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A New Amino Acid Derivative of Ursodeoxycholate, (*N*-L-Glutamyl)-UDCA (UDCA-Glu), to Selectively Release UDCA in the Colon

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Abstract. *Background: Ursodeoxycholic acid (UDCA) is chemoprotective in animal models of colon cancer but results from clinical trials have been less impressive probably because UDCA is rapidly absorbed in the small intestine and little reaches the colon. UDCA-glutamate (Glu), a novel bile acid, was synthesized with the objective of utilizing peptide bond cleavage by brush border enzymes to enhance delivery of UDCA to the colon. Materials and Methods: Qualitative and quantitative intestinal intraluminal and fecal bile acid composition measured by mass spectrometry was determined in Fisher rats after intragastric administration of UDCA, or UDCA-Glu for 5 days. The effect of UDCA and UDCA-Glu on bile flow was studied after bile duct canulation. Results: In the small intestine, UDCA was found in higher amounts when UDCA was administered compared with UDCA-Glu (1.50±0.32 vs. 0.75±0.12 mg). By contrast, UDCA-Glu administration resulted in a greater delivery of UDCA to the colon. The fecal bile acid composition resembled that of the intraluminal colonic composition and a higher mass of UDCA (unconjugated 3.39±0.30 mg; conjugated 6.40±1.03 mg) was found in rats treated with UDCA-Glu compared to those treated with UDCA (2.27±0.11 and. 0.04±0.01 mg, respectively), establishing increased delivery of UDCA to the colon. Both bile acids similarly increased bile flow but the initial effect of UDCA was greater than that of UDCA-Glu. Conclusion: Conjugation of UDCA to glutamic acid reduces its intestinal absorption and biotransformation resulting in increased colonic delivery of UDCA. UDCA-Glu may have potential application as a pro-drug for enhancing the action of UDCA in the treatment of colonic diseases.*

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Ursodeoxycholic acid (UDCA) is a hydrophilic bile acid first used for the treatment of biliary stones (1) and later proven to be beneficial in treatment of a variety of cholestatic and chronic inflammatory liver diseases (2-14). Its therapeutic value in the treatment of the inflammatory bowel diseases and colon cancer (15) has more recently been examined following preclinical studies suggesting a chemopreventive effect of UDCA in animal models of colon cancer (16-20). Results from small clinical trials of UDCA however, have proven disappointing (21-26) and this may be because UDCA is efficiently absorbed in the small bowel, thus limiting its delivery to the colon, while it also undergoes extensive biotransformation to more hydrophobic and tumor-promoting metabolites (27). In the search for new strategies for the controlled release of UDCA in the colon, the possibility of utilizing the selectivity of peptide bond cleavage exhibited by brush border enzymes, such as aminopeptidase A, offers a potentially attractive alternative for the site-specific delivery of the drug (28). It is known that aminopeptidase activity increases from the ligament of Treitz to the distal ileum (28-31). Among the host of brush border peptidases, aminopeptidase A, in particular, is able to hydrolyze peptides having an acidic *N*-terminal amino acid such as glutamic acid (29). We have shown that the physiological increment of aminopeptidase A activity from the ligament of Treitz to the distal ileum can be used to obtain high concentration of 5-aminosalicylic acid (5-ASA)

in the distal ileum (32), after the administration of its prodrug *N*-L-glutamyl-5-ASA (28). Glutamic acid plays an important role against reactive oxygen species (ROS), widely implicated in the promotion of cellular aging, inflammation and cancer development (33), and is protective on intestinal mucosa (34). This action is the result of increasing the production of glutathione, an endogenous antioxidant of which glutamic acid is a precursor. Based on the potential beneficial actions of UDCA and glutamic acid in the colon, we speculated that UDCA-glutamate (UDCA-Glu) may be a potentially attractive prodrug for delivery of both UDCA and glutamate to the colon.

The aims of this study were to examine the extent to which cleavage of the peptide bond of this novel amino acid derivative of UDCA occurred and to determine whether this UDCA analog would lead to improved delivery of UDCA to the large intestine when compared with UDCA by analysis of the intraluminal bile acid content of rats given orally both bile acids.

Materials and Methods

Chemicals and synthesis of UDCA-Glu. UDCA-Glu [*N*-(3α,7β-dihydroxy-5β-cholan-24-oyl)-glutamate] was synthesized following the procedure reported by Belleau and Malek (35). Briefly, dimethyl-L-glutamate (previously prepared by treatment of L-glutamic acid with a saturated solution of hydrochloric acid in methanol) was treated with UDCA in the presence of 2-ethoxy-1-ethoxycarbonyl-1,2-dihydroquinoline (EEDQ) in ethanol. The crude product was purified by silica gel flash chromatography to give the dimethyl glutamate derivative of UDCA (85% yield) that was then hydrolyzed with sodium hydroxide in a mixture of water/methanol. The residue was purified by silica gel flash chromatograph to give UDCA-Glu (60% yield, purity by high-performance liquid chromatography (HPLC) 98%), mp 150-152˚C, ^{1}H-NMR (D_2O) δ 0.5 (s, 3H, 18 CH_3); 0.7-0.9 (s, 6H, 19 and 21 CH3); 3.40 (s, 2H, CH-OH, C-3 and C-7); 4.00 (t, 1H, CH-(NH)-CO_2H); ^{13}C-NMR ($CDCl_3$+CD_3OD) δ 174.69; 173.24; 70.35; 55.53; 54.68; 20.66; 17.60; 11.25.

UDCA was kindly supplied by Erregierre S.p.A, Italy. L-Glutamic acid, EEDQ, sodium hydroxide and all solvents used in the synthesis and purification were purchased from Sigma-Aldrich Co. The melting point was taken on Buchi 535 apparatus. Flash chromatography was performed on silica gel 60 (spherical, particle size 0.040-0.063 mm). ^{1}H and ^{13}C NMR spectra were recorded on Varian EM 390 and Brucker AC 200. The chemical shifts are reported in parts per million (ppm) relative to trimethylsilyl (TMS). The following abbreviations are used to describe peak patterns when appropriate: s=singlet; t=triplet The analytical HPLC measurements were made on a Shimadzu (Kyoto, Japan) LC-Workstation Class LC-8A equipped with a SPD-10Avp variable-wavelength UV-vis detector and a Rheodyne 7725i injector with a 20 μl stainless steel loop. The chromatographic traces were obtained with CLASS VP (Shimadzu, version 4.3) software. The UV detection wavelength was set at 205 nm (first detection channel) and at 210 nm (second detection channel). The flow rate was 1.0 ml/min and all the analyses were performed at room temperature. A LiChrospher 100 RP-18 (Merck, Darmstadt, Germany; 250×4.0 mm, i.d. 5 μm, 100 Å) and a Ultra Aqueous C18 Restek (Bellefonte, PA, USA), 250×4.6 mm i.d., 5 μm, 100 Å) were used as analytical columns.

Animal studies. For animal studies, UDCA was purchased from Sigma Chemical (Milan, Italy) and UDCA-Glu was synthesized in the laboratory of the Institute of Chemistry and Drug Technology (University of Perugia, Italy) as described above. The purity of each compound was confirmed to be >99% by HPLC.

Male Fisher rats (body weight, 250-350 g, N=30), housed in the University of Perugia Animal Facility, were maintained on a standard diet with water *ad libitum*, and a temperature- (21˚C-23˚C) and humidity- (45%-50%) controlled room under a constant 12-h light, 12-h dark cycle for 3 days. The animals were then transferred to metabolic cages in which they were housed individually and fed the same diet. All animals received humane care according to the criteria outlined in the "Guide for the Care and Use of Laboratory Animals" prepared by the National Academy of Sciences and published by the NIH (Publication 86-23, Revised 1985).

Three separate studies were performed as follows.

Study A: Animals (n=10) were placed in metabolic cages. Half of the animals were administered a single daily bolus of UDCA (10 mg/kg bw/day; n=5) *via* an intragastric probe, and the other half were similarly administered UDCA-Glu (at the equivalent dosage of 10 mg/kg bw/day of UDCA; n=5) for five days. Feces were collected every day and pooled for qualitative and quantitative analysis of bile acid composition by fast atom bombardment ionization mass spectrometry (FAB-MS) and gas chromatography-mass spectrometry (GC-MS) (36).

Study B: Animals (n=10) were administered either UDCA (10 mg/kg bw/day) or UDCA-Glu (at the equivalent dosage of 10 mg/kg bw/day of UDCA) *via* an intragastric probe a single bolus for 5 consecutive days. On the last day, 3 h after bile acid administration, the rats were anesthetized with ketamine (*i.p.* 50 mg/kg) and phenobarbital (*i.p.* 100 mg/kg) and sacrificed. The small intestine and the colon were removed and intraluminal contents collected in glass vials and frozen at –20˚C for later analysis of intestinal tract bile acid composition by FAB-MS and GC-MS.

Study C: Animals (n=10) were anesthetized with ketamine (*i.p.* 50 mg/kg) and phentobarbital (*i.p.* 100 mg/kg). Body temperature was maintained at a constant 37.0˚C with a temperature controller lamp (Yellow Springs Instrument Co., Yellow Springs, OH, USA) to prevent hypothermic-induced alterations of bile flow. The abdomen was opened through a midline incision, a duodenal fistula was performed and the common bile duct was isolated and cannulated (PE-10, Intramedic, Clay Adams). Saline solution was infused over a 60 min period *via* the duodenal fistula using a Harvard microliter syringe pump. Through the same duodenal fistula, a single-bolus dose of UDCA (10 mg/kg bw), or UDCA-Glu (at the equivalent dosage of 10 mg/kg/bw of UDCA) was infused in order to determine the effects of these bile acids on bile flow. Bile samples were collected *via* the external biliary fistula every 15 min for up to 6.5 h and then weighed in order to determine the bile flow.

Bile acid analysis. Bile acids in intestinal contents and feces: The 5-day pooled feces from each animal and the intraluminal contents (small intestine and colon) from animals were sonicated and sequentially refluxed in 80% methanol for 2 h, followed by reflux

in chloroform/methanol (1:1) for 1 h (37). Samples were taken to dryness, and the dried extracts were resuspended in 80% methanol (20 ml). Fractions (1/20th) of the methanolic extract were taken and the internal standard nordeoxycholic acid (10 μg) was added. This extract was diluted with 0.01 mol/l acetic acid (20 ml) and passed a first through a column of Lipidex 1000 (bed size, 4×1 cm; Packard Instrument Co., Groningen, the Netherlands) and then through a Bond-Elut C_{18} cartridge. Bile acids were recovered by elution of the Lipidex 1000 column and Bond-Elut cartridge with methanol (20 ml and 5 ml, respectively), and these extracts were combined and taken to dryness. Unconjugated bile acids were isolated and separated from neutral sterols and conjugated bile acids by lipophilic anion-exchange chromatography on diethylaminohydroxypropyl Sephadex LH-20 (Lipidex-DEAP; Packard Instrument Co.) (38). Recovery of unconjugated bile acids was achieved by elution of a Lipidex DEAP column with 0.1 mol/l acetic acid in 72% ethanol (7 ml) followed by evaporation of the solvents. Total conjugated bile acids were recovered with 9 ml of 0.3 mol/l acetic acid in 72% ethanol, pH 9.6. Salts were removed by passage of the conjugated bile acid fraction through a Bond-Elut C_{18} cartridge after addition of an internal standard, nordeoxycholic acid (10 μg), and conjugated bile acids were recovered by elution with 5 ml of methanol. Solvolysis was performed with a mixture of methanol (1 ml), distilled tetrahydrofuran (9 ml), and 1 mol/l trifluoroacetic acid in dioxane (0.1 ml) and the sample was heated to 45˚C for 2 h (39). After solvolysis, unconjugated bile acids were isolated by chromatography on Lipidex-DEAP as described above.

Bile acids were converted to methyl esters by redissolving the sample in methanol (0.3 ml) and reacting for 30 min at 60˚C with 2.7 ml of freshly distilled ethereal diazomethane (40, 41). After evaporation of the reagents, the methyl ester derivatives were converted to trimethylsilyl ethers by the addition of 50 μg of Tri-Sil reagent with heating at 60˚C for 30 min (Pierce Chemicals, Rockford, IL, USA). A column of Lipidex 5000 (Packard Instrument Co.) was used to remove derivatizing reagents and to further purify the samples (42). The methyl ester-trimethylsilyl (Me-TMS) ether derivatives were separated on a 30 m × 0.25 mm DB-1-fused silica capillary column (J & W Scientific, Folson, CA, USA) using a temperature program from 225˚C to 295˚C with increment 2˚C/min and a final isothermal period of 30 min.

GC-MS analysis was performed on an Autospect GC-MS instrument (Waters, Co. Milford, MA, USA) that housed an identical gas chromatography column operated under the same conditions. Electron ionization (70 eV) mass spectra were recorded over the mass range of 50-800 Daltons by repetitive scanning of the eluting components. Identification of bile acids was made on the basis of the gas chromatographic retention index relative to a homologous series of *n*-alkanes, referred to as the methylene unit (MU) value, and the mass spectrum compared with authentic standards (43). Quantification of bile acids was achieved using gas chromatography by comparing the peak height response of the individual bile acids with the peak height response obtained from the internal standard (37).

Statistical analysis. Data are reported as the mean±SD, or as the mean values of all animals when extracts were pooled before analysis. Results from different groups were compared using paired and unpaired two-tailored Students *t*-test. *P*-values of <0.05 were considered statistically significant.

Results

Effect of UDCA and UDCA-Glu on fecal bile acid excretion. Typical FAB-MS negative-ion spectra from analysis of fecal extracts from animals fed UDCA, or UDCA-Glu are shown in Figure 1. When UDCA-Glu was administered, the major ion in the spectrum was at *m/z* 520 corresponding to the deprotonated molecular ion of unchanged UDCA-Glu. Ions at *m/z* 391 and *m/z* 375 correspond to unconjugated dihydroxy- and monohydroxy-cholanoates, respectively, and reflect the presence of the secondary bile acids deoxycholic and lithocholic acids. The negative-ion spectrum of the fecal extracts of animals fed UDCA were characterized virtually exclusively by the ions at *m/z* 407, *m/z* 391 and *m/z* 375 which are derived from unconjugated trihydroxy-, dihydroxy- and monohydroxy-cholanoates, respectively. FAB-MS, which is a qualitative technique, provides primarily molecular weight information and cannot distinguish bile acid structures based on positional or stereoisomers. For this reason, the samples were analyzed by GC-MS after further work-up that included the group separation of individual bile acid conjugates. The major bile acids identified in each fraction and the quantitative excretion (mg/day) are indicated in Table I.

Quantitative fecal bile acid excretion was greater in animals administered UDCA-Glu compared with animals given UDCA (Table I). After administration of UDCA-Glu, the total fecal bile acid recovered after 5 days was 60.36±7.07 mg in the unconjugated fraction and 7.39±1.21 mg in the conjugated fraction. By contrast these fractions yielded a recovery of 44.75±3.73 mg and 0.28±0.05 mg, respectively, when UDCA was administered. The distribution of bile acids between the unconjugated and conjugated forms also differed, with a significantly greater proportion and total excretion of bile acids being found in the conjugated fraction ($p<0.05$) when UDCA-Glu was administered (Table I and Figure 2), confirming the findings from the FAB-MS analysis. Identification of the major bile acids by GC-MS established that the principal conjugated bile acid excreted was unchanged UDCA-Glu (6.37±1.03 mg), accounting for 86% of the bile acids identified in this fraction. The excretion of unconjugated UDCA in feces was also higher in animals given UDCA-Glu compared with those administered UDCA (3.39±0.30 *vs*. 2.27±0.11 mg, $p=0.06$) consistent with a greater delivery of the bile acid through the colon (Figure 2).

Intraluminal bile acid composition after UDCA and UDCA-Glu administration. The intraluminal total bile acid content of the small intestine was similar for both groups of animals and is summarized in Table II. The majority of bile acids identified in the small intestine after administering UDCA were in conjugated form and comprised conjugated UDCA and the primary bile acids conjugates of cholic and β-muricholic acids. There was a proportionally much lower

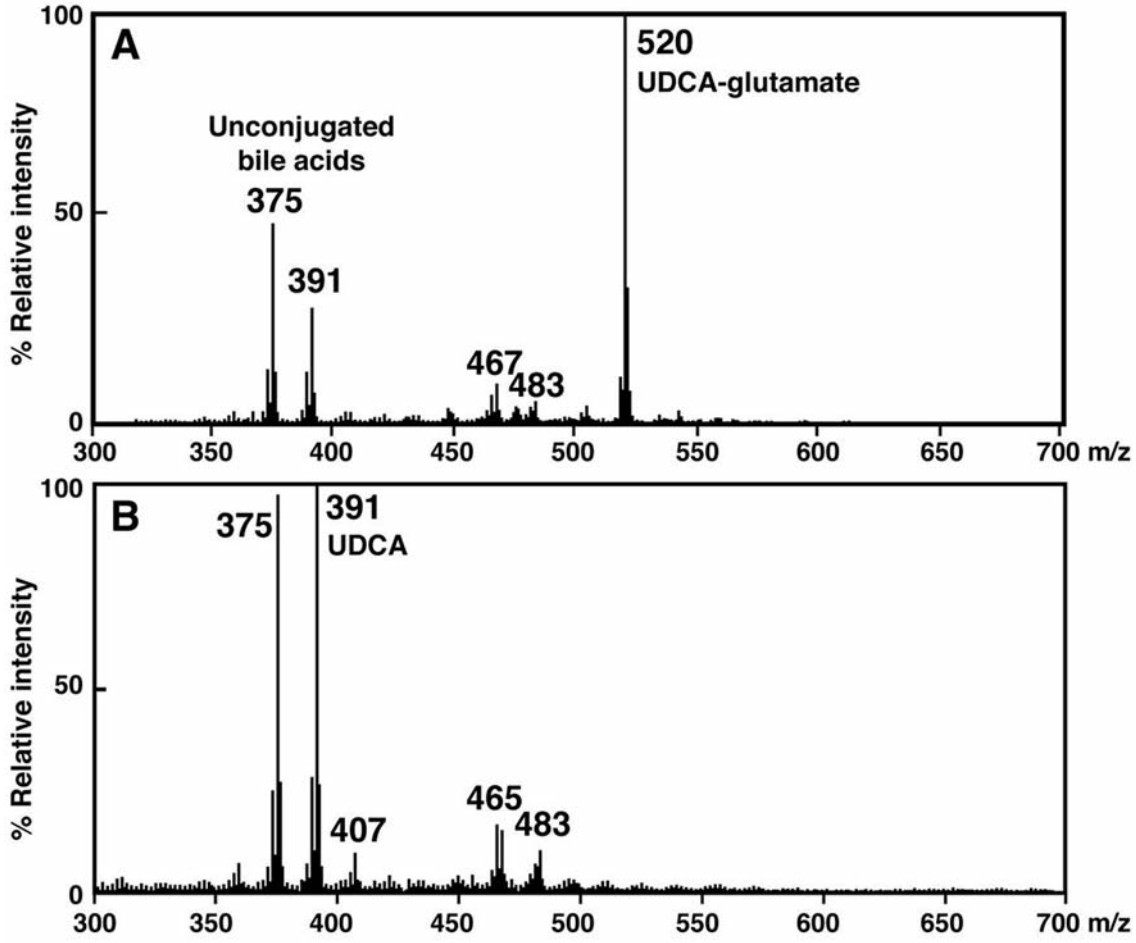

Figure 1. *Negative ion FAB-MS spectra comparing the bile acids identified feces from rats administered (A) UDCA-glutamate and (B) UDCA.*

content of secondary bile acids. Conjugated bile acids in the small intestine accounted for >80% of the total bile acids in the lumen. Unconjugated UDCA accounted for 37.50% of the total bile acids in the unconjugated fraction and likewise conjugated UDCA accounted for 46.94% of the total amount of conjugated bile acids (Table II).

In animals administered UDCA-Glu, the total intraluminal bile acid content and distribution of bile acids within the unconjugated and conjugated fractions was similar to that of UDCA-treated rats (Table II). Unconjugated UDCA (0.75±0.12 mg) accounted for 18.52% of the total amount of unconjugated bile acids in the small intestine, while unchanged UDCA-Glu (9.18±0.20 mg) accounted for 48.5% of the total amount of conjugated bile acids present. In the small intestine of rats administered UDCA-Glu, the concentration of both unconjugated and conjugated UDCA was significantly lower than in rats given UDCA ($p<0.05$), which is consistent with the known non-ionic passive diffusion of unconjugated bile acids in the proximal small bowel (1).

The intraluminal bile acid content of the colon was strikingly different from that of the small intestine after UDCA was administered (Figure 3). Quantitatively, 99.3% of the total bile acids in the colon were in the unconjugated form when UDCA was given, compared with 87% in animals administered UDCA-Glu. The total bile acid content of the colon was significantly higher when UDCA-Glu was administered, consistent with a higher proportion of the administered dose reaching the colon (Table II). Of the conjugated bile acids in the colon, unchanged UDCA-Glu (1.40±0.48 mg) accounted for 86.37% of the total. The higher proportion of lithocholic acid in the colon after administration of UDCA-Glu compared with UDCA is consistent with a greater delivery of UDCA to the colon.

Effect of UDCA and UDCA-Glu on bile flow. The mean (±SD) bile flow at baseline (the pre-infusion period) was 78.4±2.2 μl/kg min in the group of animals given UDCA-Glu and 77.4±2.4 μl/kg min in the group of animals given UDCA

Table I. *Individual and total fecal bile acid excretion (mg) in adult Fischer rats 5 days after intragastric delivery of 10 mg/kg/day of UDCA or UDCA-glutamate.*

Bile acid identified	UDCA administration		UDCA-glutamate administration	
	Unconjugated fraction	Conjugated fraction	Unconjugated fraction	Conjugated fraction
Lithocholic + iso-lithocholic acid	12.32±0.62	0.08±0.01	19.17±0.86	0.19±0.04
Deoxycholic acid	7.43±0.52	0.08±0.01	7.28±0.69	0.09±0.01
Hyodeoxycholic acid	12.67±0.76	0.01±0.00	15.73±0.62	0.16±0.06
Ursodeoxycholic acid	2.27±0.11	0.04±0.01	3.39±0.30	6.40±1.03*
6-Oxo-lithocholic acid	4.37±0.87	0.02±0.01	6.86±2.10	0.31±0.06
β-Muricholic	5.69±0.85	0.05±0.01	7.93±2.50	0.24±0.01
Total bile acids	44.75±3.73	0.28±0.05	60.36±7.07	7.39±1.21

*$p<0.05$.

Table II. *Individual and total bile acid content (mg) of the small intestine and colon in adult Fischer rats 5 days after intragastric delivery of 10 mg/kg/day of UDCA or UDCA-glutamate.*

Bile acid identified	UDCA administration		UDCA-glutamate administration	
	Unconjugated fraction	Conjugated fraction	Unconjugated fraction	Conjugated fraction
Small intestine				
Lithocholic + iso-lithocholic acid	0.04±0.08	0.45±0.00	0.01±0.11	0.05±0.00
Deoxycholic acid	0.90±0.19	0.43±0.08	0.86±0.25	0.10±0.03
Hyodeoxycholic acid	0.30±0.02	1.12±0.08	0.50±0.05	1.61±0.33
Ursodeoxycholic acid	1.50±0.32	7.76±0.05	0.75±0.12	9.18±0.20*
6-Oxo-lithocholic acid	0.09±0.01	0.32±0.02	0.12±0.02	0.02±0.16
β-Muricholic	0.67±0.81	3.15±0.09	0.93±0.15	4.98±0.11
Cholic acid	0.50±0.12	3.30±0.04	0.88±0.12	2.97±0.09
Total small intestine bile acids	4.00±1.55	16.53±0.36	4.05±0.82	18.91±0.92
Colonic				
Lithocholic + iso-lithocholic acid	2.61±0.72	0.01±0.01	3.90±0.80	0.04±0.00
Deoxycholic acid	1.39±0.35	0.02±0.00	1.32±0.50	0.03±0.00
Hyodeoxycholic acid	2.21±0.69	0.002±0.00	2.41±0.73	0.04±0.00
Ursodeoxycholic acid	0.51±0.15	0.001±0.00	0.90±0.14	1.40±0.48*
6-Oxo-lithocholic acid	1.01±0.20	0.004±0.00	1.60±0.33	0.06±0.00
β-Muricholic	1.00±0.09	0.02±0.00	1.52±0.29	0.05±0.00
Total colonic bile acids	8.73±2.20	0.057±0.01	11.65±2.79	1.62±0.48

*$p<0.05$.

(Figure 4); this difference was not statistically significant. Significantly greater bile flow was observed in the first 30 min in animals infused with UDCA compared to those given UDCA-Glu ($p<0.05$). In the first 30 min, bile flow increased to 94.0±2.7 μl/kg-min with UDCA infusion, which represented a statistically significant ($p<0.05$) increment of 21.4%, over baseline bile flow. After the initial increase in bile flow over the first 30 min infusion of UDCA, bile flow was not statistically different between the two groups of animals (74.7±7.0 μl/kg min *vs*. 81.6±5.5 μl/kg min in the UDCA-Glu *vs*. UDCA groups, respectively). After 4.5 h, bile flow tended to decline in both groups of animals; although maintenance of bile flow in this period was better with the UDCA infusion, the difference was not statistically significant.

Discussion

We described here, for the first time, metabolic fate and physiological effects of UDCA-Glu, a novel bile acid designed to effect retention of UDCA within the intestinal tract and delivery to the colon following the selective cleavage of its glutamate residue by aminopeptidase A, an enzyme localized at high concentration in the intestinal brush border of the terminal ileum (29, 28, 31). The metabolic fate of UDCA-Glu was compared to that of UDCA from detailed analysis of the intraluminal bile acid composition and fecal bile acid output in adult Fischer rats. Total fecal bile acid excretion was significantly higher when UDCA-Glu was given intragastrically compared with administration of an equimolar

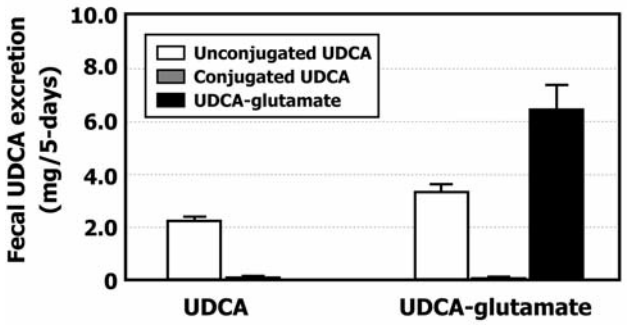

Figure 2. *Fecal ursodeoxycholic acid (UDCA) excretion in adult Fischer rats given intragastrically UDCA, or UDCA-glutamate (10 mg/kg bw/day for 5 consecutive days). The distribution of UDCA in feces according to mode of conjugation is shown.*

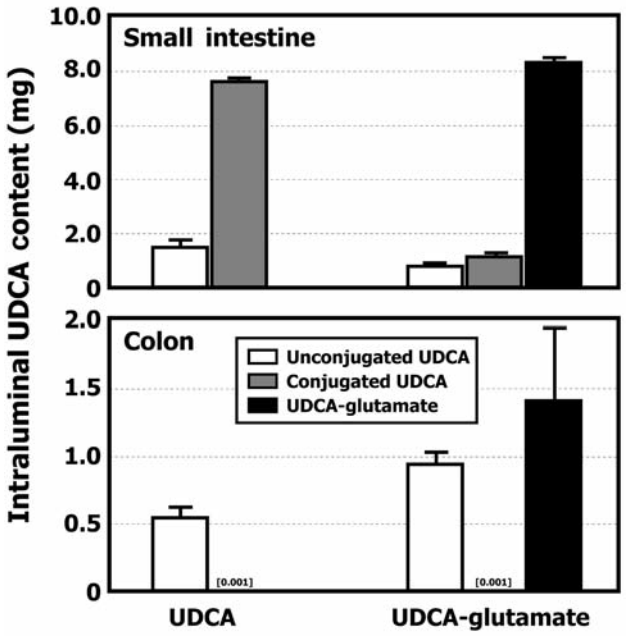

Figure 3. *Total intraluminal ursodeoxycholic acid (UDCA) content (mg) in the small intestine and colon of adult Fischer rats given UDCA, or UDCA-glutamate (10 mg/kg bw/day for 5 consecutive days) intragastrically. The distribution of UDCA within the intestinal lumen according to mode of conjugation is shown.*

dose of UDCA (Figure 2), confirming enhanced delivery of UDCA through the gastrointestinal tract when it was coupled to glutamic acid. This finding established that UDCA-Glu was either somewhat resistant to hydrolysis by the aminopeptidase A in the brush border of the small intestine, or that the dose administered exceeded the capacity for the aminopeptidase to cleave. Overall, the effect was to increase the flux of bile acid through the colon. As expected, when UDCA was administered intragastrically, very little unchanged UDCA was found in the feces; UDCA accounted for only 5.1% of the total fecal bile acids excreted (Table I) and this observation is consistent with previous findings for adult Sprague-Dawley rats fed UDCA in the diet (27).

The relative composition of bile acids within the intestinal lumen of the small intestine and large bowel was markedly different after oral administration of the two bile acids. In the small intestine of UDCA-treated animals, unconjugated and conjugated (glycine and taurine conjugated) UDCA were the predominant forms. This observation is consistent with its efficient proximal absorption, first-pass hepatic conjugation and enterohepatic recycling (1, 44). However, by the time these bile acids reached the colon, the content of UDCA was relatively small and consistent with bile acid deconjugation by intestinal bacteria. Analysis of the intraluminal bile acid composition of the colon showed the major bile acids to be 6-oxo-lithocholic, iso-lithocholic and lithocholic acids, confirming extensive biotransformation of UDCA to secondary bile acids during its passage through the intestinal tract (37). The finding of little delivery of UDCA to the colon and extensive biotransformation supports previous observations in rats fed UDCA in the diet (27) and offers a plausible explanation for the lack of success of clinical trials of UDCA for colon cancer prevention (21-26).

By contrast, the bile acid content of the small intestine and colon of animals given UDCA-Glu comprised mostly unchanged UDCA-Glu and unconjugated UDCA, consistent with retention of the molecule within the intestinal tract, and limited cleavage of the *N*-L-glutamyl bond in the distal ileum. The effect was to afford a relatively high content of UDCA and UDCA-Glu in the colon at the expense of more hydrophobic secondary bile acids. In principle, this should lead to enhanced efficacy of UDCA as an anti-inflammatory (45, 46) and cancer preventive agent in the colon (16, 47) as it would buffer the promotional effects of more hydrophobic secondary bile acids that are normally the major components in the colon (48). Lithocholic acid (LCA), the main biotransformation product of UDCA in humans and animals increases colonic cell proliferation (49) and risk of adenomatous polyps and colon cancer (50, 51), however, its harmful effects are buffered in the presence of relatively high concentrations of UDCA (52-54). A study performed by Alberts *et al*. in 1,285 patients who had undergone removal of a colorectal adenoma showed that UDCA significantly prevented the recurrence of adenoma with high-grade dysplasia (25).

In the acute study, the administration of UDCA-Glu led to statistically significant higher levels of UDCA in the colon with respect to the group of animals treated with UDCA (Figure 3). Additionally, a relevant increment of UDCA in the colon was detected after five days of total colon bile acid recovery when animals were treated with UDCA-Glu as compared to UDCA, although this value was not statistically significant. Nevertheless, the lower fecal UDCA recovery in

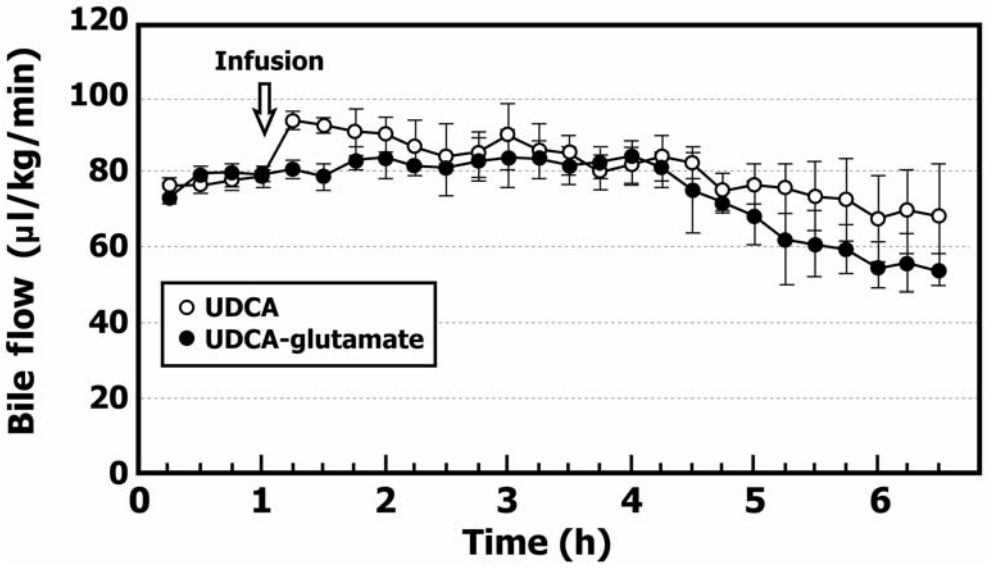

Figure 4. *Effect of intraduodenal instillation of a single equimolar dose of UDCA, or UDCA-glutamate (equivalent to 10 mg UDCA/kg bw/day), on hepatic bile flow in adult Fischer rats with bile fistula.*

the chronic as compared to the acute experiment could be due to an accumulation of UDCA-Glu over time which exceeded the capacity of the enzyme aminopeptidase A to cleave glutamic acid residues. Accordingly, high uncleaved UDCA-Glu levels were detected in the chronic experiment and further studies using lower doses of UDCA-Glu should be conducted in order to increase the concentration of unconjugated UDCA in the colon.

Furthermore, since glutamate, the cleavage product of UDCA-Glu is a specific precursor for the biosynthesis of glutathione, a key molecule responsible for the protection of intestinal mucosa and for the generation of gastrointestinal energy (55, 34), the use of UDCA-Glu may offer advantages over that of UDCA in its efficacy for treating colonic disease.

UDCA is widely used in the treatment of patients with cholestatic liver disease because it is a potent choleretic agent that rapidly increases in bile flow. Following cannulation of the bile duct and measurement of bile flow, UDCA showed an approximate 21.4% increase in bile flow within 30 min of intraduodenal administration in these animals, and increased bile flow was maintained for several hours before it slowly declined, consistent with the known rapid clearance of UDCA from the systemic circulation (1, 44, 56). The initial response to UDCA-Glu was less obvious and consistent with the finding that the small intestine contained mostly unchanged UDCA-Glu and only a low level of unconjugated UDCA. Several hours after intraduodenal instillation of UDCA-Glu, the effect on bile flow was similar to that observed for UDCA. These physiological effects are consistent with the metabolic fate of the two molecules within the intestinal lumen observed in these studies.

In conclusion, conjugation of UDCA to glutamic acid leads to reduced small intestinal absorption, decreased intestinal biotransformation to more hydrophobic bile acids that have the potential to increase colon cancer risk, and increased delivery of UDCA to the colon. This approach is analogous to the approach that was used to improve the delivery of the anti-inflammatory agent 5-ASA to the large bowel, where the enzyme aminopeptidase A is utilized to selectively cleave the conjugate. UDCA-Glu administration, as compared to UDCA, appears a promising prodrug for UDCA delivery to the colon and consequently may be a more effective than UDCA as a chemopreventive agent for inflammatory bowel diseases and colon cancer.

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