Ursodeoxycholic Acid versus Sulfasalazine in Colitis-Related Colon Carcinogenesis in Mice

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Abstract

Purpose: Inflammation influences carcinogenesis. In the current study, we investigated whether ursodeoxycholic acid (UDCA) can inhibit colitis-related mouse colon carcinogenesis and compared it with the effects of sulfasalazine.

Experimental Design: Male CD-1 mice were given a single i.p. injection of azoxymethane followed by 1-week oral exposure of 1% dextran sodium sulfate in drinking water. They are then maintained on a basal diet mixed with UDCA (0.016%, 0.08%, or 0.4%) or sulfasalazine (0.05%) for 17 weeks. At week 20, the tumor-inhibitory effects of both chemicals were assessed by counting the incidence and multiplicity of colonic neoplasms. The immunohistochemical expression of the proliferating cell nuclear antigen labeling index in colonic epithelial malignancies was also assessed. Finally, at week 5, the mRNA expressions for cyclooxygenase-2, inducible nitric oxide synthase, peroxisome proliferator-activated receptor-γ, and tumor necrosis factor-α were measured in nontumorous mucosa.

Results: Feeding the mice with UDCA at all doses significantly inhibited the multiplicity of colonic adenocarcinoma. The treatment also significantly lowered the proliferating cell nuclear antigen labeling index in the colonic malignancies. UDCA feeding reduced the expression of inducible nitric oxide synthase and tumor necrosis factor-α mRNA in the colonic mucosa, while not significantly affecting the expression of cyclooxygenase-2 mRNA and peroxisome proliferator-activated receptor-γ mRNA. Sulfasalazine caused a nonsignificant reduction in the incidence and multiplicity of colonic neoplasia and did not affect these mRNA expression.

Conclusions: Our findings suggest that UDCA rather than sulfasalazine could serve as an effective suppressing agent in colitis-related colon cancer development in mice.

Colorectal cancer is one of the most serious complications of inflammatory bowel disease (IBD), including ulcerative colitis (1). Ulcerative colitis patients are 10 times more likely to develop colorectal dysplasia and adenocarcinoma than the general population (2). The precise mechanisms involved in IBD-related carcinogenesis are largely unclear, but chronic inflammation influences the development of IBD-related carcinogenesis (3).

Epidemiologic studies suggest that environmental and dietary factors contribute to the development of colorectal cancer (4). High fats generate secondary bile acids that are known to enhance cell proliferation and promote colorectal cancer development (5). In humans, high levels of serum and fecal secondary bile acids are found in patients with colitis (6) and colorectal neoplasia (7). On the other hand, ursodeoxycholic acid (UDCA) reduces the colonic concentration of the secondary bile acids (8) and suppresses colon carcinogenesis (9–11) in animal studies.

Primary prevention of colorectal cancer in IBD has recently received more attention. A number of therapeutic agents using IBD may have chemopreventive effects and offer some hope for primary chemoprevention of colorectal cancer in IBD (12–14). Aminosalicylates, including sulfasalazine, are the most commonly prescribed anti-inflammatory agents for IBD. The intake of sulfasalazine, especially as a long-term maintenance treatment in chronic IBD, was found to be associated with a decreased risk of colorectal cancer (15). UDCA is also reported to have colon cancer chemopreventive effects in preclinical studies (9–11). A recent report has suggested that UDCA decreases the risk for developing dysplasia in ulcerative colitis patients with primary sclerosing cholangitis (16, 17).

For understanding the pathogenesis of IBD and IBD-related colorectal cancer, several animal models have been established. Most common is a mouse model with dextran sodium sulfate (DSS; ref. 18). The modifying effects of certain xenobiotics on colorectal cancer–related colon carcinogenesis have been reported (19) using this model. The model uses DSS, with or without carcinogen, and also requires a long period and repeated administration of DSS to induce colitis and colitis-related

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colorectal cancer. We therefore developed a novel colitis-related mouse colorectal cancer model initiated with a colon carcinogen azoxymethane and promoted by DSS. In this new model, colorectal cancer develops within a short-term period (20), and the expression of cyclooxygenase (COX)-2 and inducible nitric oxide synthase (iNOS) increased the colonic neoplasia (20) and human IBD (21, 22). In addition, peroxisome proliferator-activated receptors (PPAR) play important roles in both the lipid metabolism and inflammation (23). PPAR ligands attenuate the colonic inflammation in the mouse model of colitis (24), and the protein levels of PPARγ are decreased in cryptal cells from the ulcerative colitis patients (25). Using this animal model, we showed that dietary administration of COX-2 inhibitor and PPAR ligands can suppress colitis-related colonic carcinogenesis through suppression of COX-2 and iNOS expression (24).

To determine the chemoprevention ability of UDCA in colitis-related colon carcinogenesis in the present study, we examined the effect of UDCA on our mouse colon carcinogenesis model and compared with sulfasalazine used for patients with IBD.

Materials and Methods

Animals, chemicals, and diets. Male Crj:CD-1 (ICR) mice (Charles River Japan, Inc., Tokyo, Japan) ages 5 weeks were used in this study. They were maintained at Kanazawa Medical University Animal Facility according to Institutional Animal Care Guidelines. All animals were housed in plastic cages (five or six mice per cage) with free access to drinking water and a pelleted basal diet and CRF-1 (Oriental Yeast Co. Ltd., Tokyo, Japan). The mice were under controlled conditions of humidity (50 ± 10%), light (12/12-h light/dark cycle), and temperature (23 ± 2°C). They were quarantined for the first 7 days then separated randomly by body weight into experimental and control groups. A colonic carcinogen azoxymethane and sulfasalazine were purchased from Sigma-Chemical Co. (St. Louis, MO), and DSS with a molecular weight of 36,000 to 50,000 was purchased from ICN Biochemicals, Inc. (Aurora, OH). The DSS for induction of colitis was dissolved in water at a concentration of 1% (w/v). UDCA was obtained from Mitsubishi Pharma Corp. (Osaka, Japan). Experimental diets were prepared by mixing chemopreventive agents with a modified CRF-1 control diet. The compound was mixed at concentrations of 0.016%, 0.08%, and 0.4% with the powdered basal diet CRF-1. The highest dose (0.4%) was a selected to investigate the effects of UDCA on colon polyp formation as used by Narisawa et al. (10). The dose of sulfasalazine was chosen from the results of a study conducted by Suzuki et al. (26), in which a diet, including sulfasalazine, suppressed colorectal carcinogenesis.

Experimental procedure. A total of 130 male ICR mice were divided into eight experimental and control groups. The mice in groups 1 to 5 were given a single i.p. injection of azoxymethane (10 mg/kg body weight). Starting 1 week after the injection, they were administered 1% DSS in drinking water for 7 days. The mice in group 1 were maintained on the basal diet throughout the study. Mice in groups 2 to 4 were given a diet, including UDCA (0.016% in group 2, 0.08% in group 3, and 0.4% in group 4), starting 1 week after the stop of DSS administration. Group 5 was fed a diet mixed with 0.05% sulfasalazine, starting 1 week after the stop of DSS administration. Animals in groups 6 and 7 were given a diet containing 0.4% UDCA alone and 0.05% sulfasalazine alone, respectively. Group 8 was untreated. To assess the relative quantification of gene expression in colonic mucosa at week 5, samples of colon of mice (n = 5 per group) were washed with PBS, and they were quickly frozen in liquid nitrogen and stored at −80°C. All the remaining animals were sacrificed at week 20. At sacrifice, all organs were removed, and the large bowels were flushed with PBS, excised, measured in length (from ileocecal junction to the anal verge), cut open longitudinally along the main axis, and then washed with PBS. The large bowels were macroscopically inspected, and whole colon was processed for paraffin embedding. The large bowels were macroscopically inspected, cut, and fixed in 10% buffered formalin for at least 24 h. A histologic examination was then done on paraffin-embedded sections after H&E staining. Colonic neoplasms were diagnosed according to the description by Ward (27).

Immunohistochemistry of proliferating cell nuclear antigen. Immunohistochemistry for proliferating cell nuclear antigen (PCNA) was done on 4-μm-thick paraffin-embedded sections from colonic adenocarcinomas of mice in groups 1 to 5 at week 20 as previous described (24). Briefly, the sections were incubated overnight at 4°C with a primary antibody, anti-PCNA mouse monoclonal antibody (1:50 dilution; PC10; Dako, Glostrup, Denmark). To reduce the nonspecific staining of mouse tissue by the mouse antibodies, a Mouse On Mouse IgG blocking reagent (Vector Laboratories, Inc., Burlingame, CA) was applied for 1 h. Horseradish peroxidase activity was visualized through treatment with H_2O_2 and 3,3′-diaminobenzidine for 5 min. At the last step, the sections were weakly counterstained with Mayer's hematoxylin (Merck Ltd., Tokyo, Japan). For control sections, incubation with the primary antibodies was omitted. Intensity and localization of immunoreactivity against the primary antibody were examined on all colonic adenocarcinomas using a microscope (Olympus BX41, Olympus Optical Co. Ltd., Tokyo, Japan). For the determination of PCNA-incorporated nuclei, a PCNA immunohistochemistry was done according to the method described by Watanabe et al. (28). The PCNA indices were determined by counting the number of positive cells among at least 200 cells in a lesion and were indicated as percentages.

RNA extraction and synthesis of cDNA. The extraction of total RNA from frozen colonic mucosa was done using an RNeasy Mini kit (QIAGEN, Inc., Valencia, CA) following the manufacturer's directions. The concentration of total RNA was measured using a spectrophotometer. The A_{260}/A_{280} ratio of RNA was 1.8 to 2.0. Oligo (dT)20 primer and SuperScript III First-Strand Synthesis System for reverse transcription-PCR (Invitrogen/Life Technologies, Paisley, United Kingdom) was used to synthesize cDNA according to the manufacturer's protocol and stored at −30°C until analyzed.

Relative quantification of gene expression by LightCycler reverse transcription-PCR. Reverse transcription-PCR analysis showed comparable levels of glyceraldehyde-3-phosphate dehydrogenase mRNA in the non-lesional colonic mucosa of five mice from each group at week 5 after DSS administration. All real-time experiments were conducted using a LightCycler FastStart DNA Master HybProbe kit (Roche, Mannheim, Germany). Oligonucleotide primers and hybridization probes are purchased from Nihon Gene Research Labs, Inc. (Sendai, Japan) and listed in Table 1. PCR reactions contained 15 μL of master mix and 5 μL of template cDNA. The final reaction mixture contained glyceraldehyde-3-phosphate dehydrogenase, iNOS, tumor necrosis factor-α (TNF-α), 3 mmol/L $MgCl_2$, 0.5 μmol/L of the primers (forward and reverse), 0.2 μmol/L of the Fluorescein probe, 0.4 μmol/L of the LCRed probe, and 1× LightCycler FastStart DNA Master HybProbe. It also contained PPARγ, 3 mmol/L $MgCl_2$, 0.3 μmol/L of the primers (forward and reverse), 0.2 μmol/L of the probes (Fluorescein and LCRed), 1× LightCycler FastStart DNA Master HybProbe, and COX-2 based on the manufacturer's recommendations. The contents were placed in a glass capillary, capped, briefly centrifuged, and placed in the LightCycler. The denaturing process for all genes was one cycle at 95°C for 10 min. The amplification conditions were as follows: glyceraldehyde-3-phosphate dehydrogenase, 40 cycles of 95°C for 10 s, 60°C for 15 s, and 72°C for 9 s; iNOS, 40 cycles of 95°C for 10 s, 62°C for 15 s, and 72°C for 9 s; TNF-α, 40 cycles of 95°C for 10 s, 61°C for 15 s, and 72°C for 7 s; and PPARγ and COX-2, 45 cycles of 95°C for 10 s, 62°C for 15 s, and 72°C for 7 s. The amplification protocol was followed by a cooling period of one cycle at 40°C for 30 s. Data collection was done during extension and monitored through the F2/1

Table 1. Primers and probes used for real-time PCR

Gene	Primer or probe	Sequence (5′-3′)
GAPDH	Forward primer	TGAACGGGAAGCTCACTGG
	Reverse primer	TCCACCACCCTGTTGCTGTA
	Donor probe	CTGAGGACCAGGTTGTCTCCTGCGA
	Acceptor probe	TTCAACAGCAACTCCCACTCTTCCACC
iNOS	Forward primer	GCAAACCCAAGGTCTACGTT
	Reverse primer	GGAAAAGACTGCACCGAAGA
	Donor probe	TGGTAGCCACATCCCGAGCCAT
	Acceptor probe	CGCACATCTCCGCAAATGTAGAGG
TNF-α	Forward primer	CCACGTCGTAGCAAACCAC
	Reverse primer	TGGGTGAGGAGCACGTAGT
	Donor probe	TGGTGCCAGCCGATGGGTTGTAC
	Acceptor probe	TTGTCTACTCCCAGGTTCTCTTCAAGG
PPARγ	Forward primer	GAGTTCCTCAAAAACCTGCG
	Reverse primer	TGTCTTGGATGTCCTCGATG
	Donor probe	CAATGCACTGGAATTAGATGACAGTGACTTGGCTA
	Acceptor probe	ATTTATAGCTGTCATTATTCTCAGTGGAGACCGCCC
COX-2	Forward primer	CCATCTGTTCTCCTCAATAC
	Reverse primer	TTTGGTAGGCTGTGGAT
	Donor probe	ACCTTTGGAGGCGAAGTGGGTTTTAAGATC
	Acceptor probe	TCAATACTGCCTCAATTCAGTCTCTCATCTGCA

NOTE: Donor probes were labeled at the 3′ end with fluorescein. Acceptor probes were labeled at the 5′ end with LightCyler Red 640.

channel of the instrument. Data analyses were conducted using the second derivative maximum method of the LightCycler software.

Statistical analysis. All measurements were compared using Tukey's or Bonferroni's multiple comparison post test or Fisher's exact probability test. Differences were considered statistically significant at $P < 0.05$.

Results

General observation. Bloody stool was observed in a few mice that received 1% DSS, and body weight gains were slightly decreased during the course of the treatment. However, thereafter, no clinical symptoms were noted. Mean weights, mean liver weights, relative liver weights, and mean length of large bowel in all groups at the end of the study are shown in Table 2. The mean body weights, liver weights, and relative liver weights did not significantly differ among the groups. The mean length of the large bowels in groups 2 to 5 was lower than in group 1, but the differences did not reach statistical significance. Histologically, there were no pathologic alterations suggesting toxicity of UDCA and sulfasalazine in the liver, kidneys, lung, or heart. Food consumption (g/d per mice) did not significantly differ among the groups (data not shown).

Incidence and multiplicity of colonic neoplasm. Macroscopically, nodular and polypoid colonic tumors were observed in the middle and distal colon of mice in groups 1 to 5. Histopathologically, dysplasia, adenoma, and adenocarcinoma (Fig. 1A, C, E, G, and I) developed in the azoxymethane/DSS–treated mice. The mice in groups 6 to 8 did not exhibit large bowel neoplasms in any organs examined, including the colon. The incidences and multiplicity of colon neoplasma are summarized in Table 3. Group 1 (azoxymethane/DSS) induced a 65% incidence of colon tumors with a multiplicity of 3.7 ± 6.0. The incidences of colon tumors in group 2 (azoxymethane/DSS/0.016% UDCA), group 3 (azoxymethane/DSS/0.08% UDCA), and group 4 (azoxymethane/DSS/0.4% UDCA) were lower than group 1. The differences, however, were not statistically

Table 2. Body, liver, and relative liver weights

Group no.	Treatment	No. mice	Body weight (g)	Liver weight (g)	Relative liver weight (g/100 g body weight)	Length of large bowel (cm)
1	AOM/DSS	20	49.8 ± 5.6	2.98 ± 0.50	5.98 ± 0.68	14.1 ± 1.0
2	AOM/DSS/0.016% UDCA	20	44.7 ± 4.2	2.78 ± 0.40	6.21 ± 0.64	14.2 ± 1.1
3	AOM/DSS/0.08% UDCA	20	47.3 ± 6.4	2.72 ± 0.28	5.79 ± 0.46	14.0 ± 1.2
4	AOM/DSS/0.4% UDCA	20	48.9 ± 2.6	2.63 ± 0.42	5.38 ± 0.71	14.6 ± 1.1
5	AOM/DSS/0.05% SASP	20	45.0 ± 4.3	2.75 ± 0.23	6.14 ± 0.59	13.4 ± 1.2
6	0.4% UDCA	5	42.0 ± 1.8	2.35 ± 0.19	5.60 ± 0.36	13.8 ± 0.6
7	0.05% SASP	5	45.5 ± 3.4	2.80 ± 0.30	6.17 ± 0.54	14.9 ± 0.6
8	None	5	43.6 ± 3.8	2.67 ± 0.43	6.13 ± 0.95	13.4 ± 1.1

NOTE: Values are expressed as mean ± SD.
Abbreviations: AOM, azoxymethane; SASP, sulfasalazine.

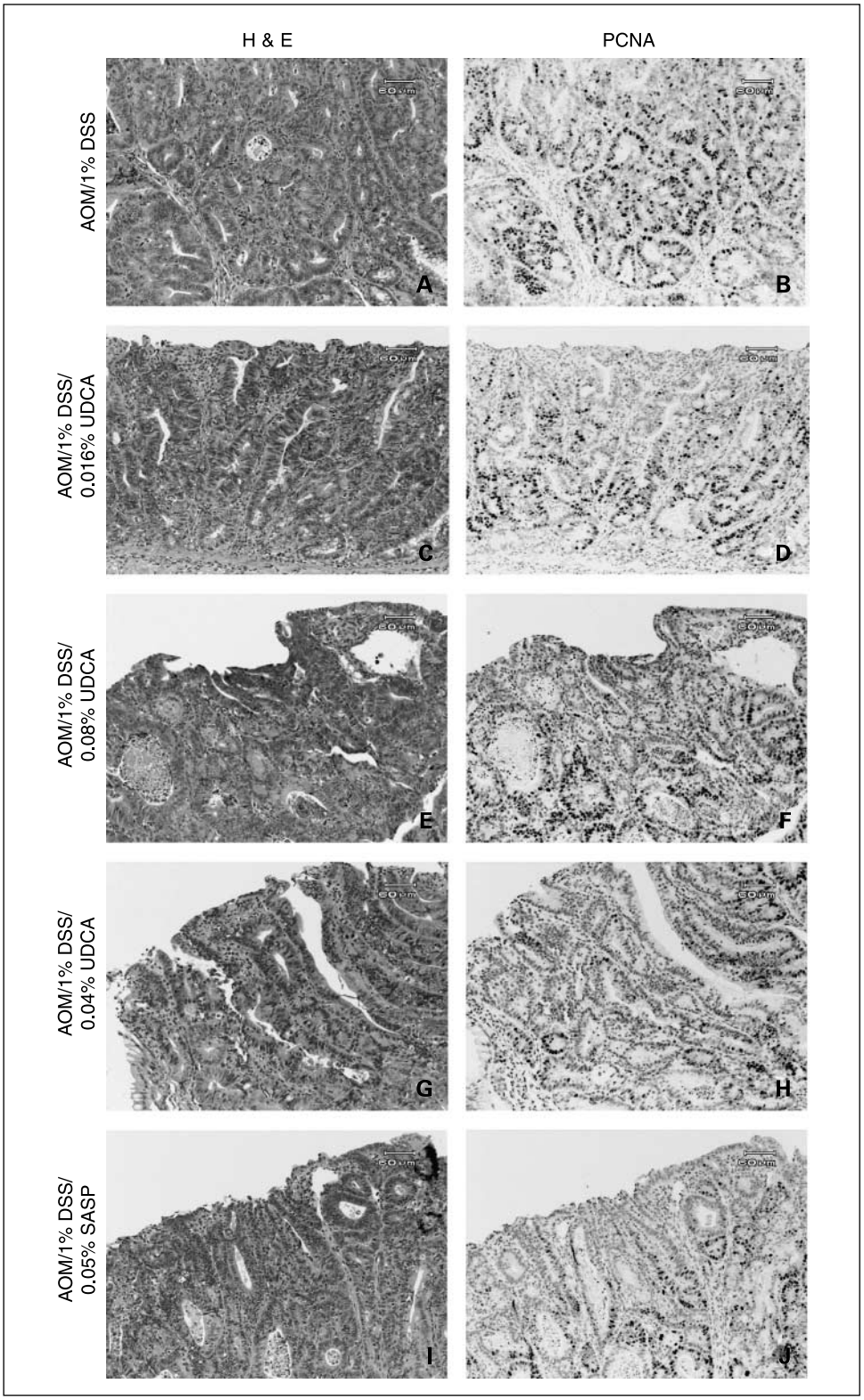

Fig. 1. Histopathology of adenocarcinomas and their corresponding immunohistochemistry of PCNA. An adenocarcinoma from a mouse of group 1 (*A* and *B*), 2 (*C* and *D*), 3 (*E* and *F*), 4 (*G* and *H*), or 5 (*I* and *J*). H&E stain (*A, C, E, G*, and *I*) and PCNA immunohistochemistry (*B, D, F, H*, and *J*). Bar, 60 μm.

significant. The multiplicities of colon tumors and adenocarcinomas in group 2 ($P < 0.05$), group 3 ($P < 0.05$), and group 4 ($P < 0.05$) were significantly lower than that of group 1. Finally, whereas the multiplicities of colon tumors and adenocarcinomas in group 5 (azoxymethane/DSS/0.05% sulfasalazine) were smaller than group 1, the differences did not reach to statistical significance.

Immunohistochemistry for PCNA in colonic adenocarcinoma. As summarized in Table 4, the PCNA labeling indices of colonic adenocarcinomas developed in group 2 ($P < 0.01$; Fig. 1D), group 3 ($P < 0.001$; Fig. 1F), and group 4 ($P < 0.001$; Fig. 1H) were significantly smaller than group 1 (Fig. 1B). Dietary sulfasalazine (group 5) did not significantly affect the PCNA

Table 3. Incidence and multiplicity of colonic neoplasia

Group no.	Treatment	No. mice	Incidence (no. mice with neoplasms)			Multiplicity (no. tumors/mice)*		
			Total	Adenoma	Adenocarcinoma	Total	Adenoma	Adenocarcinoma
1	AOM/DSS	20	13/20 (65%)	12/20 (60%)	9/20 (45%)	3.7 ± 6.0	1.5 ± 1.8	2.2 ± 4.5
2	AOM/DSS/0.016% UDCA	20	8/20 (40%)	6/20 (30%)	5/20 (25%)	0.9 ± 1.5†	0.6 ± 1.1	0.3 ± 0.4†
3	AOM/DSS/0.08% UDCA	20	9/20 (45%)	5/20 (25%)	5/20 (25%)	0.6 ± 0.8†	0.4 ± 0.7	0.3 ± 0.4†
4	AOM/DSS/0.4% UDCA	20	8/20 (40%)	8/20 (40%)	4/20 (20%)	1.0 ± 1.5†	0.7 ± 0.9	0.3 ± 0.7†
5	AOM/DSS/0.05% SASP	20	11/20 (55%)	9/20 (45%)	8/20 (40%)	1.6 ± 1.9	0.9 ± 1.1	0.7 ± 1.0
6	0.4% UDCA	5	0/4 (0%)	0/4 (0%)	0/4 (0%)	0	0	0
7	0.05% SASP	5	0/5 (0%)	0/5 (0%)	0/5 (0%)	0	0	0
8	None	5	0/5 (0%)	0/5 (0%)	0/5 (0%)	0	0	0

*Values are expressed as mean ± SD.
†$P < 0.05$, significantly different from group 1 by Tukey's multiple comparison post-test.

labeling index in the colonic adenocarcinoma (Fig. 1J) of the mice in comparison with the azoxymethane/DSS group (Fig. 1B).

Validation of selected genes by real-time quantitative reverse transcription-PCR. As shown in Table 5, the mRNA expression of COX-2, iNOS, and TNF-α in group 8 (nontreated group) were very low in the colonic mucosa, but the expression dramatically increased when the mice were given azoxymethane/DSS (group 1). PPARγ mRNA expression in mice treated with azoxymethane (groups 1–5) was higher than non-azoxymethane mice (groups 6–8), but the values were not statistically different among the groups.

The mRNA expression of iNOS in the colonic tissue of group 2 (azoxymethane/DSS/0.016% UDCA, $P < 0.001$), group 3 (azoxymethane/DSS/0.08% UDCA, $P < 0.001$), and group 4 (azoxymethane/DSS/0.4% UDCA, $P < 0.01$) was significantly lower than that of group 1 (azoxymethane/DSS). The expression of TNF-α mRNA was significantly lower in the group 2 ($P < 0.001$), group 3 ($P < 0.001$), and group 4 ($P < 0.01$) in comparison with group 1. In contrast, there were no differences in the expression of COX-2 and PPARγ in the colonic mucosa among the groups. The differences in expression of COX-2, iNOS, PPARγ, and TNF-α mRNA between group 1 (azoxymethane/DSS) and group 5 (azoxymethane/DSS/sulfasalazine) were not statistical significant.

Discussion

The results of the present study clearly indicated that UDCA effectively inhibits azoxymethane/DSS–induced colitis-related colonic carcinogenesis without any adverse effects in mice. The inhibitory effect of UDCA was closely similar at all dose levels. This correlates with the findings of other reports, in which of carcinogen-induced colon carcinogenesis by feeding with UDCA was not dose dependent (9–11). The suppressive effect of UDCA on the development of colonic adenocarcinoma closely correlated with the inhibition of the PCNA labeling index of colonic adenocarcinomas and the suppression of iNOS and TNF-α mRNA levels in the nontumorous colonic mucosa.

In both experimental animal and human studies, secondary bile acids, such as DCA, have been shown to enhance colonic epithelial cell proliferation, to be cytotoxic to colonic epithelial cells (29), and to be moderately mutagenic (30). In contrast, UDCA inhibited colorectal cancer and adenoma formation in azoxymethane-induced rat carcinogenesis models (11). Furthermore, recent reports suggested that UDCA decreases the risk for developing dysplasia and cancer in ulcerative colitis patients with primary sclerosing cholangitis (16, 17). More recently, Loddenkemper et al. (31) found in their colitis-associated model that the development of murine colorectal tumors can be inhibited by oral treatment with UDCA. Although they noted that colonic tumors were histologically mucinous adenocarcinomas or squamous carcinomas, we observed well/moderately differentiated tubular adenocarcinomas in the present study. These findings may suggest that dietary UDCA is able to suppress the development of various histologic types of colon carcinoma.

Sulfasalazine is commonly used for the treatment of IBD, such as ulcerative colitis and Chron's disease (32). Published evidence indicates that sulfasalazine prevents the development of dysplasia and colorectal cancer in patients with IBD (33). Recently, Suzuki et al. (26) reported that treatment with sulfasalazine resulted in a reduction of tumorous lesions with high-grade dysplasia in female CBA/J mice initiated with azoxymethane and promoted by three cycle administration of DSS. In this study, the suppressive effects of sulfasalazine given in the promotion/progression phase on the development of colorectal cancer were relatively weak. The discrepancy between their results and ours may be due to the differences in the treatment period of sulfasalazine and/or the strains of mice used.

Table 4. PCNA indices in colonic adenocarcinomas

Group no.	Treatment	PCNA labeling index (%)*
1	AOM + 1% DSS	61.4 ± 11.0 (16)
2	AOM + 1% DSS/0.016% UDCA	39.8 ± 9.3 (5)†
3	AOM + 1% DSS/0.08% UDCA	38.2 ± 6.1 (5)‡
4	AOM + 1% DSS/0.4% UDCA	37.1 ± 7.7 (6)‡
5	AOM + 1% DSS/0.05% SASP	52.6 ± 10.0 (14)

*Values in column are expressed as mean ± SD. Numbers in parentheses are numbers of lesions examined.
†$P < 0.01$, significantly different from group 1 by Tukey's multiple comparison post-test.
‡$P < 0.001$, significantly different from group 1 by Tukey's multiple comparison post-test.

Table 5. mRNA expression of COX-2, iNOS, PPARγ, and TNF-α in colonic mucosa at week 5

Group no.	Treatment (no. mice examined)	mRNA/GAPDH mRNA ratio ($\times 10^4$)			
		COX-2	iNOS	PPARγ	TNF-α
1	AOM + 1% DSS (5)	1.03 ± 0.46*	1.41 ± 0.69†	5.13 ± 1.00	7.98 ± 3.38†
2	AOM + 1% DSS/0.016% UDCA (5)	1.25 ± 0.40	0.15 ± 0.22‡	4.83 ± 0.87	2.60 ± 0.79‡
3	AOM + 1% DSS/0.08% UDCA (5)	0.70 ± 0.36	0.26 ± 0.21‡	6.50 ± 2.29	2.57 ± 1.06‡
4	AOM + 1% DSS/0.4% UDCA (5)	1.06 ± 0.40	0.52 ± 0.40§	4.55 ± 1.08	3.38 ± 2.18§
5	AOM + 1% DSS/0.05% SASP (5)	1.07 ± 0.89	1.19 ± 0.51	5.34 ± 0.75	5.00 ± 2.38
6	0.4% UDCA (5)	0.04 ± 0.01	0.02 ± 0.01	2.51 ± 1.08	1.00 ± 0.48
7	0.05% SASP (5)	0.08 ± 0.07	0.03 ± 0.02	2.49 ± 0.43	0.65 ± 0.32
8	None (5)	0.07 ± 0.06	0.06 ± 0.06	2.90 ± 0.83	0.79 ± 0.72

NOTE: Values are expressed as mean ± SD.
*$P < 0.05$, significantly different from group 8 by Bonferroni's multiple comparison post-test.
†$P < 0.01$, significantly different from group 8 by Bonferroni's multiple comparison post-test.
‡$P < 0.001$, significantly different from group 1 by Bonferroni's multiple comparison post-test.
§$P < 0.01$, significantly different from group 1 by Bonferroni's multiple comparison post-test.

The development of colonic neoplasia is influenced by alterations in the balance of cell renewal and cell death that regulate normal cellular homeostasis in the colon (34). UDCA inhibits the proliferation of colon cancer cell lines *in vitro* (9), while also decreasing the size and number of colon tumors induced by *N*-methylnitrosourea (10) or azoxymethane (11) in rats. In addition, Wali et al. (35) reported that UDCA inhibits cyclin D1 expression. This is an important positive cell cycle regulator (36) in aberrant crypt foci and non–aberrant crypt foci crypts in rats treated with azoxymethane in conjunction with their inhibition of crypt cell hyperplasia and tumors. In the present study, UDCA feeding significantly lowered the PCNA labeling index in the adenocarcinomas, thus suggesting that dietary UDCA therefore suppresses the abnormal proliferative activity of preneoplastic and neoplastic cells, thereby inhibiting carcinogenesis.

Inflammation-caused oxidative/nitrosative cellular damage is suspected to contribute to the development of IBD-associated colorectal neoplasms. Expression and activity of iNOS is increased in the colonic mucosa in patients with IBD (37) and colonic adenomas (38) in murine noncancerous colonic mucosa (39, 40). Numerous iNOS-positive and nitrotyrosine-positive inflammatory cells are observed in the nontumorous tissues of mice treated with DSS (40). Rao et al. (41) showed that an iNOS-selective inhibitor suppressed azoxymethane-induced colonic aberrant crypt foci development and their iNOS activity. UDCA also inhibited the induction of iNOS in the human intestinal adenocarcinoma cell lines and the colonic epithelium of rats exposed to lipopolysaccharide (42). In the current study, UDCA significantly reduced iNOS mRNA expression in the colonic mucosa at week 5. Therefore, the suppression of iNOS expression and/or activity may thus be related to the chemopreventive effects of UDCA.

TNF-α is a potent pro-inflammatory cytokine involved in the pathogenesis of IBD, which plays a crucial role in the progression inflammatory responses in the colon (43). In fact, the plasma and colonic TNF-α levels are elevated in patients with IBD (44, 45), and the colonic expression of TNF-α mRNA is also enhanced in DSS-induced colitis in mice (46). Although the results from the study on treatment with anti-TNF-α monoclonal antibody in DSS-treated mice suggested a more complex role for TNF-α in colonic inflammation (47), Myers et al. (48) reported that antisense oligonucleotide specific for murine TNF-α could prevent DSS-induced colitis. In the current study, we found that dietary UDCA thus reduces the expression of TNF-α mRNA in colonic mucosa of mice treated with azoxymethane and DSS. Based on our findings, the suppression of the iNOS and TNF-α expression and/or the activity by UDCA may thus explain its chemopreventive ability in colitis-related mouse carcinogenesis induced by azoxymethane/DSS. Taken together, UDCA rather than sulfasalazine could be a valuable approach for the chemoprevention of human IBD-related colorectal cancer.

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