Mini Review

**Dietary Tricin Suppresses Inflammation-Related Colon Carcinogenesis in Mice**

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**Summary**
Tricin present in rice and wheat exhibits antigrowth activity in several human cancer cell lines and anti-inflammatory potential. However, the chemopreventive activity has not yet been elucidated in preclinical animal models. This study was designed to determine whether dietary tricin exerts inflammation-associated colon carcinogenesis induced by azoxymethane (AOM) and dextran sulfate sodium (DSS) in mice. Male Crj: CD-1 mice were initiated with a single i.p. injection of AOM (10 mg/kg bw) and followed by a 1-week exposure to DSS (1.5%, w/v) in drinking water to induce colonic neoplasms. They were then given the experimental diet containing 50 or 250 ppm tricin. The experiment was terminated at week 18 to determine the chemopreventive efficacy of tricin. The effects of dietary tricin on the expression of several inflammatory cytokines, including tumor necrosis factor (TNF)-α, were also assayed. Feeding with tricin at both doses significantly inhibited the development of colonic tumors. Dietary tricin also significantly reduced the proliferation index and the numbers of mitoses/anaphase bridging of adenocarcinoma cells. Tricin feeding significantly suppressed the TNF-α expression in the normal appearing crypts. Our findings may suggest a potential use of tricin for clinical trials of colorectal cancer chemoprevention in the inflamed colon.

**Key Words**
rice bran, tricin, colitis-associate cancer, colorectal cancer; chemoprevention

A good example that exemplifies the association between chronic inflammation and the onset of cancer is colorectal oncogenesis (1). Colitis-associated colorectal cancer (CAC) is a malignant epithelial neoplasm that develops in the colorectum, where chronic inflammation persists (1). CAC differs from sporadic colorectal cancer (CRC), and is considered the most serious complication of inflammatory bowel disease (IBD), which includes ulcerative colitis (UC) and Crohn’s disease (CD) (1). UC patients are more likely to develop CRC (20-fold to 30-fold risk), when compared to the general population (1, 2). Additionally, the risk of developing CAC increases notably 8–10 y after the patients are diagnosed as IBD (1). Moreover, CAC occurs in patients who are younger than those with CRC, and has more malignant potential than sporadic CRC. Thus, the typically advanced stage of CAC at diagnosis reduces the duration of life expectancy (1). The pathological mechanisms, by which elevate the risk of CAC in IBD particularly UC patients, are still unclear. In spite of recent great advances in the therapy of cancer (3, 4), prevention of the diseases, including cancer, is the gold standard (5). We believe that CAC and/or CRC chemoprevention is needed and possible, when we are focusing on preventing lethal cancers (5, 6). For this purpose, a traditional approach of drug development may serve us for selection of promising chemopreventive agents. However, dietary prevention strategy using bioactive phytochemicals against cancer development is attractive because the approaches are low cost and safe (5, 6). In addition, their target includes preneoplasia in the tissues, implying that chemopreventive efficacy can be monitors by measuring biomarkers (7). Although animal models are limited in their ability to represent human cancers, but that does not negate their potential usefulness in identifying promising candidate chemopreventive agents with low toxicity.

Rice bran, rice bran oil, and defatted rice bran contain several cancer chemopreventive compounds, such as ferulic acid, caffeic acid, γ-oryzanol, phytic acid, fiber (8–13). Among them, a flavones, tricin, is produced during times of environmental stress or pathogenic attack (14). Biological activities of tricin have been reported to be anti-oxidative, anti-inflammatory, anti-viral, and anti-histamic (15). Such activities are suggestive of its potential of cancer chemopreventive effects (16, 17).

In the current study, we investigated the potential cancer chemopreventive effects of tricin on inflammation-associated colorectal carcinogenesis in mice, which we previously developed as an animal model of UC-associated CAC (18, 19). Several in vitro and in vivo studi-
ies on the effects of tricin on cancer cell growth using breast cancer have been reported (8). However, the effects of tricin on colorectal oncogenesis have not been performed.

Materials and Methods

Chemicals. Tricin (>99% pure) was isolated and prepared from the leaves of Sasa albo-marginata (Hou-polou Co. Ltd., Tokyo, Japan). AOM purchased from SIGMA-ALDRICH (St. Louis, MO) was used for tumor initiation of colorectal oncogenesis. 1.5% (w/v) of DSS (MW: 36,000–50,000, Cat. no. 160110 from MP Biomedicals, LLC, Aurora, OH) for induction of colitis was prepared shortly before use.

Animal experiment. A total of 95 male ICR mice (5-wk-old, Charles River Laboratories, Inc., Tokyo, Japan) were used in the study. They were divided into six experimental groups and one control group. The experimental and study design were approved by the Committee of Kanazawa Medical University Animal Facility under the Institutional Animal Care guideline. All handling and procedures were carried out in accordance with the appropriate Institutional Animal Care Guidelines. Mice were divided into six experimental groups and one control group. Mice in groups 1 through 3 were given a single intraperitoneal injection of AOM (10 mg/kg body weight). Beginning one week after the AOM injection, they were given 1.5% DSS in drinking water for seven days. Beginning one week following the final DSS treatment, the mice in groups 2 and 3 were fed an experimental diet containing tricin at a dose of 50 ppm and 250 ppm, respectively, for 15 wk. The mice in group 4 received the 250 ppm tricin-containing diet alone. The mice in groups 5 and 6 were given AOM alone and 1.5% DSS in drinking water, respectively. Group 7 served as untreated controls.

At week 8, four mice each from groups 1 through 3 and three mice each from groups 4 through 7 were randomly sacrificed for measuring mRNA expression of target inflammatory enzymes (COX-2, iNOS) and cytokines (TNF-α, NF-κB, IkBα, and IKKβ) in the colonic mucosa by quantitative reverse transcription-polymerase chain reaction (RT-PCR).

At week 18, complete necropsy of the remaining animals was performed for determining the incidence and multiplicity of tumors in the large bowel. The removed colorectum was fixed in 10% buffered formalin for histopathological examination. On the hematoxylin and eosin (H&E)-stained sections made from paraffin-embedded blocks colorectal lesions were diagnosed according to criteria established in a prior study (19). PCNA immunohistochemistry was also performed to determine the proliferative activity of cancer cells and surrounding mucosal crypts.

Mitotic index (MI) and anaphase bridging index (ABI) of adenocarcinoma cells. The effects of dietary tricin on chromosomal instability (20) in adenocarcinoma cells, the ABI was determined on H&E-stained sections of five adenocarcinomas each from groups 1 through 3.

Statistical analysis. Data obtained were analyzed using one-way ANOVA with Tukey-Kramer Multiple Comparisons Test or Bonferroni (GraphPad Instat version 3.05, GraphPad Software, San Diego, CA, USA). Fisher’s Exact Probability test or the Chi-square test was used for comparison of the incidence of colorectal lesions. Data on mRNA expression were compared with Mann-Whitney U test.

Results

General observation. All animals remained healthy throughout the experimental period. There were no clinical signs of toxicity of tricin.

Table 1. Inhibition rates of the incidence and multiplicity of the colorectal dysplasia by dietary feeding with tricin.

| Treatment (no. of mice) | High grade dysplasia |
|------------------------|----------------------|
|                        | Incidence | Multiplicity |
| AOM + 1.5% DSS (16)    | 100%      | 5.00 ± 3.79  |
| AOM + 1.5% DSS + 50 ppm tricin (16) | 20%      | 49%         |
| AOM + 1.5% DSS + 250 ppm tricin (15) | 27%      | 69%         |

Table 2. Inhibition rates of the incidence and multiplicity of the colorectal adenoma by dietary feeding with tricin.

| Treatment (no. of mice) | Adenoma |
|------------------------|---------|
|                        | Incidence | Multiplicity |
| AOM + 1.5% DSS (16)    | 88%      | 4.19 ± 4.22  |
| AOM + 1.5% DSS + 50 ppm tricin (16) | 50%  | 66%         |
| AOM + 1.5% DSS + 250 ppm tricin (15) | 24%      | 55%         |

Table 3. Inhibition rates of the incidence and multiplicity of the colorectal adenocarcinoma by dietary feeding with tricin.

| Treatment (no. of mice) | Adenocarcinoma |
|------------------------|----------------|
|                        | Incidence | Multiplicity |
| AOM + 1.5% DSS (16)    | 94%       | 4.63 ± 3.72  |
| AOM + 1.5% DSS + 50 ppm tricin (16) | 20%       | 31%         |
| AOM + 1.5% DSS + 250 ppm tricin (15) | 29%       | 61%         |

Incidence and multiplicity of Colorectal lesions. The incidence of macroscopic colorectal lesions, including tumors and small ulcerations, were observed in the mice belonging to the groups 1, 2, 3, and 6. The microscopic data on the incidence and multiplicity (no. of lesion/colorectum) of the lesions, including dysplastic crypts and tumors, are listed in Tables 1–3. There were no colorectal lesions in the mice of groups 4, 5, and 7. The mean number of colorectal adenoma and adenocarcinoma in group 1 were 4.19±4.22 (Table 2) and 4.63±3.72 (Table 3), respectively. The dietary feeding with 50 ppm tricin (group 2) significantly lowered the number of adenoma, when compared to group 1 (66% inhibition rate, p<0.05, Table 2). Dietary administration of 250 ppm tricin (group 3) also significantly reduced the number of adenocarcinoma, when compared to group 1 (61% inhibition rate, p<0.05, Table 3). The mean number of dysplastic crypts in groups 2 (49% inhibition rate, p<0.05) and 3 (69% inhibition rate, p<0.01) were significantly smaller than that of group 1 (Table 1).

PCNA-labeling indices of the normal crypts and adenocarcinoma cells. The dietary administration of tricin significantly reduced the PCNA-labeling index of normal crypts in groups 2 (21% inhibition rate, p<0.05) and 3 (25% inhibition rate, p<0.05), when compared to group 1 (48±11%). The PCNA-labeling indexes of adenocarcinoma cells in groups 2 (7% inhibition rate, p<0.05) and 3 (40% inhibition rate, p<0.001) were significantly smaller than in group 1 (80±8%).

The effects of tricin on the MI and ABI. Dietary feeding of tricin significantly reduced the MI in groups 2 (16% inhibition rate, p<0.05) and 3 (29% inhibition rate, p<0.001), when compared to group 1 (20.8±2.4%). Feeding with tricin also lowered the ABI in groups 2 (55% inhibition rate) and 3 (74% inhibition rate, p<0.05) in comparison to group 1 (1.10±0.57%).

Expressions of inflammatory enzymes and cytokines in colorectal mucosa. At week eight, the TNF-α expression significantly reduced in group 3, when compared to group 1 (p<0.05). While the expression of COX-2, iNOS, NF-κB, IκBα and IKKβ decreased by feeding with tricin, the differences were statistically insignificant.

Discussion

The high dose (250 ppm) of tricin significantly reduced development of adenocarcinoma induced by AOM and DSS in mice. The dietary administration of tricin also significantly affected the mucosal expression of TNF-α at week 8 and significantly lowered the PCNA-labeling index, MI and ABI in the colorectal adenocarcinoma at week 18.

Previously, the anti-tumor and chemoprevention activities of tricin have been reported in both in vitro and in vivo studies. In vivo experiments included transplanted human breast cancer cell lines in nude mice (21). Additionally, 0.2% tricin in the diet fed effectively inhibited the number of adenoma in the small intestine of ApcMin/+ mice (22). The animal model AOM/DSS-induced mice carcinogenesis model is useful for evaluation of potential chemopreventive agents for inflammation-related colon carcinogenesis (18). Interestingly, feeding with tricin lowered the number of dysplastic crypts as well as colorectal neoplasms.

We considered several mode of actions by which tricin inhibited AOM/DSS-induced colorectal oncogenesis. Dietary tricin inhibited the growth of colonic adenocarcinoma, as determined by PCNA-labeling index, suggesting that tricin possesses anti-growth ability of colorectal cancer, the findings being in agreement with the reports by Hudson et al. (23). In addition, our interesting findings are that dietary tricin significantly lowered the ABI of cancer cells, suggesting that tricin may affect the chromosomal instability of cancer cells (20).

Although dietary tricin did not significantly affect the expression of COX-2/iNOS and NF-κB-signaling pathway at week eight, the treatment significantly inhibited the expression of TNF-α in the colorectal mucosa. TNF-α is known to act as a master switch to establish an intricate link between inflammation and cancer (24, 25). Verschoyle, et al. (26) demonstrated a lack of mutagenic, clatogenic, chromosomal aberration or micronuclei activities by tricin in several assays. These findings suggest that tricin is safe for clinical application as a cancer chemopreventive agent.

Disclosure at State of COI

No conflicts of interest to be declared.

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