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# Anti-inflammatory effects of 5-aminosalicylic acid conjugates with chenodeoxycholic acid and ursodeoxycholic acid on carrageenan-induced colitis in guinea-pigs

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**Abstract**

Two epimeric bile acid conjugates, 5-aminosalicylic acid–chenodeoxycholic acid (5-ASA–CDCA) and 5-aminosalicylic acid–ursodeoxycholic acid (5-ASA–UDCA), were synthesized to deliver 5-ASA to the large intestine by oral administration. The movement of the conjugates down the gastrointestinal tract and the anti-inflammatory effects on ulcerative colitis were investigated by administering the conjugates to guinea-pigs with an inflammatory bowel disease induced by 2% degraded carrageenan solution. The conjugates were protected from deconjugation in stomach and small intestine and reached the caecum and the colon, where 5-ASA was more easily liberated from 5-ASA–CDCA than from 5-ASA–UDCA. The conjugates at doses equivalent to 50 or 150 mg $kg^{-1}$ 5-ASA were orally administered once a day for 4 weeks from the 15th day after starting carrageenan treatment. The body weights and the bleeding scores of occult blood in faeces were measured during the experiment. The number of ulcers in the caecum and the colon were counted after killing the guinea-pigs at the end of the experiment. Rapid onset of efficacy was shown by a significant reduction in bleeding scores within a week after administration of the conjugates. Treatment with the lower dose of 5-ASA–CDCA showed a recovery of body weight and a significantly decreased number of ulcers in the caecum, and the ulcers in the colon had completely disappeared by the end of the experiment. There was a good correlation found between the number of ulcers in the caecum and the bleeding scores of occult blood in faeces. The findings indicate that both conjugates were sufficiently delivered to the large intestine without deconjugation and that the lower dose of 5-ASA–CDCA is enough for treatment of ulcerative colitis in colonic inflammatory bowel diseases.

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## Introduction

5-Aminosalicylic acid (5-ASA) is widely used for achieving remission of mild to moderately active cases of inflammatory bowel disease and is also used in long-term maintenance therapy (Martin 1987; Schroeder et al 1987; Tremaine et al 1994). However, orally administered 5-ASA is rapidly and largely absorbed from the upper gastrointestinal tract, and thereafter quickly acetylated and eliminated from the blood stream (Nielsen & Bondesen 1983; Myers et al 1987). As the efficacy of 5-ASA is dependent on direct contact with the mucosal lining of the intestinal wall, it is necessary to deliver enough 5-ASA to the inflammatory regions of the intestine. Some preparations for 5-ASA delivery to the colon have been developed

and investigated for clinical use (Martin 1987). Other approaches are still under development (Steed et al 1997; Takaya et al 1997; Brøndsted et al 1998; Krishnaiah et al 1998; Rodriguez et al 1998; Ishibashi et al 1999). Salazosulfapyridine, which delivers 5-ASA specifically to the colon, produces sulfapyridine from the deconjugation and causes serious side effects (Taffet & Das 1983). Although olsalazine, consisting of two 5-ASA molecules linked together by an azo bond, produces desirable 5-ASA delivery in healthy subjects, nearly 50% of the dose is excreted in the non-degraded form in faeces of patients with inflammatory bowel disease having diarrhoea (Rijk et al 1992). A sustained-release tablet (Pentasa) and delayed-release preparations (Asacol and Salofalk) often induce the potential risk of side effects such as nephrotoxicity during long-term therapy, because a certain amount of 5-ASA is released in the small intestine, which makes systemic absorption easy (Calder et al 1972; Laursen et al 1990; Lundberg et al 1996; Stretch et al 1996). Rectal administration of 5-ASA has been established in the treatment of distal ulcerative colitis only (Sutherland et al 1987; Campieri et al 1991; Gionchetti et al 1997). It is, however, preferable for patients to use oral administration for convenience and for compliance with long-term therapy.

Recently, Batta et al (1998) prepared the conjugate 5-aminosalicylic acid–ursodeoxycholic acid (5-ASA–UDCA), which was linked by an amide bond similar to bile acid conjugates, and they evaluated its biliary secretion and intestinal metabolism. When 5-ASA–UDCA sodium salt was infused into the duodenum of rats, only 2.5% of the conjugate was recovered in the bile after 3 h. In the rat fed the chow containing 1% 5-ASA–UDCA for 14 days, UDCA constituted 95% of total bile acids excreted in faeces and 37% of excreted UDCA was found in the unconjugated form. Konishi et al (1997) synthesized 5-ASA–UDCA monophosphate for the evaluation of microbial overgrowth. It was reported that 5-ASA–UDCA monophosphate was hydrolysed by only cholylglycinehydrolase and not absorbed from the small intestine of rats either actively or passively in-vitro. Assuming that the amide bond between 5-ASA and UDCA is stable in the upper gastrointestinal tract and that 5-ASA–UDCA is poorly absorbed from the small intestine, the conjugate should reach the colon and be hydrolysed by the colonic bacterial enzymes to liberate 5-ASA. In previous studies, however, there was no evaluation of anti-inflammatory effects of the conjugate on inflammatory bowel disease.

UDCA is a 7$\beta$-hydroxy epimer of chenodeoxycholic acid (CDCA), which is a primary bile acid in humans, and both are therapeutic agents for cholesterol gallstone dissolution (Danzinger et al 1972; Salen et al 1980). Thus, 5-aminosalicylic acid–chenodeoxycholic acid (5-ASA–CDCA) may also be expected to possess similar characteristics.

In this study, we synthesized de-novo 5-ASA–CDCA as an epimer of 5-ASA–UDCA and investigated the effects of 5-ASA–CDCA and 5-ASA–UDCA on ulcerative colitis induced by degraded carrageenan in guinea-pigs to compare their use as oral agents in the treatment of inflammatory bowel disease. Guinea-pigs are highly susceptible to the development of caecal and colonic ulceration induced by carrageenan, thus they are suitable as an experimental animal model (Watt & Marcus 1975). Additionally, the microbial distribution along the gastrointestinal tract in guinea-pigs is more similar to that of humans than is that of rats or mice (Hill & Drasar 1968).

## Materials and Methods

### Chemicals

Chenodeoxycholic acid (CDCA), ursodeoxycholic acid (UDCA) and 5-aminosalicylic acid (5-ASA) were purchased from Tokyo Chemical Industries Ltd (Tokyo, Japan). $\lambda$-Carrageenan and Occult Blood Test Wako were obtained from Wako Pure Chemical Industries Ltd (Osaka, Japan). Salazosulfapyridine was purchased from Sigma Chemical (St Louis, MO). All other chemicals and solvents used were of analytical reagent grade.

### Apparatus

FAB mass spectra were measured by a JOEL JMS-HX110 mass spectrometer (JOEL Ltd, Tokyo, Japan) equipped with a JMA-DA7000 data processing system. $^1$H NMR was taken on a JOEL GX-500 interfaced with a DEC RSX-11M computer (JOEL Ltd, Tokyo, Japan) at 35°C. Samples were dissolved in dimethyl sulfoxide (DMSO)-$d_6$ and the chemical shifts were assigned based on the internal standard, tetramethylsilane. Melting points were determined by DSC (differential scanning calorimetry) (DSC 3,100, MAC SCIENCE Co. Ltd, USA). Elemental analysis was carried out using CHNO-RAPID (Heraus Co. Ltd, Germany).

### Synthesis of conjugates of 5-ASA and bile acid

5-amino salicylic acid–chenodeoxycholic acid (5-ASA–CDCA) and 5-amino salicylic acid–ursodeoxycholic acid (5-ASA–UDCA) were synthesized by a modification of the method of Bergström & Norman (1953). CDCA or UDCA (0.98 g, 2.5 mmol) was dissolved in

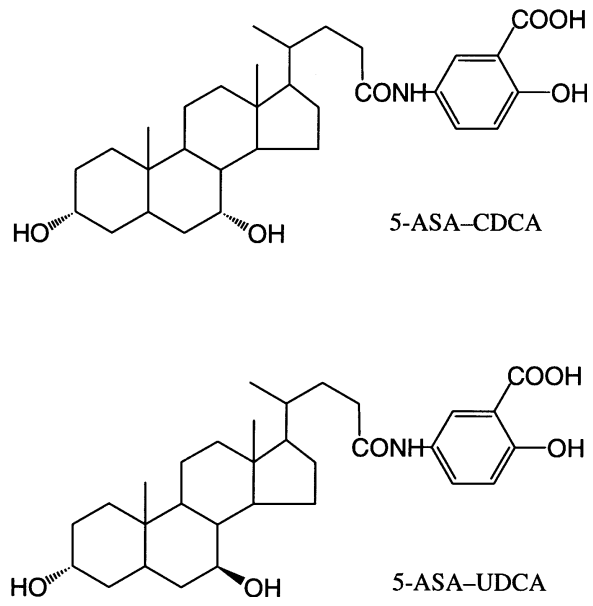


**Figure 1** Structure of 5-ASA–CDCA and 5-ASA–UDCA.

5 mL dioxane containing 0.59 mL of tri-*n*-butylamine. The solution was cooled to 10°C and 0.238 mL ethylchlorocarbonate was added. After stirring the mixture for 1 h at 10°C, a solution of 5-ASA (0.38 g, 2.5 mmol) in 2.5 mL 1 M sodium hydroxide was added and the mixture was rapidly stirred at room temperature. After 3 h when the solution had set to a gel, enough water was added to the reaction mixture to give a white suspension that was evaporated in-vacuo to a syrup; it was then redissolved in about 100 mL ethyl acetate, washed with 1 M hydrochloric acid first, then with distilled water three times until all HCl was removed, and finally alkalized to about pH 8 by addition of 0.1 M sodium hydroxide to remove impurities from the organic phase. The final extract with ethyl acetate following re-acidification with 1 M hydrochloric acid was dried over anhydrous sodium sulfate overnight, and gradually evaporated under reduced pressure to obtain a brown mass of crude crystals of the synthesized product. The crystals were dissolved in 2 mL methanol and purified by middle-pressure chromatography on a preparative reversed-phase column (Lobar LiChroprep RP-18, 25 × 310 mm, 40–63 $\mu$m particles, Merck, Germany) using a mixture of methanol–acetonitrile (1 : 10 v/v) solution as the eluent at a flow rate of 4 mL min$^{-1}$. When the pooled fractions of the product were evaporated to remove the organic solvent, a yellowish white powder was obtained in a final yield of about 25%. The chemical structures of the conjugates, 5-ASA–CDCA and 5-ASA–UDCA, are shown in Figure 1.

5-ASA–CDCA: mp 242.9°C. Negative-ion FAB-MS m/z: 526 $(M-H)^-$. $^1$H NMR (DMSO-$d_6$) $\delta$: 0.61 (3H, s, 18-$H_3$), 0.84 (3H, s, 19-$H_3$), 0.93 (3H, d, $J_{21,20}$ = 6.41 Hz, 21-$H_3$), 0.87–2.33 (26H, m, ring $H_2$; 20-H; 22-$H_2$; 23-$H_2$), 3.63 (1H, s, 7$\beta$-H), 4.05 (1H, s, 7$\alpha$-OH), 6.88 (1H, d, $J_{3',4'}$ = 8.85 Hz, 3′-H), 7.65 (1H, dd, $J_{4',3'}$ = 8.85 Hz, $J_{4',6'}$ = 2.44 Hz, 4′-H), 8.09 (1H, d, $J_{6',4'}$ = 2.45 Hz, 6′-H), 9.77 (1H, s, -CONH-). Anal. Calcd for $C_{31}H_{45}NO_6$: C, 70.56; H, 8.60; N, 2.66. Found: C, 70.31; H, 8.33; N, 2.82.

5-ASA–UDCA: mp 259.8°C. Negative-ion FAB-MS m/z: 526 $(M-H)^-$. $^1$H NMR (DMSO-$d_6$) $\delta$: 0.62 (3H, s, 18-$H_3$), 0.87 (3H, s, 19-$H_3$), 0.93 (3H, d, $J_{21,20}$ = 6.71 Hz, 21-$H_3$), 0.92–2.32 (26H, m, ring $H_2$; 20-H; 22-$H_2$; 23-$H_2$), 3.89 (1H, brs, 7$\beta$-OH), 4.28 (1H, brs, 3$\alpha$-OH), 6.88 (1H, d, $J_{3',4'}$ = 8.85 Hz, 3′-H), 7.65 (1H, dd, $J_{4',3'}$ = 8.85 Hz, $J_{4',6'}$ = 2.74 Hz, 4′-H), 8.08 (1H, d, $J_{6',4'}$ = 2.75 Hz, 6′-H), 9.76 (1H, s, -CONH-). Anal. Calcd $C_{31}H_{45}NO_6$: C, 70.56; H, 8.60; N, 2.66. Found: C, 70.67; H, 8.53; N, 2.61.

### Preparation of degraded λ-carrageenan by oxidation with 0.04 M hypochlorite

Degraded carrageenan was prepared by oxidation with sodium hypochlorite at pH 7 (Black et al 1965). λ-Carrageenan (10 g) dissolved in water (1600 mL) was mixed with 60 mL 10% sodium hypochlorite. The solution was adjusted to pH 7.0 with 1 M HCl, made up to 2.0 L with distilled water and kept in the dark at 20°C for 3 h. After oxidation, the excess hypochlorite was destroyed with 200 mL 2 M acetic acid and 20 g potassium iodide. The liberated iodine was titrated with 2 M sodium thiosulfate. The solution was dialysed first against running tap-water, then against distilled water for one day at room temperature, and was concentrated by evaporation. The degraded products were isolated by freeze-drying in a yield of 76%. The limiting intrinsic viscosity of 0.2% degraded carrageenan in 0.1 M sodium chloride was estimated at 1.31 dL g$^{-1}$ using a Brookfield viscometer. The degraded carrageenan was dissolved in drinking water and 2% carrageenan solution was freshly prepared every day.

### Induction of colitis in guinea-pigs by degraded carrageenan and anti-inflammatory experiment with 5-ASA conjugates

Young male Hartley guinea-pigs (200–250 g) were obtained from Kyudo Co. Ltd (Saga, Japan) and housed in the breeding room at a controlled temperature of

22°C under a 12-h light–dark cycle. The guinea-pigs were fed an ascorbic-acid enriched chow (CG-7; Kyudo, Japan) with free access to tap-water for a week, and were then given 2% degraded carrageenan solution instead of water daily for 6 weeks over the experimental period. The guinea-pigs were divided into the following five groups 2 weeks after the onset of the carrageenan treatment. Groups A and B were orally given 0.5% CMC (carboxymethylcellulose) solution containing 5-ASA–CDCA equivalent to 50 and 150 mg $kg^{-1}$ body weight, respectively, of 5-ASA; groups C and D were given 5-ASA–UDCA equivalent to 50 and 150 mg $kg^{-1}$ of 5-ASA in 0.5% CMC solution, respectively; and group E was orally given salazosulfapyridine equivalent to 50 mg $kg^{-1}$ body weight of 5-ASA as a positive control under the same conditions.

During the period of degraded carrageenan treatment, body weights were measured daily. Occult blood in the faeces was checked daily for the first week and then every two days for the next five weeks by an independent observer blinded to the treatment. The amount of occult blood in each sample was assessed by a comparative table accompanying the Occult Blood Test Wako, where the occult blood test was scored on a 0–6 scale based on the colour index by reagent.

The guinea-pigs were sacrificed under ether at the end of the experiment. The gastrointestinal tracts were removed to count the number of ulcers and the inner cavities of the large intestines were carefully washed with saline. After fixation in 4% formaldehyde solution, the presence of ulcerative disease was examined by transmitted light. The number of ulcers of diameter 0.5–1.2 mm was counted using a cold light viewer with magnifying lens (10 diopters) according to the method described by Watt & Marcus (1975). The counting study was performed in a randomized, blinded manner to prevent bias by an observer unaware of the treatment.

### Hydrolysis of the conjugates in the gastrointestinal tract

Seven Hartley male guinea-pigs with ulcerative colitis were administered 0.5% CMC solution containing each conjugate equivalent to 5-ASA 60 mg $kg^{-1}$ body weight by gastric intubation. Guinea-pigs were sacrificed, 4 and 12 h after 5-ASA conjugate administration, under ether anaesthesia to remove the gastrointestinal tract. The gastrointestinal tracts including the contents were segmented into stomach, small intestine, caecum and colon. The small intestine was further divided into proximal and distal segments of equal length. Each segment was cut into small pieces and was homogenized (Chemical Stirrer B-100, Tokyo RIKAKIKAI Co. Ltd, Tokyo, Japan). Then 1 g of the homogenate was diluted with 10 mL methanol to stop the hydrolysis of the conjugates and to deproteinize the samples, and centrifuged at 10000 *g* for 10 min. The amounts of the conjugates and released 5-ASA were measured by HPLC analysis.

These experiments were permitted by Experimental Animal Care and Use Committee in Fukuoka University.

### HPLC analysis

The HPLC system used consisted of a Shimadzu LC-10A pump with 20 $\mu$L loop injection, CTO-6A column oven, RF-550 fluorescence detector and C-R6A chromato integrator (all from Shimadzu Co. Ltd, Kyoto, Japan). Separation of the samples was performed at 45°C using a reversed phase Wakosil-II 5C18 column ($4.6 \times 250$ mm, 5 $\mu$m particles) in connection with a guard column (Wakosil-II 5C18 column, $4.6 \times 10$ mm, 5 $\mu$m particles), both from Wako Pure Chemical Co. Ltd (Osaka, Japan). The conjugates, 5-ASA–CDCA and 5-ASA–UDCA, were analysed using a mixture of methanol–0.02 mM phosphate buffer, pH 6.0 (70:30 v/v) as the eluent at a flow rate of 1.2 mL $min^{-1}$ and measured by fluorescence detection with 320 nm excitation and 440 nm emission wavelengths. The amount of 5-ASA was analysed using a mixture of methanol–0.02 mM phosphate buffer, pH 6.5 (20:80 v/v) containing 0.015% of tetrabutylammonium chloride as the eluent at a flow rate of 1.5 mL $min^{-1}$ and by fluorescence detection with 335 nm excitation and 490 nm emission wavelengths.

### Statistical analysis

Statistical analyses were carried out using Student's *t*-test and Kruskal-Wallis tests.

## Results

All guinea-pigs receiving 2% carrageenan solutions showed body weight losses with a slight gain. The carrageenan solutions induced a 100% incidence of ulceration in the large intestines of every guinea-pig by the end of the experiment. The ulcers were produced mainly in the caecum and modestly in the proximal colon, but they were not observed in the stomach, small

intestine, distal colon or rectum. Extensive damage to the large intestinal walls was observed in the group receiving 2% carrageenan solution. The faeces of some guinea-pigs receiving carrageenan solution were soft in form but there was no diarrhoea observed.

### Anti-inflammatory effect of 5-ASA–CDCA and 5-ASA–UDCA on ulcerative colitis in guinea-pigs

The experiment on the anti-inflammatory effect of 5-ASA conjugates was performed on five groups (A–E) of five young guinea-pigs: groups A and B were given 5-ASA–CDCA equivalent to 50 and 150 mg $kg^{-1}$ of 5-ASA, respectively; groups C and D were given 5-ASA–UDCA equivalent to 50 and 150 mg $kg^{-1}$ of 5-ASA, respectively; and group E was given salazosulfapyridine equivalent to 50 mg $kg^{-1}$ of 5-ASA. The control group was orally given 0.5% CMC solution every 24 h instead of 5-ASA. Guinea-pigs in all groups received 2% degraded carrageenan solution as drinking fluid daily during the experiment. Table 1 summarizes the body weight gains for the first 2 weeks and the last 4 weeks after carrageenan treatment, and the number of ulcers in the caecum and colon at the end of the experiment for each group of guinea-pigs. Comparing the body weight gains, for the last 4 weeks, groups A and B (given 5-ASA–CDCA) showed a significant difference in weight ($P < 0.05$) from control group. The weight gains of groups C and D (given 5-ASA–UDCA) were not significantly increased. When all guinea-pigs were killed at the end of the experiment, the number of ulcers in the caecum and colon was counted to compare the six groups. The number of ulcers in the caecum for groups A and B was significantly decreased compared with the control group ($P < 0.01$ or $P < 0.05$, respectively), indicating that the lower dose of 5-ASA–CDCA was more effective than the higher dose. By the end of the experiment, the ulcers in the colon had disappeared in both groups A and B. In the caecum and colon for groups C and D, however, there was no significant difference compared with the control group. This lack of significance could be due to the limited number of guinea-pigs in each group. There was a high degree of variance with the groups C and D. In group E, both body weight gain and the number of ulcers in the caecum differed significantly from the control group ($P < 0.05$).

Figure 2 shows the effects of 5-ASA–CDCA and 5-ASA–UDCA administration on the occult blood in faeces. The groups receiving the 2% carrageenan solutions showed a positive sign in the occult blood test within a week of commencing carrageenan treatment and the bleeding scores were sharply increased for the first 2 weeks and gradually increased to levels greater than 4. When 5-ASA conjugates were first administered to the guinea-pigs, the average bleeding score of each group was over level 3. In groups A and B, the level was lowered within a week of 5-ASA–CDCA administration, and then decreased day by day (Figure 2A). These groups showed a significant difference ($P < 0.01$) from the control group during the last two weeks. On the other hand, the bleeding score in groups C and D was not decreased markedly after administration of 5-ASA–UDCA (Figure 2B). The level was lowered from 4 to 3 ($P < 0.05$) during the last week and a good correlation ($P < 0.01$) was found between the number of ulcers in the caecum and the bleeding scores for all

**Table 1** Effects of the conjugates on body weight gain and number of ulcers induced by 2% carrageenan solution in guinea-pigs.

| Groups | No. of guinea-pigs | Body weight gain (g) after carrageenan supply | | Number of ulcers | |
|---|---|---|---|---|---|
| | | First two weeks | Last four weeks | Caecum | Colon |
| Control | 7 | 75.2±7.2 | 117±13 | 437±50 | 10±5 |
| A | 5 | 75.3±2.5 | 155±3* | 100±28** | 0 |
| B | 5 | 76.7±7.0 | 141±17* | 132±37* | 0 |
| C | 5 | 75.3±13.4 | 125±25 | 338±92 | 7±1 |
| D | 5 | 77.3±4.3 | 110±21 | 242±109 | 2±1 |
| E | 5 | 75.1±6.6 | 153±9* | 125±35* | 4±2 |

Control group received 0.5% CMC solution; group A received 5-ASA–CDCA equivalent to 50 mg $kg^{-1}$ 5-ASA; group B received 5-ASA–CDCA equivalent to 150 mg $kg^{-1}$ 5-ASA; group C received 5-ASA–UDCA equivalent to 50 mg $kg^{-1}$ 5-ASA; group D received 5-ASA–UDCA equivalent to 150 mg $kg^{-1}$ 5-ASA; group E (positive control) received salazosulfapyridine equivalent to 50 mg $kg^{-1}$ 5-ASA. Results are expressed as the mean±s.e.; *$P < 0.05$, **$P < 0.01$ vs control group.

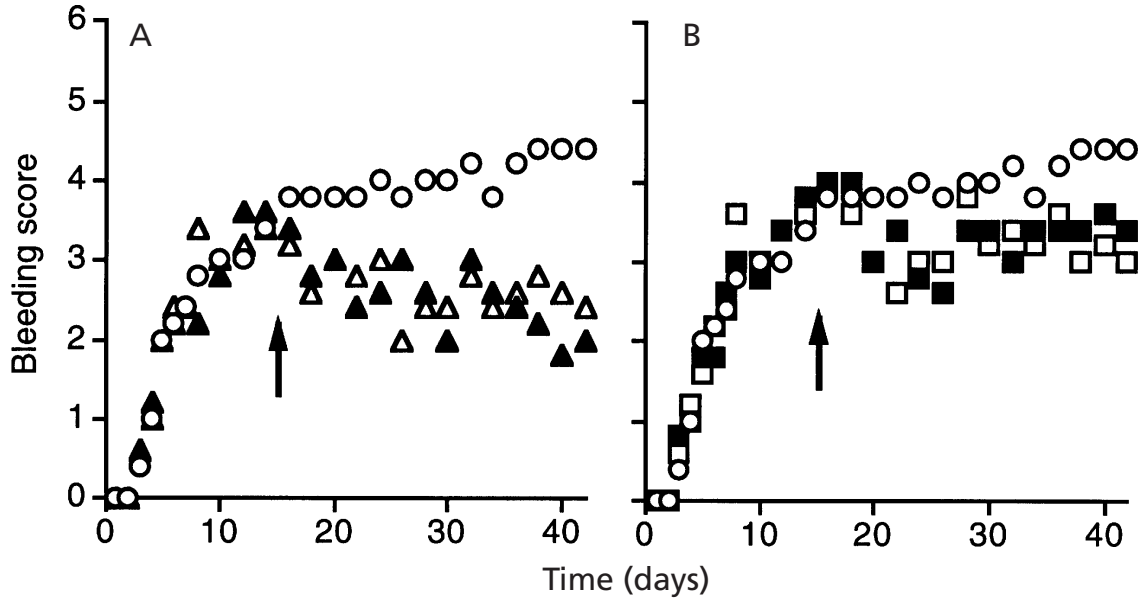


**Figure 2** Effect of 5-ASA–CDCA (A) or 5-ASA–UDCA (B) treatment on the bleeding scores (occult blood in faeces) of guinea-pigs with carrageenan-induced colitis. The conjugates were administered from 15th day after the start of carrageenan treatment, represented by arrows in the figure. During the experiment guinea-pigs received 2% carrageenan solution as drinking fluid. Each point represents the mean value of five guinea pigs. ○, control group receiving 0.5% CMC solution; ▲, group A receiving 5-ASA–CDCA equivalent to 50 mg $kg^{-1}$ 5-ASA; △, group B receiving 5-ASA–CDCA equivalent to 150 mg $kg^{-1}$ 5-ASA; ■, group C receiving 5-ASA–UDCA equivalent to 50 mg $kg^{-1}$ 5-ASA; and □, group D receiving 5-ASA–UDCA equivalent to 150 mg $kg^{-1}$ 5-ASA.

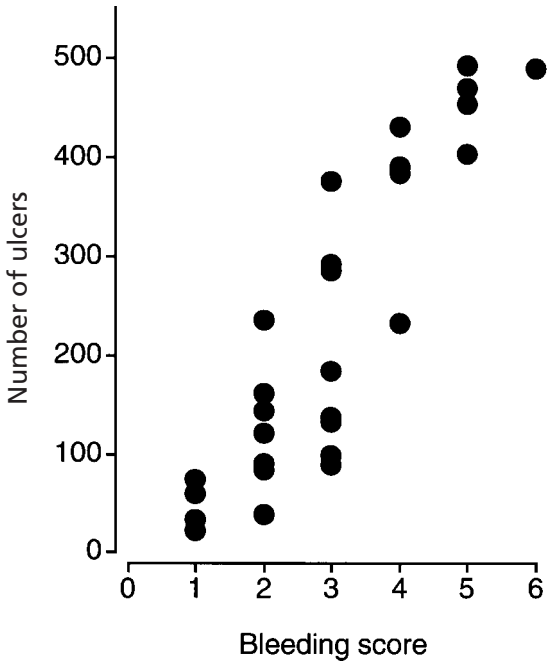


**Figure 3** Correlation between the bleeding score (occult blood in faeces) and the number of ulcers in the caecum at the end of the experiment for all guinea-pigs.

guinea-pigs (Figure 3). Throughout the experiment, no diarrhoea was observed in guinea-pigs given the conjugates.

### Gastrointestinal distribution of conjugates and liberation of 5-ASA from the conjugates

To evaluate the gastrointestinal distribution of the conjugates and liberation of 5-ASA in-vivo, their recovery in the luminal contents was determined 4 h and 12 h after oral administration of the conjugates. Figures 4 and 5 show the results of the percentage recovery for 5-ASA–CDCA and 5-ASA–UDCA, respectively.

The gastrointestinal transit time of 5-ASA–UDCA was slightly shorter than that of 5-ASA–CDCA. Although 40% remained in the stomach 4 h after administration, about 45% of the oral dose of 5-ASA–CDCA reached the large intestine, where the conjugate was hydrolysed completely (Figure 4A). On the other hand, about 70% of the oral dose of 5-ASA-UDCA reached the large intestine, where only a half of the conjugate released 5-ASA at the same period (Figure 5A). Even 12 h after administration, 46% and 38% of the doses of

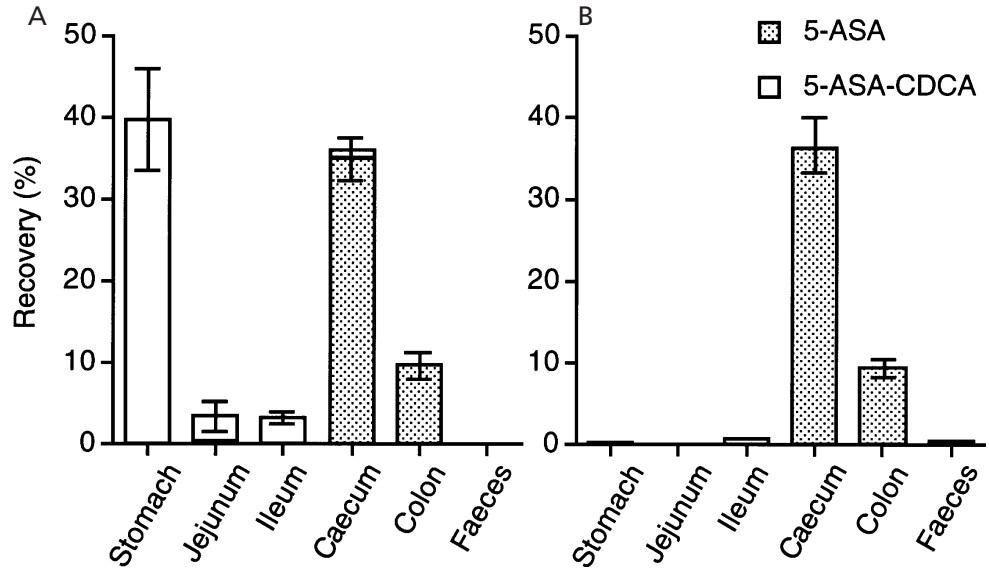


**Figure 4** Recovery of 5-ASA–CDCA and 5-ASA from luminal contents of guinea-pigs with ulcerative colitis at four (A) and twelve (B) hours after oral administration of 5-ASA–CDCA (207 mg $kg^{-1}$). Each column represents the mean ± s.e. of seven guinea-pigs.

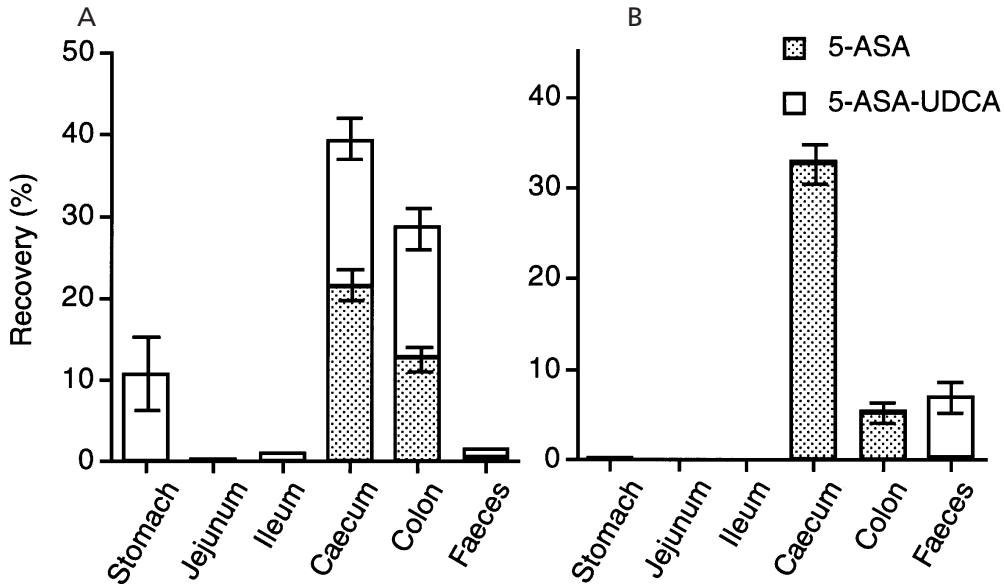


**Figure 5** Recovery of 5-ASA–UDCA and 5-ASA from luminal contents of guinea-pigs with ulcerative colitis at four (A) and twelve (B) hours after oral administration of 5-ASA–UDCA (207 mg $kg^{-1}$). Each column represents the mean ± s.e. of seven guinea-pigs.

5-ASA–CDCA and 5-ASA–UDCA, respectively, were recovered as 5-ASA in the large intestine and there was found to be a significant difference ($P < 0.05$) between the treatments (Figures 4B and 5B). Also, 7% of 5-ASA–UDCA was excreted in faeces without deconjugation. The formation of 5-ASA was recognized in neither the stomach nor the small intestine for both conjugates. These results indicate that the activity of hydrolysis of the conjugates was localized only in the large intestine. Furthermore, 5-ASA–CDCA reached the caecum and the colon and could be completely hydrolysed to 5-ASA.

## Discussion

The degraded form of carrageenan has generally been used to prepare colitis models in animals (Anver & Cohen 1976; Watt & Marcus 1975; Watt et al 1979; Marcus et al 1989; Kitsukawa et al 1992). The provision of carrageenan to guinea-pigs induces colonic lesions that are similar to those noted in ulcerative colitis in humans (Watt & Marcus 1971; Onderdonk et al 1978). In animals, however, the initial lesions involve the caecum and then progress distally, whereas the distal colon is the predominant site of disease in humans (Marcus et al 1989). An experimental animal model used in this study, with a relatively rapid onset of ulceration, provided a convenient and economic method of evaluating the relative efficiency of the administration of two conjugates. Guinea-pigs receiving 2% degraded carrageenan solution as drinking fluid showed a slight body weight gain, occult blood in faeces, and ulceration in isolated forms within the limits of the caecum and proximal colon (Table 1 and Figure 2). There was a good correlation ($P < 0.01$) between the bleeding scores for occult blood and the number of ulcers in the caecum as shown in Figure 3, suggesting that it is possible to assess the severity of the ulcerative colitis and the relative effects of the conjugates without sacrifice.

In this study, two epimeric 5-ASA–bile-acid conjugates were designed to deliver 5-ASA to the large intestine, where 5-ASA was liberated. The conjugates were shown to be stable in the stomach and small intestine, but they were quantitatively hydrolysed to 5-ASA in the large intestine (Figures 4 and 5). Given that most of the hydrolytic activity in the luminal contents is probably due to enzymes produced by the microflora of the gastrointestinal tract, these findings on the gastrointestinal distribution of 5-ASA liberated from the conjugates were predictable: the large intestine had the highest hydrolytic activity for both conjugates and no hydrolysis was seen in the stomach over 12 h. In guinea-pigs, $10^7$ $g^{-1}$, $10^6$ $g^{-1}$, $10^{7-8}$ $g^{-1}$ and $10^9$ $g^{-1}$ enterobacteria are present in the stomach, jejunum, ileum and large intestine, respectively (Drasar 1988). The bacteria mediating the deconjugation of bile-acid conjugates were considered to increase modestly in the more distal ileum and markedly in the colon. In humans, $< 10^3$ $g^{-1}$, $< 10^4$ $g^{-1}$, $10^7$ $g^{-1}$ and $10^{10-12}$ $g^{-1}$ enterobacteria are present in the stomach, jejunum, ileum and large intestine, respectively (Drasar et al 1969; Simon & Gorbach 1986). Thus, the potential efficiency of the conjugate-delivery approach for selective local delivery of 5-ASA to the large intestine is probably much greater in humans than can be demonstrated in guinea-pigs. According to Konishi et al (1997), 5-ASA–UDCA monophosphate was poorly absorbed from the intestine of rats and completely resistant to deconjugation by pancreatic enzymes, human plasma, intestinal mucosal homogenates and liver homogenates. Since the conjugates were designed to be hydrolysed by the enzymes produced by enteric bacteria, it is possible that pH and food content in the gastrointestinal tract have little influence on the delivery of 5-ASA.

From these findings, it was indicated that guinea-pigs given lower doses of 5-ASA–CDCA, equivalent to 50 mg $kg^{-1}$ of 5-ASA, would show higher efficacy or potency in colitis than any other groups under the same experimental conditions (Table 1). In the groups given 5-ASA–UDCA, there was no significant difference in body weight gain or the number of ulcers in the caecum and colon from the control group. Only the level of the bleeding score was lowered from 4 to 3 (Figure 2B). This may be due to the insufficient liberation of 5-ASA from 5-ASA–UDCA compared with from 5-ASA–CDCA in the large intestine. The deconjugation of 5-ASA–CDCA was virtually complete in the large intestine but about 7% of 5-ASA–UDCA was excreted in faeces without deconjugation (Figures 4B and 5B). Batta et al (1998) reported a similar finding, that about 60% of UDCA was present in the 5-ASA-conjugated form in faeces after 5-ASA–UDCA was orally administered to rats for 14 days.

CDCA is a primary bile acid in the guinea-pig and human (Guertin et al 1995). UDCA is a 7$\beta$-hydroxy epimer of CDCA and has quite different physicochemical characteristics, such as aqueous solubility, critical micellar concentration and lipophilicity, from CDCA (Igimi & Carey 1980; Roda et al 1990; Moroi et al 1992). Especially, the aqueous solubility of UDCA was very small compared with CDCA and other bile acids (Moroi et al 1992). UDCA was precipitated in the colon at pH $< 8.0$ but CDCA was soluble at pH $> 6.9$ (Igimi & Carey 1980). These differences would be related to the lower hydrolytic activity or anti-inflammatory efficacy of 5-ASA–CDCA in the large intestine. To remove the influence of the bile acids released from the conjugates on the activity of 5-ASA, the physicochemical properties and the rate of hydrolysis of the conjugates should be investigated. Furthermore, more studies are required to more effectively demonstrate the anti-inflammatory activity of the compound, such as microscopic histological analysis or biochemical determination of mediators involved in the inflammatory response.

In this study, it was indicated that 5-ASA–CDCA was more effective than 5-ASA–UDCA against the

ulcerative colitis experimentally induced by degraded carrageenan in guinea-pigs. During administration of 5-ASA–CDCA, the guinea-pigs recovered body weight. The bleeding scores in faeces and the numbers of ulcers in the caecum were more significantly decreased in comparison with those of the control group and the 5-ASA–UDCA administered group, plus the ulcers in the colon disappeared. Although dose dependence was not noted, the dose of 5-ASA–CDCA equivalent to 50 mg $kg^{-1}$ of 5-ASA showed a significant difference ($P < 0.01$) in the number of ulcers from the control. This seems to be because 5-ASA–CDCA was more easily hydrolysed to release 5-ASA in the caecum and colon. In the guinea-pigs given the conjugates, there was no diarrhoea in faeces as a side effect of bile acids. These conjugates, 5-ASA–CDCA in particular, may be useful as therapeutic agents and the lower dose is sufficient for the treatment of ulcerative colitis and Crohn's disease occurring in the human large intestine.

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