,23S,25-pentol, 2.56.[18]

Bile acid conjugated were deconjugated with cholylglycine hydrolase and the free bile acids were first converted into their methyl esters (100 μL methanolic hydrochloric acid, 15 minutes at room temperature, followed by evaporation of volatile solvents under N_2 at 55°C) and then converted into their TMS ether derivatives. Nor-deoxycholic acid was used as the internal recovery standard. The methyl ester-TMS ether derivatives were dissolved in 100 μL hexane and 1 to 3 μL was injected into the gas-chromatograph for quantitation. Retention times of various bile acids relative to 5α-cholestane (retention time, 13.20 minutes): lithocholic acid, 1.34; deoxycholic acid, 1.45; chenodeoxycholic acid, 1.49; cholic acid, 1.53; ursodeoxycholic acid, 1.57 and ursocholic acid, 1.62.[19]

Mass Spectrometry

The mass spectra of the bile alcohols, when needed, were obtained on a Hewlett-Packard Model 5972A mass-selective detector coupled to a Model 6890 gas chromatograph using a 25-m CP-Sil-5 CB capillary column.[20]

Isolation of Sterols and Bile Alcohols

Plasma sterols were isolated as described by Salen et al.[8,13] Briefly, plasma (0.2 mL), to which 10 μg coprostanol was added as internal recovery standard, was saponified by heating with 3 mL of 0.5N ethanolic sodium hydroxide for 2 hours at 80°C, followed by extraction of sterols with hexane (4 × 4 mL). Hexane extracts were evaporated to dryness, and residue was subjected to TMS ether formation and an aliquot was injected onto the gas-liquid chromatograph column.

Bile alcohols and bile acids were isolated from plasma (1 mL), bile (0.2 mL), and urine (2 mL). Internal standards of 24-nor-5β-cholestane-3α,7α,12α,25-tetrol (10 μg) and nor-deoxycholic acid (10 μg) were added to each sample, which was then subjected to enzymatic hydrolysis with cholylglycine hydrolase and β-glucuronidase. The liberated free bile alcohols and unconjugated bile acids were isolated by passing through a pre-washed Sep-pak column and the bile alcohols and bile acids were separated via alkali treatment as described in detail by Batta et al.[6] The fraction containing bile alcohols was subjected to TMS ether formation, dissolved in 100 μL hexane, and 1 to 5 μL was injected into the gas chromatograph. The fraction containing bile acids was first subjected to methyl ester formation followed by TMS ether derivatization. After extraction into 100 μL hexane, 1 to 5 μL was injected into the gas chromatograph. All compounds were quantitated by gas-liquid chromatography and their structures were confirmed by gas chromatography–mass spectometry.

RESULTS

Significant amounts of 5β-cholestane-3α,7α,12α,25-tetrol and 5β-cholestane-pentols were found in the plasma from all 4 untreated CTX patients, whereas these compounds were not detected in the plasma from 6 control subjects (Table 2). Similarly, all CTX patients had 10- to 15-fold higher levels of cholestanol as compared with control subjects (0.2 ± 0.2 mg/dL). Bile alcohols were also increased in the bile and urine of these patients (Tables 3 and 4) and consistent with earlier observations, biliary bile alcohols were found to mirror plasma bile alcohols in that 5β-cholestane-3α,7α,12α,25-tetrol was the predominant bile alcohol, whereas the more polar 5β-cholestane-pentols conjugated with glucuronic acid predominated in the urine from both patients.

Table 2 shows the effect of the various treatments on plasma bile alcohol and cholestanol levels in the CTX patients. Cholestanol levels did not decline after treatment with ursodeoxycholic acid in patients and tended to increase after ursocholic acid, cholestyramine, and lovastatin treatments (13.5%, 27.4%, and 8.0%, respectively), even though cholesterol levels de-

Table 3. Effect of Treatments on Biliary Bile Acid and Bile Alcohol Composition in CTX Patients

Treatment	Patient No.	NCA (%)	CA (%)	CDCA (%)	DCA (%)	UDCA (%)	UCA (%)	Other* (%)	Bile Alcohols† (%)
Patients 1 and 2									
Pretreatment	1	4.3	61.0	8.6	3.3	1.3	—	4.3	17.2
	2	4.2	68.0	8.6	3.5	0.5	—	4.3	10.8
UDCA	1	4.0	18.0	13.5	0.9	43.1	—	7.3	13.2
	2	4.8	19.0	14.7	0.9	43.1	—	6.7	10.8
UCA	1	2.4	39.3	2.4	5.9	—	27.8	5.2	17.0
	2	2.4	30.2	2.4	5.5	—	33.7	4.6	21.0
CDCA	1	tr[e]	3.0	89.0	—	6.5	—	1.0	0.5
	1	tr	3.0	82.0	—	13.5	—	1.0	0.5
Patients 3 and 4									
Pretreatment	3	2.8	77.0	3.9	2.0	—	—	7.7	6.6
	4	2.8	84.6	3.7	1.8	—	—	1.7	5.4
Cholestyramine	3	4.0	46.4	2.2	2.2	—	—	18.2	27.0
	4	4.6	40.0	2.2	2.2	—	—	22.2	28.8
Lovastatin	3	3.4	79.0	3.5	1.9	—	—	4.8	7.4
	4	2.2	82.2	4.1	1.9	—	—	4.6	5.0
CDCA	3	tr	1.0	83.4	1.0	13.0	—	1.0	0.6
	4	tr	1.0	85.6	1.0	11.0	—	1.0	0.4
Control (n = 6)									
Untreated		tr	31 ± 6	34 ± 8	27 ± 6	5 ± 3	2 ± 2	1 ± 1	tr

NOTE. Values represent the mean values from 2 measurements.

Abbreviations: NCA, nor-cholic acid; CA, cholic acid; CDCA, chenodeoxycholic acid; DCA, deoxycholic acid; UDCA, ursodeoxycholic acid; UCA, ursocholic acid.

*Contains 1 or more of the following: 1-Hydroxy-cholic acid, 1-hydroxy-deoxycholic acid, 1-hydroxy-ursodeoxycholic acid, 23α-hydroxycholic acid, hyocholic acid, lithocholic acid.

†5β-cholestane-3α,7α,12α,25-tetrol; 5β-cholestane-3α,7α,12α,23R,25-pentol; 5β-cholestane-3α,7α,12α,24S,25-pentol, and 5β-cholestane-3α,7α,12α,24R,25-pentol.

Table 4. Effect of Treatments on Urinary Bile Alcohol Output in CTX Patients[a]

Treatment	Patient No.	25-Tetrol (%)	23R-Pentol (%)	24S-Pentol (%)	24R-Pentol (%)	Total (mg/d)
Patients 1 and 2						
Pretreatment	1	8	36	30	26	275
	2	8	44	26	22	235
UDCA	1	7	43	25	25	370
	2	9	44	23	24	415
UCA	1	7	46	25	22	630
	2	3	58	19	20	710
CDCA	1	tr[c]	54	25	21	8.6
	2	tr	46	29	25	9.2
Patients 3 and 4						
Pretreatment	3	11	32	31	26	138
	4	17	28	29	26	170
Cholestyramine	3	5	55	20	20	615
	4	5	45	24	26	665
Lovastatin	3	13	30	23	34	160
	4	17	30	29	24	110
CDCA	3	tr	50	32	18	4.6
	4	tr	50	30	20	6.2
Control (n = 6)						
Untreated		ND	ND	ND	ND	

NOTE. Values represent the mean values from 2 measurements.

Abbreviations: 25-Tetrol, 5β-cholestane-3α,7α,12α,25-tetrol; 23-pentol, 5β-cholestane-3α,7α,12α,23R,25-pentol; 24S-pentol, 5β-cholestane-3α,7α,12α,24S,25-pentol; 24R-pentol, 5 β-cholestane-3α,7α,12α,24R,25-pentol; ND, not detected.

creased 13.5% with lovastatin. However, chenodeoxycholic acid feeding significantly reduced cholestanol levels to approach those seen in control subjects and tended to raise cholesterol levels such that the ratio of cholesterol/cholestanol rose in all patients to approach values in controls. In agreement with the cholestanol findings, the treatments with ursodeoxycholic acid, ursocholic acid, cholestyramine, and lovastatin did not reduce the levels of plasma bile alcohols. Moreover, plasma bile alcohols seemed to increase after 4 weeks of cholestyramine feeding to patients 3 and 4, as well as in patients 1 and 3 after feeding ursocholic acid. In contrast, treatment with chenodeoxycholic acid for 4 weeks reduced bile alcohol levels by 94% in the plasma in all 4 CTX patients.

Biliary bile acid pattern in the CTX subjects differed from controls. Thus, as seen in Table 3, cholic acid was the major bile acid in all patients, accounting for as much as 80.8% of total bile acids. Cholic acid was present mainly as the taurine conjugate as determined by thin-layer chromatography where no unconjugated cholic acid was detected in bile.[6] In addition to cholic acid, significant amounts of nor-cholic and 23-hydroxycholic acids were also found in the bile and urine of the patients.[21] Chenodeoxycholic and deoxycholic acids were only minor components in the bile. Feeding ursodeoxycholic acid or ursocholic acid to patients 1 and 2 greatly increased the proportions of the 2 fed bile acids in the bile and they became the predominant bile acids. Whereas feeding ursodeoxycholic acid also increased the proportion of biliary chenodeoxycholic acid due to intestinal conversion of ursodeoxycholic acid to chenodeoxycholic acid,[22,23] no such increase in the proportion of cholic acid was seen when ursocholic acid was fed and the proportion of deoxycholic acid was only slightly increased,[24] due to reduced bacterial 7-dehydroxylation in these patients as also evidenced by reduced proportions of deoxycholic acid in bile from untreated patients (Table 3). Both nor-cholic and the 23-hydroxycholic acids were still present in proportions similar to those seen in pretreatment samples. Lovastatin feeding did not appreciably change the biliary bile acid pattern in patients 3 and 4 from their pretreatment pattern,[13] but cholestyramine feeding significantly increased the proportions of nor-cholic acid and 23-hydroxycholic acid. As expected, on feeding, chenodeoxycholic acid became the predominant biliary bile acid in all patients although significant amounts of ursodeoxycholic acid were also detected.[22] Nor-cholic and the 23-hydroxycholic acids virtually disappeared (Table 3).

Biliary bile alcohol levels also were not reduced with any treatment regimen except when chenodeoxycholic acid was instituted in the patients (Table 3). Cholestyramine treatment significantly increased the proportions of biliary bile alcohols in the patients due to increased cholesterol 7α-hydroxylation,[25,26] while lovastatin did not reduce their proportions.[13] Although the proportions of bile alcohols did not change on feeding ursodeoxycholic acid, ursocholic acid feeding increased the proportion of all bile alcohols by 50%. Since the bile acid pool increases when these bile acids are fed to humans,[24,27,28] the absolute amounts of bile alcohols may also have been significantly increased in our patients. Only trace amounts of bile alcohols were detected when chenodeoxycholic acid was fed to the patients.

Greatly increased amounts of 23-hydroxycholic acid and nor-cholic acid were excreted in the urine of all patients (data not shown). These hydrophilic bile acids are virtually absent in urine of control subjects. Both ursodeoxycholic and ursocholic acids were the major bile acids excreted in the urine when fed to patients 1 and 2, and more than half of the fed ursocholic acid was excreted daily in the urine (Batta AK, Salen G, unpublished data). Excretion of nor-cholic and 23-hydroxycholic acids was also increased on feeding hydrophilic ursodeoxycholic and ursocholic acids. Lovastatin again had no significant effect on urinary bile acid excretion, but significantly increased amounts of 23-hydroxycholic acid were excreted when cholestyramine was fed to patients 3 and 4. Once again, chenodeoxycholic acid almost normalized urinary bile acid pattern and only trace amounts of nor-cholic and 23-hydroxycholic acids were seen in the urine.

The detrimental effect of ursocholic acid feeding was also observed on examination of urinary bile alcohol excretion when almost 2.5-fold increase in the amounts of the 5β-cholestane-pentols was seen in the urine (Table 4). Urinary bile alcohol levels tended to increase from their pretreatment levels after ursodeoxycholic acid feeding also, but lovastatin did not show any significant effect when fed to patients 3 and 4. Cholestyramine feeding resulted in almost 4-fold increase in excretion of urinary bile alcohols in patients 3 and 4 (Table 4). Chenodeoxycholic acid resulted in a 30 to 40-fold reduction in the urinary bile alcohol excretion in the patients.

DISCUSSION

The results of this investigation in 4 patients with CTX illustrate the bile acid synthetic defect and the effect of treatment with hydrophilic and hydrophobic bile acids, lovastatin, and cholestyramine on plasma, biliary, and urinary concentrations of bile alcohols and cholestanol. The bile acid synthetic defect in CTX results from inherited mutations in *CYP27* that is involved in cleavage of the cholesterol side chain.[29] As a result, C_{27}-bile alcohols are formed that can not be converted to C_{24} primary bile acids and production and pool sizes of the primary bile acids, particularly chenodeoxycholic acid, are reduced.[2] Cholestanol formation and levels rise in plasma and bile because cholesterol and 7α-hydroxycholesterol are diverted from bile acid synthesis to cholestanol.[3]

It was recognized that treatment with chenodeoxycholic acid to replace the most deficient bile acid in the pool effectively inhibited abnormal bile acid synthesis resulting in the virtual disappearance of C_{27}-bile alcohols from plasma, bile, and urine and a marked reduction in plasma cholestanol levels.[1] Coincidently, there was remarkable clinical improvement with clearing of dementia and stabilization of abnormal neurologic function.[4] Recently, the mechanism by which chenodeoxycholic acid downregulates bile acid synthesis has been investigated. According to current information, the feedback regulation of cholesterol 7α-hydroxylase, the rate-controlling enzyme in classic bile acid synthesis, is carried out through the orphan nuclear receptor, FXR.[30] In the nucleus of hepatocytes, FXR combines with RXR to form a heterodimer that requires hydrophobic bile acids for activation. Chenodeoxycholic acid is highly hydrophobic and is the most powerful ligand that activates the FXR/RXR heterodimer,[31] which in turn stimulates the

transcription of the short heterodimer partner, SHP, that binds to the transcription factor, FTF. The removal of FTF from the *CYP7A* promoter by attachment to SHP reduces *CYP7A* transcription and the formation of 7α-hydroxycholesterol for classic bile acid synthesis. Thus, in untreated CTX subjects, where the formation and pool of chenodeoxycholic acid are very deficient, cholesterol 7α-hydroxylase-mRNA and activity are upregulated and the synthesis of 7α-hydroxycholesterol is markedly increased.[32] However, because side chain cleavage is impaired, synthesis of C_{24}-bile acids, particularly chenodeoxycholic acid, is diminished with the diversion of precursors to the formation of C_{27}-bile alcohols and cholestanol. Replenishing chenodeoxycholic acid in the enterohepatic pool at a daily dose 33% greater than normal bile acid synthesis activates FXR/RXR heterodimer and downregulates *CYP7A* such that only exogenous chenodeoxycholic acid circulates in the enterohepatic bile acid pool and the C_{27}bile alcohols are virtually eliminated from plasma, bile, and urine, associated with a decline in plasma cholestanol and 7α-hydroxycholesterol levels. Thus, CTX is an example of markedly upregulated *CYP7A* with the plentiful formation of 7α-hydroxycholesterol and other bile acid precursors where synthesis of C_{24}bile acids cannot be completed because of the mutated *CYP27*. Consequently, C_{27}bile alcohols, reflecting incomplete oxidation of the cholesterol side chain, are formed and circulate in plasma and are excreted in bile and urine. It is also hypothesized that the C_{27}-bile alcohols, which are present as glucuronides,[5] damage the blood-brain barrier and allow the uptake and deposition of cholesterol and cholestanol attached to low-density lipoproteins (LDLs) in the brain with consequent nervous system damage. In this regard, it is noteworthy that *CYP27* knockout mice with a null mutation of *CYP27* do not overproduce C_{27}bile alcohols or cholestanol and do not show a CTX phenotype.[33] In these mice, in distinction to CTX subjects, the alternative C-25 side-chain cleavage pathway is upregulated 3- to 5-fold such that C_{27}bile alcohols can be converted to cholic acid by this alternative 25-hydroxylation side-chain cleavage pathway that is not increased in CTX.

In contrast to chenodeoxycholic acid, treatment with either ursodeoxycholic acid, the 7β-hydroxy epimer of chenodeoxycholic acid, or even more hydrophilic ursocholic acid, the 7β-hydroxy epimer of cholic acid, at equivalent dosage did not downregulate endogenous bile acid synthesis as evidenced by the continued substantial excretion of polyhydroxylated C_{27}bile alcohols in urine,[10] and the high concentrations of cholestanol, and as reported by Koopman et al, 7α-hydroxycholesterol,[32] in plasma. In contrast to chenodeoxycholic acid, both of these hydrophilic bile acids are poor ligands of FXR, and therefore, *CYP7A* remains upregulated as FXR is deactivated.[31] Due to reduced intestinal 7-dehydroxylation in patients with CTX, the fed ursocholic acid is not appreciably transformed into deoxycholic acid. The result is that bile alcohol formation is rather greatly increased after feeding ursocholic acid, as evidenced by the increased proportion in plasma, bile, and particularly, urine.

Two additional points require discussion. When cholestyramine, which binds bile acids in the intestine and reduces their return to the liver, was administered to 2 patients, plasma cholestanol and urinary bile alcohol levels rose. The induced bile acid malabsorption further depleted the endogenous bile acid pool, increased *CYP7A* activity, and increased formation of early bile acid precursors that could not be converted to C_{24}-bile acids.[12] Although not appropriate for therapy, cholestyramine has been suggested as a provocative test to detect mildly affected CTX homozygotes or heterozygotes who will respond to cholestyramine and increase urinary C_{27}-bile alcohol outputs and plasma cholestanol levels when challenged.[26] More importantly, the statin drug, lovastatin, was ineffective in reducing plasma cholestanol or bile alcohol levels. Lovastatin inhibits HMG-CoA reductase and increases the expression of LDL receptors but did not affect abnormal bile acid synthesis in our CTX patients. The enhanced expression of LDL receptors may increase the uptake of LDL enriched with cholestanol to increase tissue deposition while bile alcohols continue to circulate. Moreover, the ratio of cholestanol to cholesterol, which is normally 1:1,000, and rises to 1:100 or greater in untreated CTX patients, was not reduced by lovastatin and, thus, lovastatin cannot be recommended for treatment.[13,34]

In summary, CTX is due to inherited mutations of *CYP27*, which prevents the oxidation of cholesterol side chain such that polyhydroxylated C_{27}bile alcohols are formed and C_{24}-bile acids are deficient. The absence of chenodeoxycholic acid from the enterohepatic circulation deactivates FXR allowing for the upregulation of *CYP7A* and early bile acid synthesis, with the diversion of 7α-hydroxycholesterol and cholesterol to cholestanol. Replacing chenodeoxycholic acid in the enterohepatic pool activates FXR/RXR and inhibits elevated *CYP7A* to downregulate the abnormal bile acid synthetic pathway, reducing bile alcohol and cholestanol levels. Substituting 7β-hydroxy bile acids, ursodeoxycholic acid, and ursocholic acid, which are hydrophilic, does not activate FXR and does not inhibit abnormal bile acid synthesis nor reduce bile alcohol or cholestanol levels. Similarly, cholestyramine or lovastatin are ineffective in downregulating abnormal bile acid synthesis and cannot be recommended for treatment of CTX.

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