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DIETARY SEED OIL RICH IN CONJUGATED LINOLENIC ACID FROM BITTER MELON INHIBITS AZOXYMETHANE-INDUCED RAT COLON CARCINOGENESIS THROUGH ELEVATION OF COLONIC PPARγ EXPRESSION AND ALTERATION OF LIPID COMPOSITION

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Our previous short-term experiment demonstrated that seed oil from bitter melon (*Momordica charantia*) (BMO), which is rich in *cis(c)9, trans(t)11, t13*-conjugated linolenic acid (CLN), inhibited the development of azoxymethane (AOM)-induced colonic aberrant crypt foci (ACF). In our study, the possible inhibitory effect of dietary administration of BMO on the development of colonic neoplasms was investigated using an animal colon carcinogenesis model initiated with a colon carcinogen AOM. Male F344 rats were given subcutaneous injections of AOM (20 mg/kg body weight) once a week for 2 weeks to induce colon neoplasms. They also received diets containing 0.01%, 0.1% or 1% BMO for 32 weeks, starting 1 week before the first dosing of AOM. At the termination of the study (32 weeks), AOM induced 83% incidence (15/18 rats) of colonic adenocarcinoma. Dietary supplementation with 0.01% and 0.1% BMO caused significant reduction in the incidence (47% inhibition by 0.01% BMO, $p<0.02$; 40% inhibition by 0.1% BMO, $p<0.05$; and 17% inhibition by 1% BMO) and the multiplicity (64% inhibition by 0.01% BMO, $p<0.005$; 58% inhibition by 0.1% BMO, $p<0.02$; and 48% inhibition by 1% BMO, $p<0.05$) of colonic adenocarcinoma, though a clear dose response was not observed. Such inhibition was associated with the increased content of CLA (c9,t11-18:2) in the lipid composition in colonic mucosa and liver. Also, BMO administration in diet enhanced expression of peroxisome proliferator-activated receptor (PPAR) γ protein in the non-lesional colonic mucosa. These findings suggest that BMO rich in CLN can suppress AOM-induced colon carcinogenesis and the inhibition might be caused, in part, by modification of lipid composition in the colon and liver and/or increased expression of PPARγ protein level in the colon mucosa.

Key words: *conjugated linolenic acid; chemoprevention; colon carcinogenesis; bitter melon; PPARγ*

Colon cancer is the third most malignant neoplasm in the world.[1] It is well known that colorectal cancer is linked to Western lifestyle, which often includes a diet high in fat.[2] In Japan, the incidence of this malignancy, being the third leading cause of cancer death, has been increasing, possibly due to the Westernization of dietary habits, with a rising fat intake. The amount and type of dietary fat consumed are of particular importance for development of this malignancy.[3–6] Epidemiological studies indicate that high intake of fish oil-consumption and fish correlates with a reduced risk of colon cancer.[7,8] Diets containing fish oil are rich in the n-3 polyunsaturated fatty acids (PUFA), such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). Studies in humans and experimental animals indicate a protective effect of n-3 PUFA (fish, fish oils or EPA ethyl ester), and the mechanism of protection is largely thought to be related to interference with biosynthesis of 2-series prostaglandins (PGs) from arachidonic acid (AA).[9] Our short-term experiment[10] also demonstrated that tuna oil rich in DHA and vitamin D_3 inhibits azoxymethane (AOM)-induced rat aberrant crypt foci (ACF), being putative precursor lesions for colonic adenocarcinoma.[11] However, limited studies of α-linolenic acid (α-LN), which is the parent fatty acid of the n-3 family, provide some promising results. Dietary feeding of perilla and flaxseed oils, both rich sources of α-LN, could decrease chemically induced colonic neoplasms and ACF in rat colon carcinogenesis models.[12–14] These results coincide with competitive exclusion of n-6 PUFA from membrane phospholipids and associate reductions in PGE_2 concentrations in colonic mucosa.[14] Other fatty acids including n-6 PUFA and their derivatives are also suspected to have possible antitumorigenic property.

Conjugated linoleic acid (CLA) refers collectively to several positional and geometric isomers of linoleic acid (LA) in which the double bonds are in conjugation, typically at positions 9 and 11 or 10 and 12. CLA has been shown to inhibit chemically induced carcinogenesis in various organs, such as mammary glands,[15] skin[16] and forestomach.[17] When compared to studies examining the protective efficacy of CLA on mammary carcinogenesis, evidence for chemoprevention by CLA against colon cancer is less definitive, although gavage with CLA lessened the occurrence of ACF induced by heterocyclic amines.[18,19] On the other hand, the occurrence of other types of conjugated PUFA are present in some seed oils.[20,21] They include conjugated trienoic fatty acids, such as α-eleostearic (*cis(c)*9,*trans(t)*11,*t*13-CLN), in the seed oil of bitter melon (*Momordica charantia*) oil (BMO), which is an edible plant bitter melon and one of the important food materials in South-East Asia. The cytotoxic effect of *c*9,*t*11,*t*13-CLN isolated from BMO on activity of tumors cells has been indicated in our recent study.[22] In addition, we have reported the protective effect of BMO on the development of ACF with high crypt multiplicity in a short-term *in*

Abbreviations: AA, arachidonic acid; AOM, azoxymethane; ACF, aberrant crypt foci; BGO, bitter melon oil; *c,cis*; CLA, conjugated linoleic acid; CLN, conjugated linolenic acid; DHA, docosahexaenoic acid; DMH, 1,2-dimethylhydrazine; DMOX, dimethyloxazoline; IQ, 2-amino-3-methylimidazo[4,5-*f*]quinoline; LA, linoleic acid; LN, linolenic acid; PPAR, peroxisome proliferator-activated receptor; PUFA, polyunsaturated fatty acid; prostaglandin, PG; sodium dodecyl sulfate, SDS; *t*, *trans*

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vivo assay, suggesting a possible inhibitory effect of BMO on colon carcinogenesis.[23]

In our study, chemopreventive ability of BMO on large bowel tumorigenesis was investigated in a long-term *in vivo* assay using a rat colon carcinogenesis model with AOM as a carcinogen. Also, the expression of peroxisome proliferator-activated receptor (PPAR) γ in colonic mucosa and the lipid composition in the liver and colon were estimated to understand the possible mechanisms of modulatory effect of BMO in colon carcinogenesis since fatty acids might be an agonist for PPARs,[24] which suppress colon tumorigenesis.[25–27]

MATERIAL AND METHODS

Animals, chemicals and diets

A total of 82 male F344 rats, 4 weeks old, obtained from Charles River Japan, Inc. (Kanagawa, Japan), were used. 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 (3 or 4 rats/cage) with free access to drinking water and a basal diet, AIN-76A,[28] under controlled conditions of humidity (50 ± 10%), lighting (12 hr light/dark cycle) and temperature (23 ± 2°C). AOM was purchased from Sigma Chemical Co. (St. Louis, MO). BMO was extracted from seed of bitter melon according to methods described previously.[23] The fatty acid profile of the total lipids in BMO was generally in harmony with that described in other report,[29] with very high level (60.2%) of 9*c*,11*t*,13*t*-18:3 and a small amount of other CLN isomers, namely, 9*c*,11*t*,13*c*-18:3 (0.6%) and 9*t*,11*t*,13*t*-18:3 (0.3%). These lipids also contained high amount (27.2%) of 18:0 and modest amount of 18:1n-9 (5.9%) and 18:2n-6 (3.8%). Four experimental diets containing various levels of BMO (0%, 0.01%, 0.1% or 1% by weight of diet) based on the AIN-76 formulation were made on the weekly base and stored at −20°C under nitrogen atmosphere in airtight containers for no longer than a week. The composition of the diets is shown in Table I.

Experimental procedure

After quarantine for 7 days, rats aged 5 weeks were divided into 6 groups as shown in Figure 1. Beginning at 5 weeks of age, all animals were fed each of the 4 different experimental diets. At 6 weeks of age, animals in groups 1 through 4 were *s.c.* injected with AOM (20 mg/kg body weight) once a week for 2 weeks. The rats in groups 1 and 6 were fed the diet containing 5% corn oil. Group 2 was fed the diet containing 0.01% BMO and 4.99% corn oil. Group 3 was given the diet containing 0.1% BMO and 4.9% corn oil. Group 4 and 5 were fed to the diet containing 1% BMO and 4% corn oil. The dose levels of test compound were determined from previous reports.[23] All rats were provided with the experimental diets and tap water *ad libitum*, and weighed weekly. The consumption of experimental diets was also recorded weekly. At the termination of the study (week 32), all of the rats were sacrificed by ether overdose to assess the incidences of neoplastic lesions in all organs including large bowel. At autopsy, all organs, especially the intestine, were carefully inspected grossly, and all abnormal lesions were examined histologically. Colons of 5 rats from each group were randomly selected for measurement of the expression of PPARγ protein and for the lipid analysis in the nonlesional colonic mucosa after resection of tumorous lesions histopathology. Colons of remaining rats were fixed in 10% buffered formalin and processed for histopathological examination by conventional methods using hematoxylin and eosin staining. The liver was excised and weighed, and then the caudate lobe was removed and fixed in 10% buffered formalin for histological examination. Remaining lobes of the liver of all rats were analyzed fatty acid composition. All other tissues were fixed in 10% buffered formalin and histological diagnosis was made. Intestinal neoplasma were diagnosed according to the criteria described by Ward.[30]

TABLE I – PERCENTAGE COMPOSITION OF EXPERIMENTAL DIETS

Diet ingredients	Control	0.01% BMO	0.1% BMO	1% BMO
Casein	20.0	20.0	20.0	20.0
DL-methionine	0.3	0.3	0.3	0.3
Corn starch	15.0	15.0	15.0	15.0
Dextrose	50.0	50.0	50.0	50.0
Cellulose	5.0	5.0	5.0	5.0
Corn oil	5.0	4.99	4.9	4.0
BMO	0	0.01	0.1	1.0
Mineral mix, AIN-76A	3.5	3.5	3.5	3.5
Vitamin mix, AIN-76A	1.0	1.0	1.0	1.0
Choline bitartrate	0.2	0.2	0.2	0.2

Western blotting analysis of PPARγ

Tissue sample were homogenized in CelLyticTM-MT Mammalian Tissue Lysis/Extraction Reagent (Sigma Chemical Co., St. Louis, MO) with a PROTEASE INHIBITOR COCKTAIL (Sigma Chemical Co., St. Louis, MO), and insoluble materials were removed by centrifugation at 4°C. The supernatants were estimated for their protein contents using Bio-Rad protein assay reagents (Bio-Rad Laboratories, Richmond, CA) with bovine serum albumin at standard. The solubilized lysates were resolved by sodium dodecyl sulfate (SDS)-PAGE electrophoresis under reducing conditions at a concentration of 50 μg protein of each sample per lane. Detection of PPARγ protein was performed with an anti- PPARγ polyclonal antibody (Santa Cruz Biotechnology, Inc., Santa Cruz, CA) with detection accomplished with an ECL-plus kit (Amersham Bioscience Corp., NJ). Quantitative analysis was performed using Scion Image analysis soft ware (Scion Corp., Frederick, MD).

Lipid extraction and analysis

Lipids in colonic mucosa and liver were extracted with chloroform/methanol (2:1, v/v) as described previously by Folch *et al.*[31] Component peaks were identified by comparison with standard fatty acid methyl ester[32] and quantified by a Shimadzu Chromatopac C-R6A integrator (Shimadzu Seisakusho Co., Ltd., Kyoto, Japan). The identification of CLA and/or CLN isomers was confirmed by using GC-mass spectrometry after conversion of the methyl esters to dimethyloxazoline (DMOX) derivatives.[33] The

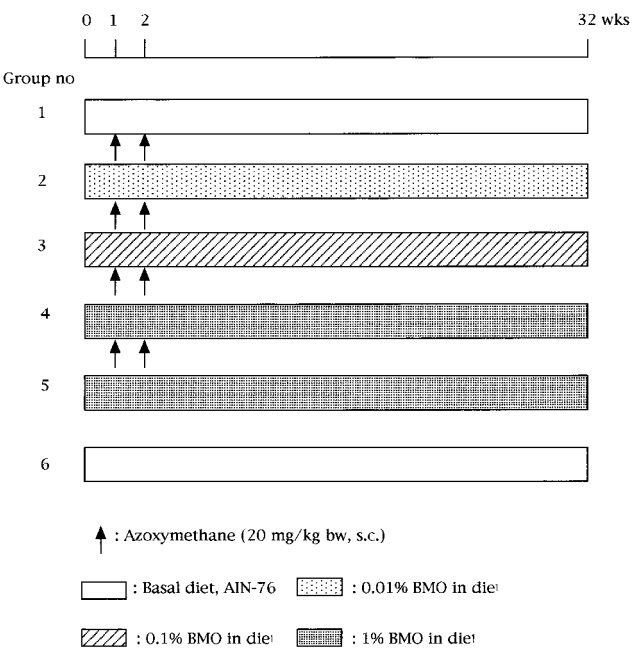

FIGURE 1 – Experimental protocol.

analysis of fatty acid composition was done more than 2 times for each sample and there was no significant difference between results for the same sample. Data are represented as means ± SD.

Statistical evaluation

Where applicable, data were analyzed using Student's *t*-test, Welch's *t*-test or Fisher's exact probably test with $p<0.05$ as the criterion of significance.

RESULTS

General observation

During the study, clinical signs of toxicity, low survival and poor condition were not observed in any groups. This was confirmed by histopathological examinations in liver, kidney, spleen, heart and lungs of the rats. Histology of liver revealed no morphological alterations, such as fatty liver. The mean daily intake of diet with or without BMO per rat was between 12.9 and 13.2 g/day/rat. Mean body, liver and % liver weights (g/100 g body weight) in all groups at sacrifice are shown in Table II. There were no significant differences among the groups.

Incidence and multiplicity of intestinal neoplasms

Macroscopic observation revealed that most tumors developed in the large intestine and some in the small intestine of rats in groups 1–4. Animals in groups 5 and 6 did not have neoplasms in any organs examined. Colon tumors were sessile or pedunculated tumors and histologically tubular adenoma, adenocarcinoma or signet ring-cell carcinoma, with a higher incidence of adenocarcinoma. The incidence and multiplicity of intestinal tumors are shown in Tables III and IV. The frequencies of large intestinal adenocarcinoma in groups 2 (44%, $p<0.02$) and 3 (50%, $p<0.05$) were significantly smaller than that in group 1 (83%). The incidence of colorectal adenocarcinoma in group 3 (69%) was lower than in group 1, but a significant difference was not present ($p=0.2758$). The incidence of small intestinal adenocarcinoma in groups 2, 3 and 4 did not significantly differ from that in group 1. As presented in Table IV, significant reduction in the multiplicities of colorectal carcinoma (number of carcinomas/rats) in groups 2 (0.69 ± 0.87, $p<0.005$), 3 (0.81 ± 1.05, $p<0.02$) and 4 (1.00 ± 0.89, $p<0.05$) was also found when compared to group 1 (1.94 ± 1.47).

Lipid analysis

The fatty acid profiles of the lipids from liver and colonic mucosa are shown in Tables V and VI, respectively. Although BMO diets contained over 60% of CLN isomer (*c*9,*t*11,*t*13-18:3), any CLN isomer was not detected in both organs of rats fed BMO diets at various doses. On the other hand, the contents of CLA (*c*9,*t*11-18:2) in the liver and colonic mucosa of rats fed BMO were increased in a dose-dependent manner.

Expression of PPARγ levels in colonic mucosa

A representative immunoblot analysis of PPARγ expression in colonic mucosa of AOM-treated animals on different dietary regimens is shown in Figure 2. Dietary administration of BMO resulted in enhanced expression of PPARγ protein levels: 1.5-fold increase in groups 2, 1.6-fold elevation in group 3 and 1.9-fold increased in group 4, when compared to rats fed the diet without BMO.

DISCUSSION

The results described here clearly indicate that dietary administration of BMO rich in *c*9,*t*11,*t*13-CLN significantly inhibits the development of colonic adenocarcinoma induced by AOM in male F344 rats without causing any adverse effects. In addition, significant reduction in the multiplicities of colorectal carcinoma (number of carcinomas/rats) in rats BMO containing diets at all dose levels (0.01%, 0.1% or 1%) was found when compared the AOM alone group. We believe that our results are the first to demonstrate the protective ability of BMO rich in *c*9,*t*11,*t*13-CLN against chemically induced colon carcinogenesis.

In our study, the protective effect of BMO against colon carcinogenesis was not dose dependent. Kimoto *et al.*[34] reported that safflower oil rich in CLA treatment protection in mammary carcinomas, without a clear dose dependence, as found in our study. Dietary CLA between 0.05% and 0.5% was found to produce a dose-dependent inhibition in mammary tumor development,[35] but the inhibitory effect of CLA reached a maximum at about 1%.[36] Thus, there may be the existence of lower threshold of conjugated fatty acids with cancer chemopreventive action.

There are a few studies that investigate the modifying effects of conjugated fatty acids on colon carcinogenesis. Although dietary CLA inhibits cancer development in including mammary gland,[15] skin[16] and forestomach[17] in rodents initiated with a variety of chemical carcinogens, the chemopreventive activity of CLA in the colon is less clear. CLA treatment inhibits the formation of colonic 2-amino-3-methylimidazo[4,5-*f*]quinoline (IQ)-DNA adducts and putative precancerous ACF in colon.[18] Recently, Park *et al.*[37] reported that dietary CLA can inhibit 1,2-dimethylhydrazine (DMH)-induced colon carcinogenesis through possibly induction of apoptosis. Cell proliferation is known to play an important role in multistage carcinogenesis with multiple genetic changes.[38] In our previous work, CLN can inhibit the growth of human and mouse cancer cells *in vitro*.[22] Feeding of BMO rich in CLN is reported to increase the number of apoptotic cells and reduce cell proliferation activity.[23] Although we did not estimate cell proliferation activity and apoptotic index in the colonic mucosa and/or neoplasms, it may be possible that inhibitory effect of BMO may be due to, in part, modification of cell proliferation and/or apoptosis induction.

In the fatty acid profiles of the lipids from colonic mucosa and liver, we did not detect any CLN isomer in the liver lipids from rat fed the BMO diets, which contained over 60% of CLN isomer (*c*9,*t*11,*t*13-18:3). On the other hand, CLA was found in these lipids and the content of the CLA isomer (*c*9,*t*11-18:2) was significantly greater in rats fed the BMO diets in a dose-dependent manner. This may indicate that part of *c*9,*t*11,*t*13-18:3 in the CLN would be enzymatically converted to *c*9,*t*11-18:2. CLA is known to be a possible chemopreventive agent against ACF formation[18,19] and colon carcinogenesis.[37] Whereas CLA used in published studies[15,17,35,39] contains a mixture of positional and geometrical iso-

TABLE II – BODY, LIVER AND RELATIVE LIVER WEIGHTS

Group number	Treatment (number of rats examined)	Body weight (g)	Liver weight (g)	Relative liver weight (g/100 g body weight)
1	AOM (18)	365 ± 23[1]	11.9 ± 1.7	3.26 ± 0.39
2	AOM + 0.01% BMO (16)	374 ± 20	12.7 ± 1.7	3.40 ± 0.40
3	AOM + 0.1% BMO (16)	360 ± 18	11.8 ± 1.6	3.27 ± 0.36
4	AOM → 1% BMO (16)	368 ± 27	11.6 ± 1.8	3.15 ± 0.37
5	1% BMO (8)	365 ± 24	11.1 ± 1.4	3.06 ± 0.29
6	None (8)	376 ± 20	12.2 ± 1.2	3.24 ± 0.27

[1]Mean ± SD.

TABLE III – INCIDENCE OF LARGE BOWEL TUMORS IN EACH GROUP

Group number	Treatment (number of rats examined)	Number of rats with tumors at					
		Small intestine			Large intestine		
		Total	AD[1]	ADC	Total	AD	ADC
1	AOM (18)	4 (22%)	0 (0%)	4 (22%)	15 (83%)	7 (39%)	15 (83%)
2	AOM + 0.01% BMO (16)	6 (38%)	1 (6%)	5 (31%)	14 (88%)	10 (63%)	7[2] (44%)
3	AOM + 0.1% BMO (16)	4 (25%)	2 (13%)	3 (19%)	15 (94%)	10 (63%)	8[3] (50%)
4	AOM + 1% BMO (16)	3 (19%)	1 (6%)	2 (13%)	14 (88%)	8 (50%)	11 (69%)
5	1% BMO (8)	0	0	0	0	0	0
6	None (8)	0	0	0	0	0	0

[1]AD = adenoma; ADC = adenocarcinoma.–[2,3]Significantly different from group 1 by Fisher's exact probability test ([2]$p < 0.02$ and [3]$p < 0.05$).

TABLE IV – MULTIPLICITY OF LARGE BOWEL TUMORS IN EACH GROUP

Group number	Treatment (number of rats examined)	Multiplicity (number of tumors/rat) of intestinal tumors at					
		Small intestine			Large intestine		
		Total	AD[1]	ADC	Total	AD	ADC
1	AOM (18)	0.28 ± 0.57[2]	0.00 ± 0.00	0.28 ± 0.57	2.50 ± 1.79	0.56 ± 0.98	1.94 ± 1.47
2	AOM + 0.01% BMO (16)	0.38 ± 0.50	0.06 ± 0.25	0.31 ± 0.48	1.56 ± 0.89	0.88 ± 0.81	0.69 ± 0.87[3]
3	AOM + 0.1% BMO (16)	0.31 ± 0.60	0.13 ± 0.34	0.19 ± 0.40	1.56 ± 1.09	0.76 ± 0.68	0.81 ± 1.05[4]
4	AOM + 1% BMO (16)	0.19 ± 0.40	0.06 ± 0.25	0.13 ± 0.34	1.69 ± 0.95	0.69 ± 0.79	1.00 ± 0.89[5]
5	1% BGO (8)	0	0	0	0	0	0
6	None (8)	0	0	0	0	0	0

[1]AD = adenoma; ADC = adenocarcinoma–[2]Mean ± SD–[3–5]Significantly different from group 1 by Student's *t*-test ([3]$p < 0.005$, [4]$p < 0.02$ and [5]$p < 0.05$).

TABLE V – EFFECTS OF BMO DIETS ON FATTY ACID COMPOSITION OF LIVER LIPIDS

Group number	Treatment	Fatty acids (wt%)									
		16:0	18:0	16:1n-7	18:1n-7	18:1n-9	18:2n-6	18:2(c9,t11)	20:4n-6	22:5n-3	22:6n-3
1	AOM	25.4 ± 1.5[1]	11.5 ± 2.2	6.2 ± 0.7	5.2 ± 0.3	19.5 ± 1.1	11.3 ± 0.6	ND[2]	12.8 ± 0.9	1.9 ± 0.1	1.0 ± 0.0
2	AOM + 0.01% BMO	27.9 ± 0.9	8.8 ± 0.4	7.6 ± 0.6	5.4 ± 0.1	20.4 ± 1.0	11.2 ± 0.4	0.04 ± 0.00	11.1 ± 0.9	1.4 ± 0.1	0.9 ± 0.1
3	AOM + 0.1% BMO	24.4 ± 2.4	10.1 ± 1.3	4.4 ± 2.0	5.0 ± 0.6	19.9 ± 1.9	15.0 ± 2.6	0.20 ± 0.02	12.6 ± 1.6	1.9 ± 0.6	1.0 ± 0.1
4	AOM + 1% BMO	23.2 ± 2.5	10.4 ± 2.3	4.1 ± 1.9	4.7 ± 0.4	21.5 ± 4.7	14.8 ± 3.3	1.68 ± 0.30	11.7 ± 2.5	1.6 ± 0.5	0.8 ± 0.2
5	1% BMO	25.7 ± 0.9	10.6 ± 0.8	6.4 ± 0.5	5.8 ± 0.3	20.3 ± 1.1	10.7 ± 0.8	1.56 ± 0.18	12.1 ± 0.7	1.3 ± 0.1	0.7 ± 0.0
6	None	23.3 ± 0.9	11.6 ± 2.7	5.2 ± 1.0	6.0 ± 0.1	19.3 ± 0.8	14.5 ± 2.0	ND	13.5 ± 1.0	1.4 ± 0.2	0.8 ± 0.0

[1]Mean ± SD.–[2]ND, not detected.

TABLE VI – EFFECTS OF BMO DIETS ON FATTY ACID COMPOSITION OF COLONIC MUCOSA

Group number	Treatment	Fatty acids (wt%)								
		16:0	18:0	16:1n-7	18:1n-7	18:1n-9	18:2n-6	18:2(c9,t11)	20:4n-6	22:5n-3
1	AOM	22.8 ± 0.6[1]	2.9 ± 0.4	5.1 ± 0.9	5.7 ± 0.3	31.4 ± 0.9	25.0 ± 1.8	ND[2]	1.5 ± 0.5	0.0 ± 0.0
2	AOM + 0.01% BMO	22.9 ± 0.7	3.5 ± 0.7	4.4 ± 0.7	6.0 ± 0.3	30.5 ± 0.7	24.5 ± 1.3	0.06 ± 0.03	2.3 ± 1.1	0.0 ± 0.0
3	AOM + 0.1% BMO	23.2 ± 0.7	3.7 ± 0.6	4.7 ± 1.0	5.5 ± 0.3	30.5 ± 0.8	24.4 ± 1.4	0.40 ± 0.04	2.2 ± 0.7	0.0 ± 0.0
4	AOM + 1% BMO	22.0 ± 0.6	4.6 ± 0.4	4.3 ± 1.1	5.9 ± 0.3	29.3 ± 1.7	21.4 ± 1.8	3.30 ± 0.23	2.8 ± 1.0	0.3 ± 0.1
5	1% BMO	23.3 ± 0.2	3.3 ± 0.2	5.2 ± 0.3	6.5 ± 0.2	30.9 ± 0.6	21.1 ± 0.9	3.66 ± 0.12	1.4 ± 0.2	0.2 ± 0.0
6	None	22.4 ± 0.6	3.6 ± 0.4	4.4 ± 0.3	6.6 ± 0.3	30.0 ± 1.3	24.5 ± 0.9	ND	2.3 ± 1.0	0.0 ± 0.0

[1]Mean ± SD.–[2]ND, not detected.

mers, *c*9,*t*11-18:2 isomer is considered to be the active constituent. Therefore, the suppressing effect of BMO on colon carcinogenesis in the current study may be attributed to *c*9,*t*11-18:2 isomer derived from *c*9,*t*11,*t*13-18:3 in the BMO diets. Furthermore, in our study, the contents of linoleic acid (18:2n-6) in the liver lipids of rats fed the BMO containing diets were significantly lower than that of rats fed the diet without BMO. This reduction in the content of linoleic acid may also contribute to the inhibitory effect of BMO on colon carcinogenesis. However, judging from the powerful inhibitory activity of BMO at lower dose levels found in our study, other factors, such as direct action of *c*9,*t*11,*t*13-18:3, should be considered.

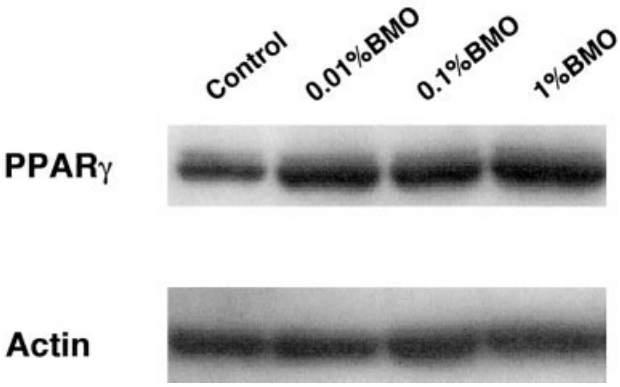

FIGURE 2 – Expression of PPARγ and β-actin proteins analyzed by immunoblot of protein extracts from the colonic mucosa.

Recently, CLA was shown to act a high affinity ligand and activator of PPARα and PPARγ.[40–42] McCarty[41] suggested that part of anticarcinogenic activity of CLA is mediated by PPAγ activation in susceptible tumors. In our study, dietary feeding of BMO enhanced PPARγ expression in nonlesional colonic mucosa. These results are of interest, since we recently demonstrated that synthetic ligands for PPARα and PPARγ effectively inhibit AOM-induced ACF in rats,[26,43] and the findings were confirmed by Osawa *et al*.[27] Thus, it may be possible that feedings with BMO suppresses colon carcinogenesis *via* altering PPARγ expression in colonic mucosa.

The antioxidant activity of *c*9,*t*11,*t*13-18:3 is another possible explanation for inhibitory effect of colon carcinogenesis by feeding of BMO diet. Ha *et al*.[17] demonstrated that CLA may act by antioxidant mechanisms cytotoxicity. In addition, Dhar *et al*.[44] reported that *c*9,*t*11,*t*13-18:3 from BMO acts as an antioxidant. In compounds with more than 2 conjugated double bond, conjugation increases the rate of oxidation. Thus, in the *in vivo* study, conjugated trienoic fatty acids are also likely to be more rapidly oxidized than linoleates by picking up more free radicals, thereby eliminating or reducing the formation of hydroperoxides. Although we did not determine these parameters, it may be possible that in our study BMO reduces the formation of hydroperoxides by lowering the generation of free radicals and peroxidation of PUFA occurring in cell membrane and other lipids. Indeed, BMO treatment could effectively inhibit colitis-related colon carcinogenesis, where production of free radicals increases (manuscript in preparation).

In our study, estimated CLN intake in rats treated with 0.01%, 0.1% and 1% BMO was 2.12, 21.5 and 212.4 mg/kg body weight per day, respectively. Whereas CLA used in animal studies usually contained a mixture of position and geometrical isomers, *c*9,*t*11-18:2 is considered to be the most active constituent.[15,17,39] As shown in our study, only the *c*9,*t*11-18:2 isomer was accumulated in the colonic mucosa and liver when rats were fed BMO containing diets. The finding that even at the 0.1% dose level of BMO, which is greater than the average CLA consumption (approximately 1g/person /day) in the United States,[45] BMO feeding exerted cancer chemopreventive activity may suggest that BMO, a good dietary resource of CLA, is one of promising cancer preventive substances against colon cancer development.

In conclusion, the results of our study suggest that dietary BMO in rich CLN has a beneficial effect on chemically induced rat colon carcinogenesis, providing an effective dietary chemopreventive approach to disease management. Our findings also suggest that BMO could be applied to preclinical studies of the prevention of colon cancer.

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