RESEARCH COMMUNICATION

Inhibitory Effects of Chlorogenic Acid on Azoxymethane-induced Colon Carcinogenesis in Male F344 Rats

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Summary

Modifying effects of chlorogenic acid (CA) on carcinogen-induced large bowel carcinogenesis was examined in rats. A total of 150 male F344 rats, 4 weeks old, were divided into 5 groups. At 6 weeks of age, groups 1-3 were given subcutaneous injections of AOM (15 mg/kg body weight) once a week for three weeks. Group 2 was given the diet mixed with CA at the dose of 250 ppm during the initiation phase (5 weeks), and group 3 was exposed to the same diet during the post-initiation phase (32 weeks). Group 4 received the diet with CA throughout the experiment. Group 5 was maintained on the basal diet alone and served as a control. At the termination of the experiment (36 weeks after the start), the incidence of colon tumors in group 2 and 3 demonstrated a tendency for decrease as compared with group 1 although this did not attain significance At this time, the multiplicity of colon tumors of group 2 was significantly smaller than in group 1. In this study, the anti-proliferating cell nuclear antigen (PCNA) indices for non-neoplastic cells of the colon mucosae in groups 2 and 3 were also smaller than in group 1. The data suggest that CA has chemopreventive potential against colon carcinogenesis in rats like that showen in a hamster model with use of methylazoxymethanol acetate.

Key Words: Cholorogenic acid - azoxymethane - rats - colon carcinogenesis

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Introduction

Dietary factors are regarded as major determinants for the occurrence of human cancers and a number of natural food ingredients are considered to have cancer-preventive potential (Ames, 1983; Davis, 1989). Chlorogenic acid (CA), a major constituent of coffee beans, acts as an effective electrophilic trapping agent like other phenolics (Newmark, 1984). Several phenolic compounds are known to be potent inhibitors of mutagenesis and carcinogenesis of polycyclic aromatic hydrocarbons (Newmark, 1987; Stich and Rosin,1984; Wattenberg and Loub, 1978). Previously, our group reported inhibitory effects of CA on methylazoxymethanol (MAM) acetate-induced colon carcinogenesis in hamsters (Mori et al.,1986). In a subsequent study, a regressive effect of this phenolic compound on azoxymethane(AOM)-induced formation of aberrant crypt foci (ACF) in rat colon was shown (Morishita et al., 1997). These data imply that CA is a promising chemopreventive agent, especially for colorectal cancers. However, in the hamster study, CA was administered to the animals without distinction of initiation or post-initiation phases (Mori et al.,1986). Therefore, we attempted to examine the modifying effect of this phenolic compound in the rat model with use of AOM to confirm the chemopreventive effect on colon carcinogenesis and to ascertain the mode of action of this agent. The effect of CA on carcinogen-induced large bowel carcinogenesis in rats was therefore separately examined with exposure during the post-initiation as well as the initiation phases.

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Materials and Methods

Animals and Chemicals

Male F344 rats (Shizuoka Laboratory Animal Center, Shizuoka, Japan), 4-weeks-old, were used. All animals were housed in wire cages (3 or 4 rats/cage) with free access to the drinking water and basal diet, CE-2 (CLEA Japan Inc., Tokyo, Japan), under controlled environmental conditions of humidity (50±10%), lighting (12 h light/dark cycle) and temperature (23±2°C). AOM was purchased from Sigma Chemical Co. (St Louis, MO, U.S.A) and CA was purchased from Tokyo Kasei Co. (Tokyo, Japan).

Experimental Procedure

A total of 150 rats were randomized into 5 groups. At 6 weeks of age, rats of groups 1-3 received subcutaneous injections of AOM, once a week for 3 weeks, at a dose of 15 mg/kg body weight. Starting a week before the first AOM injection, rats of group 2 were given the basal diet mixed with CA at the dose of 250 ppm for 5 weeks. The concentration of CA in the diet was set following the hamster experiment (Mori et al.,1986). Rats of group 3 were given the diet with 250 ppm CA from one week after the last AOM injection to the termination of experiment. Rats of group 4 were kept on the diet with 250 ppm CA throughout the experiment. Rats of group 5 were maintained on the basal diet alone and served as untreated controls (Fig. 1). At the termination of the experiment, all rats were killed by decapitation. At autopsy, the intestines were excised, opened longitudinally, flushed clean with saline and examined for the presence of tumors. Other organs were also examined histologically. Colons after fixation in 10% buffered formalin, were processed for histopathological examination by conventional methods. Intestinal neoplasms were diagnosed according to the criteria described by Ward (1974).

Anti-proliferating cell nuclear antigen (PCNA) immunohistochemistry

One section (4 mm thick) of each colonic mucosa was obtained from paraffin blocks. The sections were subjected to immunohistochemical examination to check cell proliferation of tissues using a PCNA antibody. Briefly, endogenous peroxidase activity was blocked by immersing the sections in methanol with 3% hydrogen peroxide for 5 min, and then the sections were rinsed 3 times with phosphate-buffered saline (PBS, pH 7.4). They were incubated in PBS with 1% bovine serum albumin (BSA) for 1 h at room temperature and then incubated with PCNA (DAKO, Denmark) at a 1:100 dilution in PBS-BSA for 1 h at room temperature. They were rinsed in PBS, and PCNA staining was achieved by the labeled streptavidin biotin (LSAB) method using an LSAB kit (DAKO, Carpinteria, CA). Peroxidase binding sites were detected by staining with 3,3'-diaminobenzidine in PBS. Finally, counterstaining was

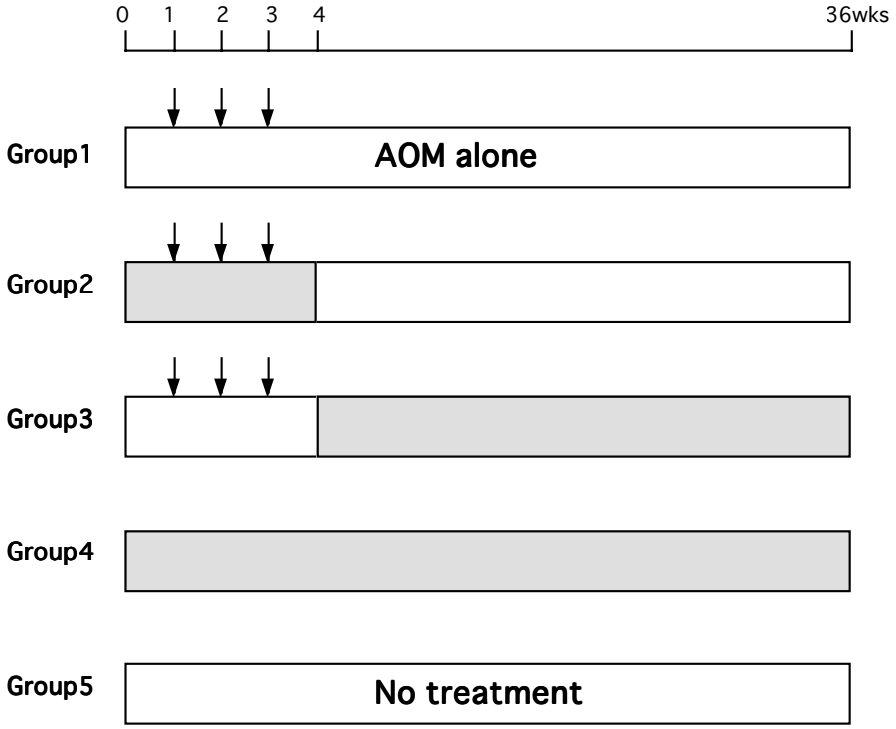

Figure 1. Azoxymethane 15 mg/kg Body Weight ↓ 250 ppm Chlorogenic Acid

performed using Mayer's hematoxylin. The PCNA-positive or -negative cells were scored in 10 colonic mucosae per rat at random, in 10 slides per group from groups 1-5 (Mori et al., 1992; Wang et al., 1993).

Statistical analysis

The Fisher's exact probability test, Student's t-test or Welch's method were used for statistical analysis. A value of $P<0.05$ was considered significant.

Results

Colon tumors were recognized in groups 1-3. Histologically, all colon tumors were diagnosed as carcinomas. They were well differentiated or poorly differentiated adenocarcinomas, and some were signet-ring cell carcinomas or mucinous carcinomas.

Data for the incidences and multiplicity of colonic neoplasms in each group are summarized in Table 1. The incidences of colonic neoplasms of groups 2 and 3 were rather lower than of group 1, although the differences were not significant. The multiplicity of colonic neoplasms of group 2 (1.11±0.33) was significantly smaller than in group 1 (1.54±0.97)($P<0.05$), and the value for group 3 showed a tendency for decrease as compared to group 1.

The PCNA indices of mucosal cells in the non-neoplasmic colonic epithelium of groups 2 and 3 (10.3±0.93 and 9.56±2.3, respectively) were significantly smaller than in group 1 (11.6±1.2)($P<0.05$).

Discussion

In the present study, CA decreased the multiplicity of AOM-induced colon tumors in rats. The PCNA index of mucosal cells in the group exposed to CA during the initiation phase was significantly lower than of the group given the carcinogen alone (group 1). Our group has demonstrated that CA inhibits: 1) MAM acetate-induced large bowel carcinogenesis in hamsters; 2) 4NQO-induced oral carcinogenesis in rats; 3) MNU-induced glandular stomach carcinogenesis in rats (Mori et al.,1986; Tanaka et al., 1993; Shimizu et al., 1999). We also proved that the phenolic compound suppresses MAM-acetate-induced development of hepatocellular altered foci in hamsters and has an inhibitory effect on AOM-induced development of colorectal ACF in rats (Mori et al.,1986; Morishita et al., 1997).

Regarding the mode of actions of CA, its antioxidative properties are considered important. CA has potent action against lipid peroxidation (Mori et al., 1997). The phenolic acid is an inhibitor of 8-hydroxydeoxyguanosine formation (Kasai et al., 2000). One of the mechanisms of chemopreventive action of CA on colon carcinogenesis would be expected to be the suppression of metabolic activation and enhancement of detoxification. AOM is known to be metabolically activated to MAM, a proximate metabolite by cytochrome P450IIE-1, mainly in the liver and colon (Sohn et al., 1991). Phytochemicals, including CA, are reported to have varied effects on the enzyme activity of isoforms of cytochrome P450, and therefore, they may have diverse effects on metabolism (Teel and Huynh,1998). A related polyphenol compound, ferulic acid is known to increase activity of detoxification enzymes glutathione S-transferase and quinone reductase in the liver and/or colonic mucosa (Kawabata et al., 2000). In this study, chemopreventive effect of CA was apparent with exposure during the initiation phase, suggesting that CA acted as a blocking agent rather than a suppressing agent. Such an effect of CA is thus probably related to inhibition of Phase I enzymes and its antioxidative properties.

At the same time, induction of cellular apoptosis is regarded as one of mechanisms of chemopreventive agents. We have examined the potential to generate cellular apoptosis of CA in human colorectal cells. However, no of activity was observed, although suppression of cell proliferation was recognized (Mori et al., 2001). It is suggested that control of hyper-proliferation of cells in the target organ is important for the mode of action of chemopreventive agents (Mori et al., 1997). In the present study, an inhibitory effect of CA on AOM-induced development of colorectal neoplasms in rats was demonstrated. The results suggest that potential chemopreventive effects of this agent might be exerted in the human situation, so that further assessment of protection against digestive organ carcinogenesis is warranted.

Table 1. Tumour Incidences and Multiplicity and PCNA Labeling Indices.

Groups	Treatment	No. of rats	Incidence (%)	Multiplicity (Mean No/ rat)	PCNA index
1	AOM alone	26	13 (50)	1.54±0.97[a]	11.6±1.2
2	AOM + 250 ppm CA	29	10 (35)	1.11±0.33[b]	10.3±0.9[c]
3	AOM → 250 ppm CA	28	11 (39)	1.18±0.40	9.6±2.3[d]
4	250 ppm CA	27	0(0)	0	8.9±1.6
5	Non treatment	30	0(0)	0	8.7±2.1

[a] : Mean ± S.D. +: Exposure during the initiation phase. → : Exposure during the postinitiation phase.
[b] : Significantly different from the value of group 1 by Welch's test ($P<0.05$)
[c] : Significantly different from group 1 by Student's t-test ($P<0.001$)
[d] : Significantly different from group 1 by Welch's test ($P<0.001$)

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