Extract of Kurosu, a Vinegar From Unpolished Rice, Inhibits Azoxymethane-Induced Colon Carcinogenesis in Male F344 Rats

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Abstract: The modifying effects of administering an ethyl acetate extract of Kurosu (EK), a vinegar made from unpolished rice, in drinking water on the development of azoxymethane (AOM)-induced colon carcinogenesis were investigated in male F344 rats. Animals were given 2 weekly subcutaneous injections of AOM (20 mg/kg body weight). They also received drinking water containing 0%, 0.05%, or 0.1% EK for 35 wk, starting 1 wk after the last dosing of AOM. EK administration significantly inhibited the incidence and multiplicity of colon adenocarcinoma (P < 0.05), compared with those in the AOM alone group. These findings suggest that EK may be effective for inhibiting colon carcinogenesis.

Introduction

Colon cancer is the third most common cause of cancer-related deaths in Japan (1). Because dietary factors are considered to play an important role in the development and prevention of colon cancer (2), primary prevention using dietary modification is one of the most useful strategies in fighting colon cancer development (3).

Vinegar, which can be made from rice, apple, wine, and various other materials, is a widely used acidic seasoning. Kurosu is a vinegar produced from unpolished rice through static surface acetic acid fermentation (4) and is characterized by a higher content of amino acids, organic acids, etc., than other vinegars. Kurosu is known to prevent hypertension in rats (5) and is recognized as a health food in Japan. An extract of Kurosu has been found to possess stronger antioxidative activity in a 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging system than other vinegars (6). Kurosu contains the antioxidative compounds dihydroferulic acid (DFA) and dihydrosinapic acid (DSA), which are derivatives of ferulic acid (FA), at higher concentrations than rice vinegar, a commonly found vinegar in Japan (7).

Unpolished rice, which is the raw material of Kurosu, contains rice germ and rice bran. Phenolic compounds found in rice bran, such as FA, are reported to inhibit the growth of human breast and colon cancer cells (8). Rice germ has an inhibitory effect on azoxymethane (AOM)-induced colon carcinogenesis in rats (9). A processed food prepared by fermenting brown rice and rice bran, known as FBRA, inhibited aberrant crypt foci (ACF) formation and suppressed the incidence and multiplicity of colon adenocarcinoma in rats treated with AOM (10). An ethyl acetate extract of Kurosu (EK) has been found to have an antipromoting activity in mouse skin (6). EK has also been shown to suppress cell growth of a variety of human cancer cells in vitro (11). These findings led us to investigate the possible modifying effects of EK on carcinogenesis. In a series of studies on the biological function of EK, we have recently demonstrated the inhibitory effects of EK on the development of ACF (12), which is considered a precursor lesion for colonic adenocarcinoma (13), suggesting that EK may potentially have chemopreventive activity in colon carcinogenesis.

The present long-term experiment was designed to confirm our previous finding that EK is effective in the prevention of early stages of colon tumorigenesis (12).

Materials and Methods

Animals, Diet, and Chemicals

The following experiments were performed with 5-wk-old male F344 rats (Charles River Japan Inc., Kanagawa, Japan). The animals were maintained in the Kanazawa Medical University Animal Facility according to the Institutional Animal Care Guidelines. All animals were housed in plastic cages (4 rats per cage) with free access to drinking water and basal diet CE-2 (CLEA Japan Inc., To-

kyo, Japan), with precise control over humidity (50% ± 10%), lighting (12-h light–dark cycle), and temperature (23 ± 2°C). Following a 7-day quarantine period, the animals were randomized by body weight into experimental and control groups. AOM (Sigma, St. Louis, MO) was administered to induce colonic neoplasms. Kurosu was made by Tamanoi Vinegar Co., Ltd. (Nara, Japan). Amberlite XAD-4 was purchased from Organo Co., Ltd. (Tokyo, Japan).

Extraction of Kurosu

EK, an ethyl acetate extract of Kurosu, was extracted according to a previously described method (12). Approximately 640 g of EK, obtained from 1,600 L of Kurosu, was used in the present experiment.

Experimental Procedure

Seventy-six male F344 rats were divided into 5 experimental and control groups. Groups 1–3 were initiated with AOM by two weekly subcutaneous injections (20 mg/kg body weight) to produce colonic neoplasms. Rats in groups 2 and 3 were given distilled water containing EK at 0.05% and 0.1%, respectively, for 35 wk, starting 1 wk after the last dosing of AOM. Group 4 was given drinking water containing 0.1% EK alone during the experiment (35 wk). Group 5 served as an untreated control. Rats were sacrificed at week 37 by light ether anesthesia, and the incidence of colonic tumors was assessed. The rats underwent careful necropsy, with emphasis on the colon, liver, and kidney. All grossly abnormal lesions in any tissue, as well as organs such as liver and kidney, were fixed in 10% buffered formalin solution. The intestine was excised, opened longitudinally, flushed clean with saline, and examined for the presence of tumors. Neoplasms in the intestine were diagnosed on hematoxylin and eosin-stained sections of 3 µm, according to the criteria described by Ward (14).

Statistical Evaluation

Where applicable, data were analyzed using one-way analysis of variance, followed by a Fisher's PLSD post hoc test with $P < 0.05$ as the criterion of significance.

Results

General Observations

All animals remained healthy throughout the experimental period. Body and liver weights, relative liver weights (g/100 g body weight), and drinking-water intake measurements with or without EK (ml/day/rat) are shown in Table 1. Water intake and body-weight gain did not differ significantly among the groups, suggesting that water containing 0.05% or 0.1% EK was well tolerated and supported normal growth in rats without any adverse effects. Mean and relative liver weights in group 1 (AOM alone) and group 4 (0.1% EK) were significantly higher than in group 5 (untreated) and those of rats in group 2 (AOM → 0.05% EK) and group 3 (AOM → 0.1% EK) were significantly lower than in group 1.

Incidence and Multiplicity of the Intestinal Tumors

Macroscopically, most tumors developed in the large intestine, and some developed in the duodenum of rats in groups 1–3. No tumors were found in any organs of rats in groups 4 and 5. The incidence and multiplicity of colonic tumors are shown in Table 2. The incidence of colonic tumors, including both adenoma and adenocarcinoma, in group 2 (55%, $P < 0.05$) and group 3 (50%, $P < 0.05$) was significantly lower than in group 1 (85%). The multiplicity (number of tumors per rat) in groups 2 (0.90 ± 0.97) and 3 (0.80 ± 0.95) was also lower than in group 1 (1.50 ± 1.24), but statistical significance was not present. When colonic adenocarcinoma was considered separately, the incidence

Table 1. Body and Liver Weights, Relative Liver Weights, and Intake of Drinking Water in Each Group[a]

Group No.	No. of Rats Examined	Treatment	Body Weight (g)	Liver Weight (g)	Relative Liver Weight (g/100 g Body Weight)	Daily Intake of: Drinking Water (ml/day/rat)	Daily Intake of: EK (mg/day/rat)
1	20	AOM	345.5 ± 18.4	12.53 ± 0.91[b]	3.63 ± 0.17[b]	23.0 ± 1.7	—
2	20	AOM → 0.05% EK	347.0 ± 18.4	11.22 ± 1.26[c]	3.23 ± 0.30[d]	22.8 ± 1.5	11.4
3	20	AOM → 0.1% EK	326.2 ± 52.1	10.57 ± 1.43[d]	3.29 ± 0.40[e]	21.5 ± 1.4	21.5
4	8	0.1% EK	346.4 ± 19.7	11.41 ± 1.22[f]	3.29 ± 0.19[g]	21.9 ± 2.0	21.9
5	8	None	350.2 ± 14.9	10.01 ± 0.99	2.85 ± 0.19	22.9 ± 1.7	—

a: Body weight, liver weight, relative liver weight, and daily intake are expressed as mean ± standard deviation. Abbreviations are as follows: AOM, azoxymethane; EK, ethyl acetate extract of Kurosu.
b: Significantly different from group 5 by StatView, followed by Fisher's PLSD post hoc test, $P < 0.0001$.
c: Significantly different from group 1 by StatView, followed by Fisher's PLSD post hoc test, $P < 0.005$.
d: Significantly different from group 1 by StatView, followed by Fisher's PLSD post hoc test, $P < 0.0001$.
e: Significantly different from group 1 by StatView, followed by Fisher's PLSD post hoc test, $P < 0.0005$.
f: Significantly different from group 5 by StatView, followed by Fisher's PLSD post hoc test, $P < 0.05$.
g: Significantly different from group 5 by StatView, followed by Fisher's PLSD post hoc test, $P < 0.005$.

Table 2. Effect of Dietary Kurosu Extract on the Development of Colonic Tumors in Male F344 Rats[a]

Group No.	No. of Rats Examined	Treatment	Incidence (%)			Multiplicity		
			AD	ADC	Total	AD	ADC	Total
1	20	AOM	1/20 (5%)	16/20 (80%)	17/20 (85%)	0.05 ± 0.22	1.45 ± 1.28	1.50 ± 1.24
2	20	AOM→0.05% EK	7/20[b] (35%)	10/20[b] (50%)	11/20[b] (55%)	0.35 ± 0.49^b	0.55 ± 0.60^c	0.90 ± 0.97
3	20	AOM→0.1% EK	7/20[b] (35%)	7/20[d] (35%)	10/20[b] (50%)	0.35 ± 0.49^b	0.45 ± 0.69^d	0.80 ± 0.95
4	8	0.1% EK	0/8 (0%)	0/8 (0%)	0/8 (0%)	0	0	0
5	8	None	0/8 (0%)	0/8 (0%)	0/8 (0%)	0	0	0

a: Abbreviations are as follows: AD, adenoma; ADC, adenocarcinoma.; AOM, azoxymethane; EK ethyl acetate extract of Kurosu. Multiplicity is expressed as mean ± standard deviation.
b: Significantly different from group 1 by StatView, followed by Fisher's PLSD post hoc test, $P < 0.05$.
c: Significantly different from group 1 by StatView, followed by Fisher's PLSD post hoc test, $P < 0.01$.
d: Significantly different from group 1 by StatView, followed by Fisher's PLSD post hoc test, $P < 0.005$.

and multiplicity in groups 2 (50%, $P < 0.05$; 0.55 ± 0.60, $P < 0.01$) and 3 (35%, $P < 0.005$; 0.45 ± 0.69, $P < 0.005$) were significantly lower than in group 1 (80%, 1.45 ± 1.28).

Discussion

This study has confirmed that EK can inhibit AOM-induced colon carcinogenesis of male F344 rats in a long-term experiment. In particular, the incidence and multiplicity of adenocarcinoma were significantly inhibited by the administration of EK in drinking water during the promotion phase of carcinogenesis. When these results and our previous finding that EK inhibits AOM-induced ACF of male F344 rats (12) are taken together, we have provided evidence for both blocking and suppressing effects of EK. In addition, it is important that there was no indication of EK toxicity, even after 35 wk.

In this study, the incidence and multiplicity of colonic adenocarcinoma were decreased by the treatment with EK, whereas those of colonic adenoma in groups 2 and 3 were more than those in group 1. This may suggest that EK treatment blocks an adenoma-carcinoma sequence in rats treated with AOM (15) or other chemical carcinogens (16,17).

The expression of cyclooxygenase (COX)-2 has been shown to increase in carcinogen-induced rat colon tumors (18). Selective inhibitors of COX-2, which catalyze the synthesis of prostanoids, would be good candidates as chemopreventive agents against colon cancer (19). High levels of prostaglandin E_2 (PGE_2), which promotes cell proliferation (20), have been detected in a variety of human cancers (21). In our ACF assay, EK inhibited the production of PGE_2 in colonic mucosa (12). These findings lead to the possibility of assessing whether EK may prevent colon tumorigenesis through suppression of COX-2 expression. Additional studies on the effects of EK on COX-2 expression during chemically induced colon carcinogenesis are under way in our laboratories.

FA, an antioxidative compound, inhibits the development of ACF in AOM-induced F344 rats and is also known to inhibit colon carcinogenesis over the course of a long-term ex-

periment (22). Glutathione S-transferase (GST) and quinone reductase (QR) activities in the rat liver and colon were significantly elevated by gavage of FA (22). EK also has antioxidative activity, elevates GST and QR activities, and inhibits the development of ACF (12). Therefore, the chemopreventive activity of EK found in this study may be caused by the biological actions found in case of FA. In addition, in our in vitro study, EK induced apoptosis in a human colon cancer cell line (11). Apoptosis induction may also contribute to the inhibitory effects of EK on AOM-induced colon carcinogenesis.

In conclusion, administration of EK prevented AOM-induced rat colon carcinogenesis. Kurosu, which is made by fermenting unpolished rice, contains other phenolic compounds besides FA. In the future, these compounds should be assessed for their ability in cancer prevention, and their exact mechanisms of inhibition should be elucidated.

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