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

Ashok K. Batta, G. Stephen Tint, Guorong Xu, Sarah Shefer, and Gerald Salen

Department of Medicine and Liver Center, University of Medicine and Dentistry-New Jersey Medical School, Newark, NJ 07103, and Department of Veterans Affairs Medical Center, East Orange, NJ 07018

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 cholyglycine 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.

Supplementary key words Ursodeoxycholic acid • ursodeoxycholic acid-5-aminosalicylic acid conjugate • intestinal bacteria • colon • inflammatory bowel disease • ulcerative colitis

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-cholanic acid); DCA, deoxycholic acid (3α,12α-dihydroxy-5β-cholanic acid); CDCA, chenodeoxycholic acid (3α,7α-dihydroxy-5β-cholanic acid); CA, cholic acid (3α,7α,12α-trihydroxy-5β-cholanic acid); H2O2, hydrogen peroxide; 3α,12α-dihydroxy-5β-cholanic acid; β-MC, β-muricholic acid (3α,6β,7β-trihydroxy-5β-cholanic acid), w-MC, ω-muricholic acid (3α,6α,7β-trihydroxy-5β-cholanic acid); TLC, thin-layer chromatography; IR, infrared 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.

1To whom correspondence should be addressed.
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 cholecystokinin hydrolyase [from C. perfringens (wet cells)] was from Sigma Chemical Co. (St. Louis, MO). Sil-Prep (hexamethyldisilazane: trimethylchlorosilane: pyridine, 3:1:9) used for making trimethylsilyl ether (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 purified before use.

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 conditions were as described in a previous 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 (Milford, 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 C18 reversed-phase column (100 × 8 mm i.d., 5 µm particle size) was used for the chromatography. A guard column (Waters Associates) with C18 reversed-phase material was placed before the separation column. Five–10 µl 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. 103 × 105 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 13C-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 13C 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-
Hydrolysis of UDCA-5-ASA conjugate 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% ammonium 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, Hachev, 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.

### TABLE 1. 13C signals in the 50.3 MHz 13C-NMR spectrum of UDCA-5-ASA conjugate

<table>
<thead>
<tr>
<th>Carbon</th>
<th>13C-Signal</th>
<th>Carbon</th>
<th>13C-Signal</th>
</tr>
</thead>
<tbody>
<tr>
<td>δ (ppm)</td>
<td>δ (ppm)</td>
<td>δ (ppm)</td>
<td>δ (ppm)</td>
</tr>
<tr>
<td>1</td>
<td>34.755</td>
<td>17</td>
<td>55.795</td>
</tr>
<tr>
<td>2</td>
<td>30.155</td>
<td>18</td>
<td>11.931</td>
</tr>
<tr>
<td>3</td>
<td>69.364</td>
<td>19</td>
<td>23.218</td>
</tr>
<tr>
<td>4</td>
<td>37.603</td>
<td>20</td>
<td>34.930</td>
</tr>
<tr>
<td>5</td>
<td>42.103</td>
<td>21</td>
<td>18.415</td>
</tr>
<tr>
<td>6</td>
<td>37.173</td>
<td>22</td>
<td>31.524</td>
</tr>
<tr>
<td>7</td>
<td>69.620</td>
<td>23</td>
<td>33.165</td>
</tr>
<tr>
<td>8</td>
<td>42.854</td>
<td>24</td>
<td>170.861</td>
</tr>
<tr>
<td>9</td>
<td>38.979</td>
<td>1’</td>
<td>157.819</td>
</tr>
<tr>
<td>10</td>
<td>33.641</td>
<td>2’</td>
<td>118.018</td>
</tr>
<tr>
<td>11</td>
<td>20.778</td>
<td>3’</td>
<td>115.487</td>
</tr>
<tr>
<td>12</td>
<td>40.135</td>
<td>4’</td>
<td>124.664</td>
</tr>
<tr>
<td>13</td>
<td>20.778</td>
<td>5’</td>
<td>129.266</td>
</tr>
<tr>
<td>14</td>
<td>54.659</td>
<td>6’</td>
<td>124.664</td>
</tr>
<tr>
<td>15</td>
<td>26.672</td>
<td>-COOH</td>
<td>172.094</td>
</tr>
<tr>
<td>16</td>
<td>28.115</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a 50.4 MHz spectra. Chemical shifts (δ) in ppm referenced to tetramethylsilane signal.
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 infrared spectrum at 3342 and 1661 cm⁻¹ and the amide group appeared at 1640 cm⁻¹. In the mass spectrum of the undersativized 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 δ5.59 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 (J1 = 9.0 Hz; J2 = 2.8 Hz), the doublet due to the g-coupling (J = 9.0 Hz) was further split by m-coupling due to the C-6’ proton (21). In the 13C-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 toglycoursodeoxycholic acid on both TLC and reversed-phase HPLC. The Rf 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 cholyglycine 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>
<thead>
<tr>
<th>Rat</th>
<th>DCA</th>
<th>α-MC</th>
<th>CA</th>
<th>UDCA</th>
<th>β-MC</th>
<th>ω-MC</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>2.4 ± 0.4</td>
<td>95 ± 2.5</td>
<td>65.2 ± 4.5</td>
<td>2.4 ± 1.1</td>
<td>17.5 ± 4.4</td>
<td>3.0 ± 1.0</td>
<td>4.48 ± 0.58</td>
</tr>
<tr>
<td>TauroUDCAb</td>
<td>1.0 ± 0.5</td>
<td>2.9 ± 1.1</td>
<td>29.1 ± 5.6</td>
<td>60.0 ± 5.9</td>
<td>5.9 ± 2.1</td>
<td>1.1 ± 1.0</td>
<td>9.03 ± 1.23</td>
</tr>
<tr>
<td>UDCA-5-ASAc</td>
<td>1.7 ± 0.5</td>
<td>11.2 ± 2.3</td>
<td>60.9 ± 6.4</td>
<td>4.9 ± 1.0d</td>
<td>18.2 ± 3.2</td>
<td>3.1 ± 0.8</td>
<td>4.63 ± 0.46</td>
</tr>
</tbody>
</table>

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.
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 choledochal 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 1H- and 13C-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, -η, 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.  

This work was supported by a grant from the Veterans Affairs Research Service, Washington, DC, and the National Cancer Research Service, Washington, DC, and the National Cancer Institute.

---

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

<table>
<thead>
<tr>
<th>Rat</th>
<th>LCA</th>
<th>DCA</th>
<th>α-MC</th>
<th>UDCA</th>
<th>β-MC</th>
<th>ω-MC</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>%</td>
<td>mg/day</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>0.1 ± 0.1</td>
<td>1.9 ± 0.6</td>
<td>8.4 ± 4.6</td>
<td>65.8 ± 6.7</td>
<td>2.7 ± 1.7</td>
<td>18.3 ± 4.6</td>
<td>2.8 ± 1.8</td>
</tr>
<tr>
<td>UDCA-5-ASA</td>
<td>0.2 ± 0.1</td>
<td>1.2 ± 0.4</td>
<td>5.4 ± 2.6</td>
<td>35.8 ± 5.8</td>
<td>40.0 ± 4.3</td>
<td>15.7 ± 3.2</td>
<td>1.7 ± 1.5</td>
</tr>
</tbody>
</table>

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.

---

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

<table>
<thead>
<tr>
<th>Rat</th>
<th>LCA</th>
<th>DCA</th>
<th>α-MC</th>
<th>CA</th>
<th>UDCA</th>
<th>β-MC</th>
<th>ω-MC</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>%</td>
<td>mg/day</td>
<td></td>
<td>%</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>0.1 ± 0.1</td>
<td>1.9 ± 0.6</td>
<td>8.4 ± 4.6</td>
<td>65.8 ± 6.7</td>
<td>2.7 ± 1.7</td>
<td>18.3 ± 4.6</td>
<td>2.8 ± 1.8</td>
<td>5.47 ± 0.47</td>
</tr>
<tr>
<td>UDCA-5-ASA</td>
<td>0.2 ± 0.1</td>
<td>1.2 ± 0.4</td>
<td>5.4 ± 2.6</td>
<td>35.8 ± 5.8</td>
<td>40.0 ± 4.3</td>
<td>15.7 ± 3.2</td>
<td>1.7 ± 1.5</td>
<td>8.18 ± 0.91</td>
</tr>
</tbody>
</table>

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.

---

**REFERENCES**

1. Batta et al. *5-Aminosalicylic acid conjugate of ursodeoxycholic acid* 1645

2. Control bile 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.

3. 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.

4. 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.

5. Free UDCA as judged by HPLC analysis.

6. Contains free UDCA, 35%, and UDCA-5-ASA, 65%, as judged by HPLC analysis.
REFERENCES


