Synthesis and intestinal metabolism of ursodeoxycholic acid conjugate with an antiinflammatory agent, 5-aminosalicylic acid

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Abstract **5-Aminosalicylic acid conjugate of ursodeoxycholic acid was synthesized in above 90% yield by adding a basic solution of 5-aminosalicylic acid into the mixed anhydride formed with ursodeoxycholic acid and ethyl chloroformate. The 5-aminosalicylic acid conjugate of ursodeoxycholic acid was poorly secreted into the bile and was deconjugated with cholylglycine hydrolase and *Clostridium perfringens*, that deconjugate naturally occurring glycine and taurine conjugates of bile acids. However, ursodeoxycholic acid 5-aminosalicylic acid conjugate was not absorbed from the duodenum but was concentrated in the colon where it was partially hydrolyzed by the intestinal bacteria to ursodeoxycholic acid and 5-aminosalicylic acid. We believe that this unique conjugation of ursodeoxycholic acid with 5-aminosalicylic acid may facilitate the transport of both 5-aminosalicylic acid and ursodeoxycholic acid to the colon and may be useful for the treatment of colonic inflammatory bowel diseases, ulcerative colitis and Crohn's disease.—**Batta, A. K., G. S. Tint, G. Xu, S. Shefer, and G. Salen. **Synthesis and intestinal metabolism of ursodeoxycholic acid conjugate with an antiinflammatory agent, 5-aminosalicylic acid. *J. Lipid. Res.* 1998.** 39: **1641–1646.**

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5-Aminosalicylic acid (5-ASA) has been widely used for the treatment of active inflammatory bowel disease, ulcerative colitis, and Crohn's disease, and prevention of their relapses (1). However, because significant amounts of free 5-ASA are absorbed from the small intestine and excreted into the urine, the effective colonic dose is reduced (2). In order to target this drug to the colon, 5-ASA is conjugated with sulfapyridine (sulfasalazine) or other amines (e.g., olsalazine), via an azo bond, the azo compound being poorly absorbed from the intestine and cleaved by colonic bacteria to release 5-ASA in the colon. However, due to poor tolerance to sulfasalazine, in particular, allergy to sulfapyridine that is released by the bacterial action and absorbed from the colon (3), other modifications of 5-ASA have been used, e.g., enterocoated time release formulation, rectal suppositories, or as a dimer with two units of 5-ASA combined by a diazo bond (4–6). The diazo compound is poorly absorbed from the small intestine, and the compound is transported to the colon where the 5-ASA is released by the intestinal bacteria (4–6). We considered bile acids as vehicles to target 5-ASA to the colon, as bile acids are natural bile constituents in animals and some bile acids are used therapeutically for cholelithiasis and liver disease (7–9).

Bile acids are the end products of cholesterol metabolism in the liver and they represent the major catabolic pathway for body cholesterol. They also facilitate fat absorption and control cholesterol synthesis by exerting negative feedback inhibition on the rate-controlling hepatic enzyme, 3-hydroxy-3-methylglutaryl coenzyme-A reductase. Once formed in the liver, bile acids are conjugated with the amino acids, glycine and taurine before secretion into the bile. These bile acid conjugates are effectively reabsorbed from the ileum during their intestinal passage, but approximately 1–2% escape reabsorption during each transit and seep into the colon. There, the bile acid conjugates are hydrolyzed by colonic anaerobic bacteria and further metabolized into secondary bile acids. Although bile acids mainly function in intestinal absorp-

Abbreviations: 5-ASA, 5-aminosalicylic acid; UDCA, ursodeoxycholic acid (3α,7β-dihydroxy-5β-cholanoic acid); LCA, lithocholic acid, (3α-hydroxy- 5β-cholanoic acid); DCA, deoxycholic acid (3α,12α-dihydroxy-5β-cholanoic acid); CDCA, chenodeoxycholic acid (3α,7α-dihydroxy-5β-cholanoic acid); CA, cholic acid (3α,7α,12α-trihydroxy-5β-cholanoic acid); HyoDC, hyodeoxycholic acid (3α,6α-dihydroxy-5β-cholanoic acid); α-MC, α-muricholic acid, (3α,6β,7α-trihydroxy-5β-cholanoic acid); β-MC, β-muricholic acid (3α,6β,7β-trihydroxy-5β-cholanoic acid); ω-MC, ω-muricholic acid (3α, 6α, 7β-trihydroxy-5β-cholanoic acid); TLC, thin-layer chromatography; IR, infra-red spectroscopy; GLC, gas–liquid chromatography; HPLC, high-performance liquid chromatography; EIMS, electron-impact mass spectrometry; NMR, nuclear magnetic resonance spectrometry; MHz, megahertz; ppm, parts per million; DEPT, distortionless enhancement by polarization transfer; TMS, trimethylsilyl.

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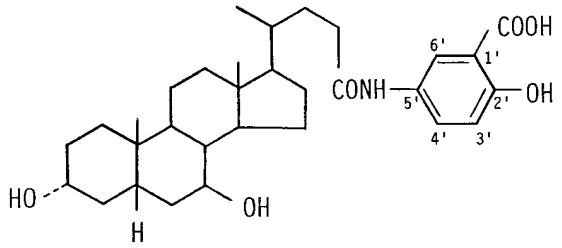

Fig. 1. Chemical structure of the 5-aminosalicylic acid conjugate of ursodeoxycholic acid.

tion of lipids during food ingestion and their transport to the liver, recent studies suggest that they may be used to transport and target drugs to the liver (10, 11).

We report the synthesis, biliary secretion, and intestinal metabolism of the conjugate of ursodeoxycholic acid (UDCA) with 5-ASA (UDCA-5-ASA; **Fig. 1**) and believe that this conjugated bile acid will release 5-ASA in the colon. Our hypothesis is that this dianionic bile acid conjugate will not be actively absorbed from the ileum and will be excreted into the colon and the intestinal bacteria will release the 5-ASA in the colon, its site of action as an anti-inflammatory agent. In addition, the liberated UDCA may also be cytoprotective (12). UDCA has been shown to protect hepatocytes from toxic effects of detergent bile acids (12, 13) and this mechanism is considered to be beneficial in hepatobiliary diseases. Further, the UDCA liberated in the colon may be beneficial in colonic polyp reduction (14) and it may be partially absorbed from the colon and also circulate in the enterohepatic circulation.

MATERIALS AND METHODS

Materials

5-Aminosalicylic acid was purchased from Aldrich Chemical Co. (Milwaukee, WI) and UDCA was a gift from Tokyo Tanabe, Japan. Sep-Pak cartridges were purchased from Waters Associates (Milford, MA). The acetone powder of cholylglycine hydrolase [from *C. perfringens* (*welchii*)] was from Sigma Chemical Co. (St. Louis, MO). Sil-Prep (hexamethyldisilazane: trimethylchlorosilane: pyridine, 3:1:9) used for making trimethylsilyl ether was purchased from Alltech Associates, Inc., Deerfield, IL. All reagents and solvents used were reagent grade and were purchased from Aldrich Chemical Co. Deuterated dimethylsulfoxide was purchased from Aldrich Chemical Co. and was above 99% pure.

The elemental analysis of the synthesized compound was performed at the Spang Microanalytical Laboratory (Eagle Harbor, MI). Melting point was determined on a Thermolyne 12,000 apparatus and is uncorrected. Infra-red spectrum was obtained on a Perkin-Elmer model 421 spectrophotometer as KBr disk.

Thin-layer chromatography (TLC)

TLC of the bile acid conjugates was carried out on precoated silica-gel plates (0.25 mm thickness, Analabs, New Haven, CT). Plates were developed in a solvent system of chloroform–methanol–acetic acid 40:5:3 (v/v/v). After development, spots were visualized by spraying the plates with phosphomolybdic acid (3.5% in isopropanol) followed by a spray with 10% sulfuric acid and subsequent heating at 110°C for 2 min.

Gas–liquid chromatography (GLC)

A Hewlett-Packard model 5890A gas chromatograph equipped with a flame ionization detector and an injector with a split/splitless device for capillary columns was used. The chromatographic column consisted of a chemically bonded fused silica CP-Sil-5 CB (stationary phase, 100% dimethylsiloxane) capillary column (25 m × 0.22 mm I.D.) (Chrompack, Inc., Raritan, NJ) and helium was used as the carrier gas at a flow rate of 1 ml/min. The GLC operating conditions were as described in earlier publication (15). The methyl ester of UDCA was formed with 3% anhydrous methanolic hydrochloric acid and was then reacted with Sil-Prep to obtain the trimethylsilyl (TMS) ether derivative (15). A 2 μl aliquot of the derivative in hexane was injected into the GLC column.

High-performance liquid chromatography (HPLC)

HPLC of UDCA-5-ASA conjugate was performed on a Waters Associates (Millford, MA) Model M-6000 reciprocating pump and a Model UK6 loop injector. A Waters Associates Model 401 differential refractometer was used and the detector response was recorded with a Spectra-Physics (San Jose, CA) Model SP 4290 integrator. A Waters Associates Radial-Pak μBondapak C_{18} reversed-phase column (100 × 8 mm I.D., 5 μm particle size) was used for the chromatography. A guard column (Waters Associates) with C_{18} reversed-phase material was placed before the separation column. Five–10 μg of the conjugate dissolved in 5 μl methanol was injected into the HPLC column. A solvent system consisting of water–methanol–acetic acid 350:650:33 (v/v/v), whose pH was brought to 4.7 with addition of 10 N sodium hydroxide, was used (16) and the flow rate was maintained at 2 ml/min (operating pressure, ca 10.3×10^3 Kpa).

Mass spectrometry

The electron impact mass spectrum (EIMS) of synthesized ursodeoxycholic acid 5-aminosalicylic acid conjugate was performed on a Hewlett-Packard Model 5988 gas chromatograph–mass spectrometer in the direct injection probe (DIP) mode at a probe temperature of 100°C increased at a rate of 35°C/min to a final temperature of 220°C. The mass spectrum for the peak at 4.60 min in the total ion current versus time plot was obtained.

Nuclear magnetic resonance spectroscopy (NMR)

The high-resolution proton NMR spectrum of the bile acid conjugate was obtained at 400 MHz in deuterated dimethylsulfoxide on a Varian Associates XL-400 spectrometer equipped with Fourier transform mode and tetramethylsilane was used as the internal standard. The ^{13}C-NMR spectra were performed at 50.4 MHz in deuterated dimethylsulfoxide as solvent and tetramethylsilane was used as the internal standard. The chemical shifts (δ) are expressed in parts per million (ppm) relative to tetramethylsilane and are accurate to ±0.05 ppm. The spectra were recorded in a proton noise-decoupled mode in order to measure the exact chemical shifts of all ^{13}C nuclei present. In order to obtain carbon multiplicities, 90° and 135° DEPT (distortionless enhancement by polarization transfer) spectra were recorded. In this mode, only the primary, secondary, and tertiary carbons appeared, the primary and tertiary carbons above the baseline while the secondary carbons were below the baseline. The positions of the quaternary carbons were determined by subtraction from the noise-decoupled spectra.

Synthesis of UDCA-5-ASA

UDCA (20 g) was dissolved in 200 ml of dioxane at 15°C and 8 ml triethylamine was added. To the cold solution, 5 ml of ethyl chloroformate was added and the contents were kept at 15°C for 15 min. A solution of 5-ASA (11 g) in 70 ml 1 N sodium hydroxide was then added to the suspension and the clear solution ob-

TABLE 1. ^{13}C signals in the 50.3 MHz ^{13}C-NMR spectrum of UDCA-5-ASA conjugate[a]

Carbon	^{13}C-Signal	Carbon	^{13}C-Signal
	δ (ppm)		*δ ppm*
1	34.755	17	55.795
2	30.155	18	11.931
3	69.364	19	23.218
4	37.603	20	34.930
5	42.103	21	18.415
6	37.173	22	31.524
7	69.620	23	33.165
8	42.854	24	170.861
9	38.979	1′	157.819
10	33.641	2′	118.018
11	20.778	3′	115.487
12	40.135	4′	121.533
13	42.103	5′	129.266
14	54.659	6′	124.664
15	26.672	–COOH	172.094
16	28.115		

[a] 50.4 MHz spectra. Chemical shifts (δ) in ppm referenced to tetramethylsilane signal.

tained was stirred for 2 h at room temperature (17). The contents were poured into 1 liter ice-cold water and acidified with 50% hydrochloric acid to pH 1. The grey-colored precipitate was filtered and washed thoroughly with water. The product (26.5 g) was poured over a column of silica gel (600 g) and eluted with ethyl acetate followed by increasing proportions of methanol. Elutions with ethyl acetate (2 liter) and ethyl acetate–methanol (99:1; 2 liter) yielded 800 mg of unreacted UDCA. Further elution of the column with ethyl acetate–methanol (96:4; 6 liter) yielded 24.8 g (92% overall yield) of a light grey solid which was crystallized from methanol–ethyl acetate to cream colored microscopic crystals, single spot on TLC, R_f, 0.65; melting point, 232–236°C (decom.). Anal. calc. for $C_{31}H_{45}O_5N$: C, 70.59; H, 8.53 and N, 2.66%. Found: C, 70.48; H, 8.59 and N, 2.63%. IR (cm^{-1}): 3372, 3342 (–OH), 1661 (–COOH), 1640 (–CONH–). EIMS (*m/z*): 527 (M^+, 0.1%), 491 (M^+–2 × H_2O, 0.5%), 374 (M^+–5-ASA, 0.9%), 356 (*m/z* 374–H_2O, 2.2%), 341 [*m/z* 374–(H_2O + CH_3), 1.8%], 323 (*m/z* 341–H_2O, 1.0%), 255 (M^+–side chain, 5.1%), 213 (*m/z* 255–rind D, 6.7%), 153 (5-ASA moiety + H, 38.8%), 151 (5-ASA moiety–H, 40.0%), 135 (aromatic ring ($C_7H_5O_3$–2H, 71%), 79 (100%). It showed the following signals in the ^1H-NMR (δ, ppm) spectrum: 0.62 (3H, s, 18–CH_3), 0.87 (3H, s, 19–CH_3), 0.93 (3H, d, J = 6.4 Hz, 21–CH_3), 3.84 (2H, m, 3β-H and 7α-H), 6.63 (1H, d, J = 8.8, 3′–H), 7.45 (1H, dd, J_1 = 2.8; J_2 = 9.0, 4′–H), 7.88 (1H, d, J = 2.8, 6′–H), 9.59 (1H, s, –CONH–). The ^{13}C-NMR signals are given in **Table 1**.

Hydrolysis of UDCA-5-ASA conjugate with cholylglycine hydrolase

Sodium salt of UDCA-5-ASA was prepared by dissolving UDCA-5-ASA (100 mg) in 1.25 ml of 0.2 N sodium hydroxide in methanol and precipitating the solution into chilled anhydrous ether. The cream-colored precipitate was filtered, washed with ether, and dried. The sodium salt of UDCA-5-ASA (2 mg) was dissolved in a solution containing acetate buffer, pH 5.6 (1 ml), 0.87% mercaptoethanol (0.5 ml), and 1.86% EDTA (0.5 ml) and 15 units of cholylglycine hydrolase were added (18). The incubation mixture was shaken at 37°C for 18 h and then passed through a pre-washed Sep-Pak and the bile acid was eluted with 5 ml methanol. After evaporation of the solvent under N_2 at 55°C, the product was subjected to TLC. The incubation product was then treated with 0.2 ml of 3% anhydrous methanolic hydrochloric acid for 2 h, solvent evaporated at 55°C under reduced pressure, and an aliquot was silylated (15) and subjected to gas–liquid chromatography.

Hydrolysis of UDCA-5-ASA with *C. perfringens*

A pure culture of *C. perfringens* was anaerobically incubated at 37°C in chopped meat broth containing 0.2% sodium salt of UDCA-5-ASA for 18 h (18). The products were then passed through a pre-washed Sep-Pak and bile acids were eluted with methanol. An aliquot of the extract was subjected to TLC while another aliquot was subjected to methylation with 3% anhydrous methanolic hydrochloric acid followed by trimethylsilylation and GLC.

Biliary excretion of UDCA-5-ASA in bile fistula rat

Three hours after creation of bile fistula in four rats, a bolus of 40 mg of sodium salt of UDCA-5-ASA was infused into the duodenum in 1 ml saline solution. Hourly bile was collected 2 h before and 3 h after infusion of the compound and analyzed for free UDCA and its 5-ASA conjugate by a combination of HPLC and GLC before and after rigorous alkaline hydrolysis. For comparison, in another group of four bile fistula rats, 40 mg of sodium salt of tauroUDCA was infused and bile was collected and analyzed in a similar fashion.

Intestinal metabolism of UDCA-5-ASA in the rat

The sodium salt of UDCA-5-ASA was mixed in rat chow to a proportion of 1% and fed to 4 rats for a period of 14 days while another four rats were fed the rat chow without added bile acid and were used as controls. Rats ate on an average 28 *g* of chow per day (equivalent to 280 mg of UDCA-5-ASA per day). Feces were collected on the last day, weighed, and freeze-dried until bile acid analysis. At the end of the study, bile fistula was constructed and bile was collected for a period of 1 h. Each dried fecal sample (from the bile acid-fed and the control animals) was thoroughly ground and 500 milligram of each specimen was thoroughly extracted overnight with 1% ammoniacal solution of ethyl alcohol. After removal of solvent, the residue was taken up in methanol and divided into two equal aliquots and each aliquot was analyzed for free and conjugated UDCA by a combination of HPLC and GLC. Thus, one aliquot was analyzed by HPLC to calculate the proportions of free as well as conjugated (with glycine, taurine and 5-ASA) UDCA and the other aliquot was first subjected to alkaline hydrolysis (4 N sodium hydroxide, 3 h at 115°C) and the liberated total free bile acids were quantitated by GLC. Bile samples from experimental rats as well as control rats were similarly analyzed for free and conjugated bile acids by HPLC and GLC both before and after alkaline hydrolysis.

RESULTS

The synthesis of UDCA-5-ASA was attempted by methods developed by Tserng, Hachey, and Klein (19) and by Norman (20) that are commonly used for the synthesis of the glycine and taurine conjugates of bile acids. However, the method of Tserng et al. (19) failed due to apparent insolubility of 5-ASA in ethyl acetate used as solvent. Use of dimethylformamide as the solvent produced only poor yield of the compound. On the other hand, the mixed anhydride method of Norman (20) produced the 5-ASA conjugate in almost quantitative yield. Thus, the mixed anhydride of UDCA and ethyl chloroformate was stirred with a basic aqueous solution of 5-ASA for 3 h followed by acidification to pH 1 and the light grey precipitate was filtered.

The 5-ASA conjugate isolated in this way was purified by column chromatography to a final yield of over 90%. The structure of the compound was confirmed from analysis of its spectral data. Thus, the chelated hydroxyl and carboxyl groups were indicated by the infra-red spectrum at 3342 and 1661 cm^{-1} and the amide group appeared at 1640 cm^{-1}. In the mass spectrum of the underivatized compound, a weak molecular ion peak was observed at *m/z* 527. The ion peak at *m/z* 491 was due to loss of two water molecules, while ion peak at *m/z* 374 resulted from cleavage of the C–N bond of the amide moiety and ion peaks at *m/z* 151 and 153 were due to the 5-ASA moiety. Ion peaks at *m/z* 356, 341, and 323 may have resulted after further loss of 18 mass units, followed by loss of a methyl group and another 18 mass units from ion peak at *m/z* 374. Cleavage of complete side chain resulted in ion peaks at *m/z* 255 and at *m/z* 213 after cleavage of ring D. In the ^1H NMR spectrum, the resonances due to the C–18 and C–19 methyl protons appeared at δ0.62 and 0.87 ppm, respectively, and the resonance due to the C–21 protons appeared as a doublet at δ0.93 ppm (J = 6.4 Hz). The –CONH– proton appeared downfield at δ9.59 ppm as a singlet. As expected, the aromatic proton at C–3′ appeared as a doublet (J = 8.8 Hz) and the C–6′ proton, flanked by the carbons carrying the –COOH and the –CONH– groups, appeared downfield at δ7.88 ppm as a doublet (J = 2.8 Hz), due to meta coupling by C–4′ proton. The proton at C–4′ appeared at δ7.45 ppm as a doublet of doublet (J_1 = 9.0 Hz; J_2=2.8 Hz), the doublet due to the o-coupling (J = 9.0 Hz) was further split by *m*-coupling due to the C–6′ proton (21). In the ^{13}C-NMR spectrum, the resonances due to carbons 1–23 of the bile acid moiety appeared at their expected positions (22, 23) and the C–24 amide carbon appeared at δ170.861 ppm. The carbons of the 5-ASA moiety were assigned from published data (21).

The 5-ASA conjugate of UDCA behaved similarly to glycoursodeoxycholic acid on both TLC and reversed-phase HPLC. The R_f value of the compound on TLC was slightly higher than that of glycoursodeoxycholic acid (0.65 vs. 0.60) and it was eluted later than glycoursodeoxycholic acid on HPLC, (8.3 min vs. 7.6 min), thereby suggesting that this conjugate may be of similar hydrophilicity as glycoursodeoxycholic acid. The amide bond was cleaved with strong alkali and when incubated with cholylglycine hydrolase under conditions known to cleave the glycine and taurine conjugates of bile acids (24), free UDCA was obtained with no detectable amounts of the unreacted compound. Similarly, when a pure strain of *C. perfringens* was grown in chopped meat broth containing UDCA-5-ASA conjugate, free UDCA was quantitatively recovered. Thus, the amide bond in this unnatural conjugate of UDCA was susceptible to cleavage with intestinal bacteria.

To study the biliary secretion of UDCA-5-ASA in the rat, the sodium salt of UDCA-5-ASA was infused into the duodenum of bile fistula rats and bile was collected before and after bile acid infusion and analyzed for free UDCA and UDCA-5-ASA. As shown in **Table 2**, only 2.5% of infused dose of the compound was recovered in the bile in 3 h and no free UDCA was found. Under the same conditions, 90% of infused tauroUDCA was recovered in the bile in 3 h and constituted 60% of the biliary bile acids. The major portion of the ingested UDCA-5-ASA was found to end up in the colon, as shown below, so that urinary excretion, if any, may be small.

In order to study the intestinal metabolism, UDCA-5-ASA was fed to four rats in the chow (1% by weight of the diet) for 14 days. Feces were collected on the last day and analyzed for free and conjugated bile acids (**Table 3**). In control rats, the secondary bile acids hyodeoxycholic acid and deoxycholic acid were the major fecal bile acids (50% and 20%, respectively, of the total fecal bile acids). However, after treatment with UDCA-5-ASA, UDCA was the major fecal bile acid and constituted 95% of the total bile acids excreted in the feces. Total fecal output increased from 10.2 mg/day to almost 215 mg/day after bile acid feeding (Table 3). Further, 37% of the excreted UDCA was found to be unconjugated while 63% was present as the 5-ASA conjugate. Other bile acids like hyodeoxycholic acid, deoxycholic acid, lithocholic acid, and β- and ω-muricholic acids amounted to a total of 5% of the excreted bile acids.

Whereas cholic acid was the major biliary bile acid in control rats and constituted almost 68% of total bile acids, UDCA became the predominant bile acid in the bile after UDCA-5-ASA was fed to the rats and its proportion rose from 2.7% to 40.0%. Contrary to the fecal UDCA which was over 60% conjugated with 5-ASA, the biliary UDCA

TABLE 2. Biliary bile acids after bolus infusion of UDCA-5-ASA into bile fistula rats

Rat	DCA	α-MC	CA	UDCA	β-MC	ω-MC	Total
			%				*mg/ml*
Control[a]	2.4 ± 0.4	9.5 ± 2.5	65.2 ± 4.5	2.4 ± 1.1	17.5 ± 4.4	3.0 ± 1.0	4.48 ± 0.58
TauroUDCA[b]	1.0 ± 0.5	2.9 ± 1.1	29.1 ± 5.6	60.0 ± 5.9	5.9 ± 2.1	1.1 ± 1.0	9.03 ± 1.23
UDCA-5-ASA[c]	1.7 ± 0.5	11.2 ± 2.3	60.9 ± 6.4	4.9 ± 1.0[d]	18.2 ± 3.2	3.1 ± 0.8	4.63 ± 0.46

Values reported are mean of 4 animals. DCA, deoxycholic acid; α-MC, α-muricholic acid; CA, cholic acid; UDCA, ursodeoxycholic acid; β-MC, β-muricholic acid; ω-MC, ω-muricholic acid.

[a] Two h after creation of bile fistula, bile was collected for 1 h and analyzed by HPLC and GLC.

[b] Bile was collected for 3 h immediately after infusion of tauroUDCA into the duodenum of bile fistula rats and analyzed by HPLC and GLC.

[c] Bile was collected for 3 h immediately after infusion of UDCA-5-ASA into the duodenum of bile fistula rats and analyzed by HPLC and GLC.

[d] Contains equal proportions of tauroUDCA and UDCA-5-ASA as determined by HPLC.

TABLE 3. Fecal bile acids in rats after treatment with UDCA-5-ASA for 14 days

Rat	LCA	DCA	HyoDC	α-MC	UDCA	β-MC	ω-MC	Total
				%				*mg/day*
Control[a]	1.0 ± 1.0	20.0 ± 5.3	50.0 ± 7.3	5.0 ± 2.5	5.0 ± 1.5[b]	12.0 ± 3.2	8.0 ± 2.5	10.2 ± 2.3
UDCA-5-ASA[c]	0.5 ± 0.2	0.5 ± 0.2	1.0 ± 0.2	—	95.0 ± 5.0[d]	2.0 ± 0.2	1.0 ± 1.0	215.1 ± 15.5

Values reported are mean of 4 animals. LCA, lithocholic acid; DCA, deoxycholic acid; hyoDC, hyodeoxycholic acid; α-MC, α-muricholic acid; CA, cholic acid; UDCA, ursodeoxycholic acid; β-MC, β-muricholic acid; ω-MC, ω-muricholic acid.

[a] Feces were collected on the last day of feeding rat chow for 14 days and freeze-dried and an aliquot was used for bile acid analysis.

[b] Free UDCA as judged by HPLC analysis.

[c] UDCA-5-ASA (1% by weight) was uniformly mixed with rat chow and fed to rats for 14 days. Feces were collected on the last day and freeze-dried and an aliquot was used for bile acid analysis.

[d] Contains free UDCA, 35%, and UDCA-5-ASA, 65%, as judged by HPLC analysis.

was predominantly taurine-conjugated; thus, UDCA-5-ASA constituted 5% of total bile acids while tauroUDCA was 35% of total biliary bile acids. UDCA-5-ASA was not found in the control bile samples. The total bile acid output in the bile increased by almost 50% after treatment with UDCA-5-ASA, the increase being mainly due to conjugates of UDCA (**Table 4**). The relative proportion of β-muricholic acid and all bile acids other than UDCA was reduced from the pretreatment values, although the total amounts did not change significantly.

DISCUSSION

UDCA, the bacterial product of chenodeoxycholic acid, has been found to have application in gallstone dissolution and the treatment of cholestatic liver diseases (25, 26). Recent studies have also shown that UDCA may be beneficial in colon polyp reduction (14). 5-ASA, on the other hand, is an antiinflammatory drug that is used combined with sulfapyridine (sulfasalazine) or other amines (e.g., olsalazine) or in enterocoated or slow-release formulations for treatment of active ulcerative colitis and prevention of relapses (1–6). The conjugation of 5-ASA with UDCA is considered to be a good way to direct 5-ASA to the colon without intestinal absorption. The conjugated bile acid was synthesized according to the mixed anhydride method of Norman (20) when the pure compound was obtained in above 90% isolated yield. The structure of UDCA-5-ASA was completely compatible with the mass spectrum and the ^{1}H- and ^{13}C-NMR spectra. UDCA-5-ASA seems to possess the desired properties of a compound to carry both UDCA and 5-ASA to the colon. Our feeding experiments show that UDCA-5-ASA is poorly absorbed from the intestine and is targeted to the colon where it is partially hydrolyzed to UDCA and 5-ASA while a portion of UDCA-5-ASA escapes bacterial cleavage. Part of the released UDCA is absorbed from colon, enters the enterohepatic circulation, is converted into the taurine conjugate by hepatic enzymes, and is secreted into the bile. We believe that conjugation of UDCA with 5-ASA is an effective mechanism to target UDCA and 5-ASA to the colon while allowing some UDCA to circulate in the enterohepatic circulation. Both 5-ASA and UDCA may exhibit their anti-inflammatory and cytoprotective effects in the colon as well as liver. In the light of the recent finding that UDCA inhibits polyp formation in experimental rats via suppression of specific isoforms of protein kinase C (isoforms -α, -β_{II}, and -ζ) (27) or via inhibiting the production of nitric oxide in the colon (28), UDCA-5-ASA may be an ideal drug in that both UDCA and 5-ASA moieties may be independently beneficial and smaller doses may be needed. Furthermore, as patients with ulcerative colitis are at greater risk for primary sclerosing cholangitis (PSC) (29), and as UDCA has been reported to be beneficial in PSC (30), the enterohepatic circulation of the UDCA generated in the colon may be cytoprotective to the hepatocyte in these patients.

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TABLE 4. Biliary bile acids in rats after treatment with UDCA-5-ASA for 14 days

Rat	LCA	DCA	α-MC	CA	UDCA	β-MC	ω-MC	Total
				%				*mg/day*
Control[a]	0.1 ± 0.1	1.9 ± 0.6	8.4 ± 4.6	65.8 ± 6.7	2.7 ± 1.7[b]	18.3 ± 4.6	2.8 ± 1.8	5.47 ± 0.47
UDCA-5-ASA[c]	0.2 ± 0.1	1.2 ± 0.4	5.4 ± 2.6	35.8 ± 5.8	40.0 ± 4.3[d]	15.7 ± 3.2	1.7 ± 1.5	8.18 ± 0.91

Values reported are mean of 4 animals. LCA, lithocholic acid; DCA, deoxycholic acid; α-MC, α-muricholic acid; CA, cholic acid; UDCA, ursodeoxycholic acid; β-MC, β-muricholic acid; ω-MC, ω-muricholic acid.

[a] Bile fistula was created on the last day after feeding rat chow for 14 days and bile was collected for 1 h and an aliquot was used for bile acid analysis.

[b] TauroUDCA as judged by HPLC analysis.

[c] Bile fistula was created on the last day of feeding UDCA-5-ASA (1% by weight) in rat chow to rats for 14 days. Bile was collected for 1 h and an aliquot was used for bile acid analysis.

[d] Contains UDCA-5-ASA, 12.5%, and tauroUDCA, 87.5%, as judged by HPLC analysis.

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