Auraptene Decreases the Activity of Matrix Metalloproteinases
in Dextran Sulfate Sodium-Induced Ulcerative Colitis in ICR Mice

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Previously we reported that auraptene was a potent suppressant for matrix metalloproteinase (MMP)-7 expression in HT-29 human colon cancer cells. In the present study, we examined the effects of auraptene on MMP-2, -7, and -9 expression in colonic mucosa from dextran sulfate sodium (DSS)-induced ulcerative colitis mice. Auraptene remarkably suppressed the DSS-induced gelatinolytic activity of MMP-7 as well as the expression of MMP-2 and -9, suggesting that it might be useful in anti-metastatic therapies via the targeting of MMPs.

Key words: matrix metalloproteinase; auraptene; ulcerative colitis; dextran sulfate sodium

Matrix metalloproteinase (MMP)-7, also referred as matrilysin, is thought to play important roles in cancer progression and metastasis, suggesting that MMP-7 suppressants might be beneficial agents in chemoprevention and chemotherapy strategies. To achieve this goal, we investigated and found that citrus nobiletin and auraptene suppressed MMP-7 expression in HT-29 cells via targeting transcription and translation steps respectively.

Previously we identified auraptene (7-geranyloxycoumarin) as an inhibitor of 12-O-tetradecanoylphorbol-13-acetate-induced Epstein-Barr virus activation. This compound occurs in a variety of citrus fruits, such as natsumikan (Citrus natsudaidai), hassaku orange (C. hassaku), and grapefruit (C. paradisi), and can be used as a food additive, based on the recent establishment of an easily performed method of producing large quantities.

Inflammatory bowel disease (IBD), such as ulcerative colitis (UC) and Crohn’s disease, is a potent risk factor for colorectal cancer. MMP-7 was induced in mucosa from DSS-administered mice, which are used as the animal model of UC. Hence, we examined the effect of oral feeding of auraptene on MMP-7 activity using this model.

Five-week-old female ICR mice were purchased from Japan SLC (Shizuoka, Japan). All animals were fed commercial rodent MF pellets (Oriental Yeast, Kyoto, Japan), given tap water ad libitum, and maintained in a room kept at 22–26 °C with a relative humidity of 55–65% under a 12-h light/dark cycle (06:00–18:00). The mice were treated in accordance with the “Guidelines for the Regulation of Animals,” drafted by the Experimentation Committee of Kyoto University. After a 1-week quarantine, the mice in the DSS group (n = 8) were allowed free access to MF pellets for 2 weeks. In the auraptene group (n = 8), 0.1% (w/w) auraptene was added to the MF pellets for 2 weeks. During the second week, colitis was induced by adding 3% (w/v) DSS to drinking water. The animals in the control group (n = 8) were given MF pellets and fresh tap water ad libitum, changed twice a week, for 2 weeks.

At the end of each experiment, all mice were killed by cervical dislocation. The mucosa was scraped from the large intestine without the cecum and homogenized (100 mg/ml) in ice-cold PBS supplemented with 1% (v/v) protease inhibitor cocktail. Aliquots (10 μg protein) of the supernatants were subjected to electrophoresis in 12% polyacrylamide gels containing 0.6 mg/ml gelatin in the presence of sodium dodecyl sulfate under non-reducing conditions. Following electrophoresis, the gel was washed with gel washing buffer (50 mM Tris, 5 mM calcium chloride, 1 μM zinc chloride, 0.02% w/v sodium azide, 2.5% v/v Triton X-100, and 6 mg/ml heparin) with gentle shaking at room temperature for 1 h, and incubated in incubation buffer (50 mM Tris, 5 mM calcium chloride, 1 μM zinc chloride, 0.02% w/v sodium azide) at 37 °C for 90 min. Following incubation, the gel was stained with 0.25% (w/v) Coomassie brilliant blue. The proteolytic activity of MMPs was visualized as a clear zone. The band intensities were quantified using

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Abbreviations: AUR, auraptene; CTL, control; DSS, dextran sulfate sodium; IBD, inflammatory bowel disease; MMP, matrix metalloproteinase; UC, ulcerative colitis.
NIHImage. β-Actin, detected by Western blotting, as previously described, was used as the internal standard.

Auraptene (> 95% purity, Fig. 1A) was purified as previously reported. Antibody against β-actin (C-11) was purchased from Santa Cruz Biotechnology (Santa Cruz, CA). DSS, with a molecular weight of 40,000, was from ICN Biomedicals (Aurora, OH). Trizma® Base (Tris), protease inhibitor cocktail, calcium chloride, zinc chloride, Triton® X-100, and heparin sodium salt were obtained from Sigma-Aldrich (St. Louis, MO). All other reagents used were purchased from Wako Pure Chemicals (Osaka, Japan), unless specified otherwise.

The animals treated with DSS developed symptoms of ulcerative colitis, such as body weight loss and colorectum shortening. As shown in Fig. 1B, the body weight of mice in both DSS and auraptene groups declined throughout the experiment, and there were statistically significant differences between the control and DSS-treated groups after day 6, while the intake of diet and drinking water showed no statistically significant dif-

Fig. 1. Effects of Auraptene on Symptomatic Changes of DSS-Induced Colitis.

In the control group (CTL, □), the animals were given MF pellets and fresh tap water ad libitum for 2 weeks. In the DSS group (■), the mice were fed MF pellets for 2 weeks, and DSS was added to their drinking water during the second week to induce colitis. In the auraptene group (AUR, ◦), 0.1% (w/w) auraptene was added to the MF pellets for 2 weeks, and the mice were exposed to DSS during the second week. A, Chemical structure of auraptene; B, Body weight; C, diet intake; D, drinking water intake; E, colon length; F, spleen weight. Data are shown as the mean ± SD. The statistical significance of differences between groups was assessed by Student’s t-test. *p < 0.0001 vs. CTL; †p < 0.05 vs. DSS; ‡p < 0.0005 vs. CTL.
ferences among the experimental groups (Fig. 1C and D). On the other hand, the length of the colorectum, a reliable marker of colitis, in the DSS group was remarkably shortened, by 30% \((p < 0.0001)\) as compared with that of the control group, while auraptene significantly suppressed the length, by 30% \((p < 0.05)\) (Fig. 1E). Spleen weights, which also reflect the development of colitis, \(1,11\) in both the DSS and auraptene groups increased as compared with those of the control group, and auraptene tended to decrease it, but not significantly (Fig. 1F). These results indicate that the effect of oral feeding of auraptene on colitis remains to be fully determined in the future.

Although slight MMP-7 activity in the control group was detected, DSS treatment increased it by 3-fold, whereas auraptene down-regulated it to the basal level (Fig. 2). In addition, DSS induced proMMP-2 and -9 expression by 13- and 31-fold, and auraptene dramatically suppressed expression by 100% and 82%, respectively.

We observed that auraptene showed substantial suppression of DSS-induced MMP-7 activity as well as both proMMP-2 and -9 expression in mouse colonic mucosa (Fig. 2). At this moment, we do not know whether auraptene suppresses the induction or activity of these MMPs, but recent cellular experiments by us indicate that this compound targets extracellular signal-regulated kinase 1/2 in disrupting the protein translation of MMP-7.\(5\) The level of MMP-7 expression was associated with the severity of ulcerative colitis and the grade of ulcerative colitis-related dysplasia and carcinoma.\(12,13\) Also, expression of MMP-2 and -9 was enhanced in the intestinal tissue of patients with IBD.\(14\) In conclusion, these observations and ours support the potential usefulness of auraptene in the prevention of colon carcinogenesis and metastasis.

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AUR Decreases MMP Activities in Colon Mucosa


