Pomegranate seed oil rich in conjugated linolenic acid suppresses chemically induced colon carcinogenesis in rats

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Pomegranate (*Punica granatum* L.) seed oil (PGO) contains more than 70% *cis*(*c*)9,*trans*(*t*)11,*c*13-18:3 as conjugated linolenic acids (CLN). Our previous short-term experiment demonstrated that seed oil from bitter melon (*Momordica charantia*) (BMO), which is rich in *c*9,*t*11,*t*13-CLN, inhibited the occurrence of colonic aberrant crypt foci (ACF) induced by azoxymethane (AOM). In this study, we investigated the effect of dietary PGO on the development of AOM-induced colonic malignancies and compared it with that of conjugated linoleic acid (CLA). To induce colonic tumors, 6-week old male F344 rats were given subcutaneous injections of AOM (20 mg/kg body weight) once a week for 2 weeks. One week before the AOM treatment they were started on diet containing 0.01%, 0.1%, or 1% PGO or 1% CLA for 32 weeks. Upon termination of the bioassay (32 weeks) colon tumors were evaluated histopathologically. AOM exposure produced colonic adenocarcinoma with an incidence of 81% and multiplicity of 1.88±1.54 at week 32. Administration of PGO in the diet significantly inhibited the incidence (AOM+0.01% PGO, 44%, $P<0.05$; AOM+0.1% PGO, 38%, $P<0.01$; AOM+1% PGO, 56%) and the multiplicity (AOM+0.01% PGO, 0.56±0.73, $P<0.01$; AOM+0.1% PGO, 0.50±0.73, $P<0.005$; AOM+1% PGO, 0.88±0.96, $P<0.05$) of colonic adenocarcinomas, although a clear dose-response relationship was not observed at these dose levels. CLA feeding also slightly, but not significantly, reduced the incidence and multiplicity of colonic adenocarcinomas. The inhibition of colonic tumors by PGO was associated with an increased content of CLA (*c*9,*t*11-18:2) in the lipid fraction of colonic mucosa and liver. Also, administration of PGO in the diet elevated expression of peroxisome proliferator-activated receptor (PPAR) γ protein in the non-tumor mucosa. These results suggest that PGO rich in *c*9,*t*11,*c*13-CLN can suppress AOM-induced colon carcinogenesis, and the inhibition is associated in part with the increased content of CLA in the colon and liver and/or increased expression of PPARγ protein in the colon mucosa. (Cancer Sci 2004; 95: 481–486)

Colon cancer is one of the leading causes of cancer deaths in Western countries. Globally, more than 875,000 men and women were afflicted with this cancer in 1996 and more than 510,000 died in the same year.[1)] Diet, especially fat intake, has been regarded as the most important nutritional influence on colon cancer development. Colorectal cancer development is known to be linked to Western lifestyle, which often includes a diet high in fat.[2)] The amount and type of dietary fat consumed are of particular importance for development of this type of malignancy.[3–6)] Epidemiological investigations indicate that high intake of fish and fish oil rich in $n-3$ polyunsaturated fatty acids (PUFAs) correlates with a reduced risk of colorectal malignancy.[7,8)] Laboratory animal model studies indicate that the $n-3$ PUFAs are protective, whereas the $n-6$ PUFAs promote colon carcinogenesis.[9)] Our previous study also demonstrated that tuna oil, rich in docosahexaenoic acid and vitamin D_3, inhibits azoxymethane (AOM)-induced aberrant crypt foci (ACF),[10)] the putative precursor lesions for colonic adenocarcinoma.[11)] In this connection, it is noteworthy that conjugated linoleic acid (CLA), a mixture of positional and geometric isomers of linoleic acid (LA), found mainly in milk fat and dairy products[12)] inhibits chemically induced tumorigenesis, particularly in rat mammary bioassays,[13)] mouse skin carcinogenesis,[14)] and mouse forestomach neoplasia.[15)] Compared with investigations on the protective efficacy of CLA in mammary carcinogenesis, evidence for chemoprevention by CLA in colon tumorigenesis is less definitive. CLA did not reduce tumorigenesis in $Apc^{Min/+}$ mouse.[16)] However, oral administration of CLA inhibited the occurrence of chemically induced ACF.[17–19)] The significance of various types of CLA as modulators of pathological processes encouraged us to explore new sources of naturally occurring CLA as chemopreventive agents against colon carcinogenesis.[20)] On the other hand, other types of conjugated PUFA are present in some seed oils.[21,22)] These include pomegranate seed oil (PGO) from pomegranate seeds (*Punica granatum* L.); PGO contains punicic acid, another form of conjugated linolenic acid (CLN). Studies from our laboratory and elsewhere have demonstrated the cytotoxic effect of *c*9,*t*11,*c*13-CLN isolated from PGO and tung oil on a variety of human cancer cell lines, including colon cancer cells.[23,24)] In addition, we have reported the protective effect of *c*9,*t*11,*t*13-CLN against the aggressive development of putative precursor lesions for colonic adenocarcinoma, ACF (with high crypt multiplicity), in a short-term *in vivo* assay.[25)] These findings suggest a possible inhibitory effect of CLN on colon carcinogenesis.

Peroxisome proliferator-activated receptors (PPARs) are a subfamily of nuclear receptors and ligand-responsive transcription factors that participate in many processes important for cell and tissue homeostasis.[26)] To date, three different isotypes have been described in various species, i.e., PPARα, PPARδ (also called PPARβ), and PPARγ, each exhibiting distinct patterns of tissue distribution and ligand specificity. PPARα regulates numerous aspects of fatty acid catabolism, whereas PPARγ controls adipocyte differentiation, systemic glucose levels, and lipid homeostasis.[27,28)] The activity of the PPARs can also be modulated by PUFAs, including LA, linolenic acid, arachidonic acid, and certain eicosanoids (prostaglandin J).[29,30)] Houseknecht *et al*. have reported that CLA activates the PPARγ transcription factor *in vitro* in CV-1 cells transfected with a PPARγ expression vector.[31)] Several studies indicate that synthetic PPARγ ligands inhibit proliferation and induce differentiation in colon cancer cell lines.[32,33)] We have shown that synthetic ligands for PPARγ prevent the development of carcinogen-induced preneoplastic ACF in the colon of rats.[34,35)] Our recent investigation has confirmed these findings: a synthetic

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PPARγ ligand effectively inhibits AOM-induced colon carcinogenesis in rats (Hirose, Y., et al., manuscript in preparation).

The present study was designed as an initial step to evaluate the inhibitory activity of PGO against colon carcinogenesis in male F344 rats. Also, the expression of PPARγ in colonic mucosa and the lipid composition of hepatic and colon tissues were determined to understand the possible mechanisms through which PGO suppresses colon tumorigenesis, since PPARs might be activated by fatty acids.[36]

Materials and Methods

Animals, chemicals, and diets. We used a total of 104 male F344/Ducrj rats, obtained from Charles River Japan, Inc. (Kanagawa), at the age of 4 weeks. The rats 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,[37] under controlled conditions of relative humidity (50±10%), lighting (12-h light/dark cycle) and temperature (23±2°C). AOM was purchased from Sigma Chemical Co. (St. Louis, MO). PGO was extracted from the seeds of pomegranate according to the methods described previously.[25] The fatty acid profile of the total lipids in PGO was similar to that reported.[38] The composition of CLN isomers was as follows: *c*9,*t*11,*c*13-18:3 (75.3%), *c*9,*t*11,*t*13-18:3 (4.2%), *t*9,*t*11,*c*13-18:3 (1.6%), and *t*9,*t*11,*t*13-18:3 (0.5%). CLA was obtained from Rinoru Oil Mills Co., Ltd., Tokyo. CLA contained *c*9,*t*11-18:2 (30.9%) and *t*10,*c*12-18:2 (33.5%). All experimental diets containing various levels of PGO (0%, 0.01%, 0.1%, or 1% by weight of diet) and CLA (1% by weight of diet) were prepared weekly in our laboratory and stored at −20°C under a nitrogen atmosphere in airtight containers for no longer than a week. PGO and CLA were added to the experimental diets at the expense of corn oil. The rats were fed fresh diet every day and the peroxide value of the lipids in the fresh diets was less than 3.0 meq/kg lipid.

Experimental procedure. After quarantine for 7 days, male F344 rats, 5 weeks of age were assigned to one of eight groups. At the time, and throughout the assay, all rats were fed the control and experimental diets containing PGO or CLA. At 6 weeks of age, the rats in groups 1 through 5, designated for carcinogen treatment, were subcutaneously injected with AOM (20 mg/kg body weight) once a week for 2 weeks and those scheduled to receive vehicle treatment received equal volumes of normal saline. The unmodified diet was the control AIN-76A diet containing 5% corn oil. Group 2 was fed this AIN-76A diet containing 0.01% PGO. Group 3 was given the modified diet containing 0.1% PGO and 4.9% corn oil. Groups 4 and 6 were fed the modified diet containing 1% PGO (4% corn oil). Groups 5 and 7 were fed the modified diet containing 1% CLA (4% corn oil). All rats were provided with experimental diet and tap water *ad libitum*, and weighed weekly. The food intake was also recorded weekly. At the termination of the study (week 32), all rats were sacrificed using an overdose of ether. At autopsy, all organs, especially the intestine, were carefully examined grossly, and all abnormal lesions were examined histologically. Colons of five rats from each group were randomly selected for measurement of the expression of PPARγ protein and for lipid analysis in the non-lesional colonic mucosa. Colons of the remaining rats were fixed in 10% buffered formalin and processed for histopathological examination by conventional methods using hematoxylin and eosin staining. Intestinal neoplasms were diagnosed according to the criteria described by Ward.[39] The liver was excised and weighed, 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 for fatty acid composition. All other tissues were fixed in 10% buffered formalin and submitted to histological examination.

Western blotting analysis of PPARγ. Colon samples were homogenized in CelLyticTM-MT Mammalian Tissue Lysis/Extraction Reagent (Sigma Chemical Co.) with a protease inhibitor cocktail (Sigma Chemical Co.), and insoluble materials were removed by centrifugation at 4°C. The supernatants were used to determine protein contents using Bio-Rad protein assay reagents (Bio-Rad Laboratories, Richmond, CA) with bovine serum albumin as a standard. The solubilized lysates were resolved by sodium dodecyl sulfate-PAGE electrophoresis under reducing conditions at a concentration of 50 μg protein of sample per lane. Detection of PPARγ protein was performed with an anti-PPARγ polyclonal antibody (Santa Cruz Biotechnology, Inc., Santa Cruz, CA) utilizing an ECL-plus kit (Amersham Bioscience Corp., NJ). Quantitative analysis was performed using Scion Image analysis software (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.*[40] Component peaks were identified by comparison with standard fatty acid methyl ester [41] and quantified by a Shimadzu Chromatopac C-R6A integrator (Shimadzu Seisakusho Co., Ltd., Kyoto). The identification of CLA and/or CLN isomers was confirmed by using GC-Mass spectrometry after conversion of the methyl esters to dimethyloxazoline derivatives.[42] The analysis of fatty acid composition was done in duplicate for each 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 probability test with $P<0.05$ as the criterion of significance.

Results

General observations. Experimental diet containing different levels of PGO and CLA did not produce any observable toxicity or any gross change in any organ examined. This was confirmed by histopathological examinations in liver, kidney, spleen, heart, and lungs of the rats. Histology revealed no morphological evidence of fatty liver. The mean daily intake of diet with or without PGO was between 13.1 and 13.5 g/day/rat. Mean weights of body and liver (g/100 g body weight) in all groups at sacrifice are shown in Table 1. 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 1–5. Rats treated with saline and fed either control or experimental diet showed no evidence of tumor formation in any organ examined. Colon tumors were sessile or pedunculated and histologically tubular adenomas, adenocarcinomas, or signet ring-cell carcinomas with a preponderance of adenocarcinomas. The incidence (% animals with intestinal tumors) and multiplicity (number of tumors/rat) of intestinal tumors are summarized in Tables 2 and 3, respectively. Administration of 0.01% and 0.1% PGO in the diet significantly suppressed the incidence (44% in the AOM+0.01% PGO group, $P<0.05$ and 38% in the AOM+0.1% PGO group, $P<0.01$) and multiplicity (0.56±0.73 in the AOM+0.01% PGO group, $P<0.01$ and 0.50±0.73 in the AOM+0.1% PGO group, $P<0.005$) of adenocarcinomas in the colon, when compared the AOM alone group (81% incidence and 1.88±1.54 multiplicity). Whereas 1% PGO in the diet had no significant effect on the incidence of adenocarcinomas, it significantly inhibited the multiplicity of adenocarcinomas in the colon (0.88±0.96 multiplicity, $P<0.05$). Another important observation is that 1% CLA in the diet had no effect on the incidence and multiplicity of colon carcinomas.

Table 1. Body, liver and relative liver weights

Group no.	Treatment (no. of rats examined)	Body weight (g)	Liver weight (g)	Relative liver weight (g/100 g body weight)
1	AOM (16)	366±21[1)]	12.0±1.6	3.28±0.37
2	AOM+0.01% PGO (16)	375±17	11.8±1.4	3.13±0.27
3	AOM+0.1% PGO (16)	362±30	11.5±1.1	3.17±0.25
4	AOM+1% PGO (16)	369±26	11.4±1.5	3.10±0.35
5	AOM+1% CLA (16)	368±26	11.4±1.4	3.11±0.57
6	1% PGO (8)	377±28	11.8±1.1	3.13±0.33
7	1% CLA (8)	380±10	12.7±1.3	3.34±0.35
8	None (8)	377±19	12.2±1.1	3.24±0.25

1) Mean±SD.

Table 2. Incidence of intestinal tumors in each group

Group no.	Treatment (no. of rats examined)	No. of rats with intestinal tumors at:								
		Entire intestine			Small intestine			Large intestine		
		Total	AD[1)]	ADC	Total	AD	ADC	Total	AD	ADC
1	AOM[2)] (16)	13 (81%)	7 (44%)	13 (81%)	4 (25%)	0 (0%)	4 (25%)	13 (81%)	7 (44%)	13 (81%)
2	AOM+ 0.01% PGO (16)	12 (75%)	8 (50%)	9 (56%)	6 (38%)	2 (13%)	4 (25%)	10 (63%)	7 (44%)	7[3)] (44%)
3	AOM+ 0.1% PGO (16)	12 (75%)	10 (63%)	9 (56%)	7 (44%)	3 (19%)	4 (25%)	10 (63%)	9 (56%)	6[4)] (38%)
4	AOM+ 1% PGO (16)	14 (88%)	8 (50%)	11 (69%)	7 (44%)	2 (13%)	5 (31%)	12 (75%)	7 (44%)	9 (56%)
5	AOM+ 1% CLA (16)	14 (88%)	5 (31%)	11 (69%)	4 (25%)	0 (0%)	4 (25%)	12 (75%)	5 (31%)	8 (50%)
6	1% PGO (8)	0	0	0	0	0	0	0	0	0
7	1% CLA (8)	0	0	0	0	0	0	0	0	0
8	None (8)	0	0	0	0	0	0	0	0	0

1) AD, adenoma; ADC, adenocarcinoma.
2) AOM, azoxymethane.
3, *4*) Significantly different from group 1 by Fisher's exact probability test ([3)]$P<0.05$ and [4)]$P<0.01$).

Table 3. Multiplicity of intestinal tumors in each group

Group no.	Treatment (no. of rats examined)	Multiplicity (no. of tumors/rat) of intestinal tumors at:								
		Entire intestine			Small intestine			Large intestine		
		Total	AD[1)]	ADC	Total	AD	ADC	Total	AD	ADC
1	AOM (16)	2.81 ±2.01[3)]	0.63 ±1.02	2.19 ±1.72	0.31 ±0.60	0.00 ±0.00	0.31 ±0.60	2.50 ±1.90	0.63 ±1.02	1.88 ±1.54
2	AOM+ 0.01% PGO (16)	1.50[4)] ±1.21	0.69 ±0.70	0.80[4)] ±0.83	0.38 ±0.50	0.13 ±0.34	0.25 ±0.45	1.13[4)] ±1.26	0.56 ±0.73	0.56[5)] ±0.73
3	AOM+ 0.1% PGO (16)	1.75 ±1.57	1.00 ±1.03	0.75[5)] ±0.86	0.44 ±0.51	0.19 ±0.40	0.25 ±0.45	1.31[4)] ±1.35	0.81 ±0.83	0.50[6)] ±0.73
4	AOM+ 1% PGO (16)	2.00 ±1.16	0.81 ±0.98	1.19 ±1.05	0.38 ±0.50	0.13 ±0.34	0.31 ±0.48	1.56 ±1.15	0.69 ±0.87	0.88[4)] ±0.96
5	AOM+ 1% CLA (16)	1.63 ±1.02	0.38 ±0.62	1.25 ±1.06	0.25 ±0.45	0.00 ±0.00	0.25 ±0.45	1.38 ±1.09	0.38 ±0.62	1.00 ±1.10
6	1% PGO (8)	0	0	0	0	0	0	0	0	0
7	1% CLA (8)	0	0	0	0	0	0	0	0	0
8	None (8)	0	0	0	0	0	0	0	0	0

1) AD, adenoma; ADC, adenocarcinoma.
2) AOM, azoxymethane.
3) Mean±SD.
4–6) Significantly different from group 1 by Student's *t* test or Welch's *t* test ([4)]$P<0.05$, [5)]$P<0.01$, and [6)]$P<0.005$).

Lipid analysis. The fatty acid profiles of the lipids from liver and colonic mucosa are shown in Tables 4 and 5, respectively. Although PGO diets contained over 70% of CLN isomer *c*9,*t*11,*c*13-18:3, this isomer was not detected in the liver and colon of rats fed PGO 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 PGO were elevated in a dose-dependent manner. Rats receiving the diet containing CLA showed *c*9,*t*11-18:2 and *t*10,*c*12-18:2 in both the liver and colonic mucosa.

Expression of PPARγ levels in colonic mucosa. A representative immunoblot analysis of PPARγ expression in colonic mucosa of AOM-treated rats on different dietary regimens is shown in

Table 4. Effects of PGO-containing CLN diets on fatty acid composition of liver lipids

Group no.	Treatment	Fatty acids (wt %)						
		16:0	18:0	18:1*n*−9	18:2*n*−6	18:2(*c*9,*t*11)	18:2(*c*9,*t*11)	20:4*n*−6
1	AOM	26.0±0.8[1)]	10.4±0.2	19.6±0.9	11.4±0.5	ND	ND	12.8±0.7
2	AOM+0.01% PGO	26.3±0.7	8.6±0.1	20.9±0.7	12.5±0.6	0.06±0.05	ND	11.1±0.5
3	AOM+0.1% PGO	25.2±1.0	10.5±1.7	18.0±3.1	16.9±4.9	0.24±0.02	ND	12.5±1.3
4	AOM+1% PGO	26.4±0.7	9.0±1.3	20.9±1.6	12.4±1.2	2.54±0.28	ND	11.0±1.6
5	AOM+1% CLA	25.5±1.2	11.5±0.3	18.1±0.8	10.8±0.4	1.09±0.04	0.30±0.04	13.2±0.3
6	1% PGO	23.0±1.9	12.4±1.1	18.4±3.0	10.9±1.4	1.94±0.13	ND	15.1±2.5
7	1% CLA	23.8±0.3	12.5±0.3	17.6±0.0	12.0±1.0	1.18±0.08	0.30±0.02	14.5±0.3
8	None	22.9±0.5	10.4±0.9	19.4±0.7	14.6±1.6	ND	ND	13.5±0.8

1) Mean±SD.

Table 5. Effects of PGO-containing CLN diets on fatty acid composition of colonic mucosa lipids

Group no.	Treatment	Fatty acids (wt %)						
		16:0	18:0	18:1*n*−9	18:2*n*−6	18:2(*c*9,*t*11)	18:2(*c*9,*t*11)	20:4*n*−6
1	AOM	22.6±0.4[1)]	2.8±0.2	31.4±0.8	26.0±0.8	ND	ND	1.3±0.3
2	AOM+0.01% PGO	22.4±0.5	3.2±0.4	30.3±0.3	24.8±1.1	0.11±0.02	ND	2.2±0.4
3	AOM+0.1% PGO	22.2±1.3	4.7±2.3	27.6±3.6	23.3±1.9	0.38±0.12	ND	4.0±3.0
4	AOM+1% PGO	22.8±0.7	4.1±0.9	28.9±1.9	20.6±0.9	4.10±0.23	ND	2.9±1.0
5	AOM+1% CLA	24.6±0.6	3.5±0.7	29.0±1.1	21.1±1.4	2.46±0.17	0.95±0.13	1.8±0.8
6	1% PGO	23.3±0.6	2.8±0.4	30.0±0.8	21.6±0.8	4.44±0.13	ND	1.3±0.7
7	1% CLA	23.5±0.9	4.2±0.9	27.7±1.8	20.1±1.6	2.18±0.28	0.89±0.16	3.6±1.1
8	None	22.4±0.6	3.4±0.5	30.0±1.2	24.6±1.6	ND	ND	2.4±1.0

1) Mean±SD.

Fig. 1. Dietary administration of PGO resulted in enhanced expression of PPARγ protein as compared to the control, namely, a 2.4±0.8-fold increase in group 2, a 2.3±0.8-fold elevation in group 3, and a 1.8±0.7-fold increase in group 4. Feeding of CLA also increased the expression of PPARγ protein (2.1±0.6-fold) as compared to the control diet.

Discussion

The results described here clearly indicate that dietary administration of PGO, which is rich in *c*9,*t*11,*c*13-CLN, significantly inhibits the development of AOM-induced colonic adenocarcinomas in male F344 rats without causing any adverse effects. In addition, there was a significant reduction of the multiplicity of carcinomas in the colon of rats fed PGO at any dose level (0.01%, 0.1%, or 1%) as compared with the AOM-induced rats. Previous studies have shown that CLA does not alter CYP expression.[43, 44)] However, it is possible that PGO may affect liver CYPs, including CYP2E1, which activates AOM, since dietary CLN suppressed AOM-induced ACF when fed during AOM exposure.[25)] The results described here suggest that dietary feeding of PGO suppressed progression of these lesions through adenoma to malignant neoplasm in the post-initiation phase. We believe that our results are the first to demonstrate the chemopreventive efficacy of PGO, which is rich in *c*9,*t*11,*c*13-CLN, against chemically induced colon carcinogenesis.

Recent evidence suggests that pharmacological activation of PPARγ may prevent cancer.[45)] PPARγ might exert its anticancer effect through the modification of cell growth[32)] and the regulation of cyclooxygenase-2.[46)] The highly potent and specific PPARγ ligand GW7845 significantly reduces rat mammary carcinogenesis.[47)] We and others have also demonstrated that synthetic ligands for PPARα and PPARγ effectively inhibit AOM-induced ACF in rats.[34, 35, 48)] CLA was shown to act as a high-affinity ligand and activator of PPARα and PPARγ.[31, 49, 50)] McCarty[50)] suggested that a part of the anticarcinogenic activity of CLA is mediated by PPARγ activation in susceptible tumors.

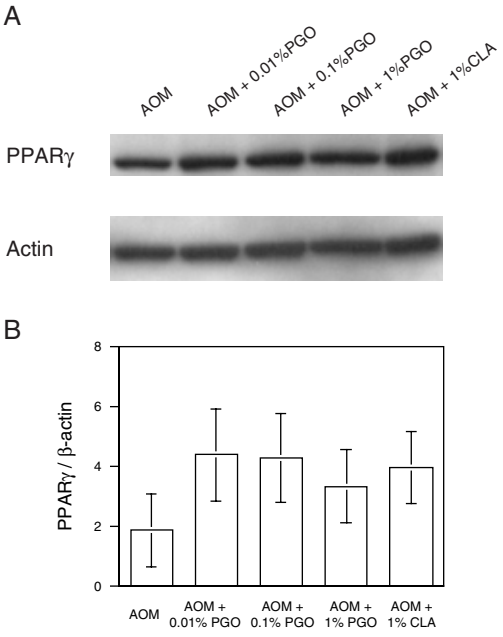

Fig. 1. Expression of PPARγ in colonic mucosa. (A) PPARγ and β-actin proteins analyzed by immunoblotting of protein extracts from the colonic mucosa. (B) Quantitative analysis using Scion Image analysis software

In the current study, dietary administration of PGO enhanced PPARγ expression in non-lesional colonic mucosa of rats, and thus, induction of PPARγ activity may account for the diminished colon tumor incidence and multiplicity that we observed.

We did not detect any CLN isomer in the lipids extracted from the livers of rats fed the PGO diets, although the PGO contained over 70% of the *c*9,*t*11,*c*13-CLN isomer. On the other hand, CLA was found in the extracted lipids: the CLA

isomer (*c*9,*t*11-18:2) was significantly and dose-dependently higher in extracts from rats fed the PGO diets. These findings are in accordance with other reports.[51, 52] This may indicate that a part of *c*9,*t*11,*c*13-18:3 in the PGO diets was enzymatically converted to *c*9,*t*11-18:2. Therefore, the suppressing effect of PGO on colon carcinogenesis in the current study may be attributed to the *c*9,*t*11-18:2 isomer derived from *c*9,*t*11,*c*13-18:3 in the PGO diets. However, feeding 1% PGO enhanced the accumulation of *c*9,*t*11-CLA, the active component of the CLA isomer. On the other hand, the suppressive effect against colon carcinogenesis of PGO at the 1% level was weaker than that of lower dose administration (0.01% and 0.1%). Ip *et al*.[53] found that dietary CLA at between 0.05% and 0.5% produced a dose-dependent inhibition of mammary tumor development, but the inhibitory effect of CLA reached a maximum at about 1%.[54] Thus, there may exist an optimal dose of conjugated fatty acids to exert an optimal cancer chemopreventive action.

The present study also demonstrates that dietary feeding of CLA enhanced PPARγ expression in non-lesional colonic mucosa. However, the protective effect of CLA against colon carcinogenesis was relatively weaker than that of PGO. The differences in effect on PPARγ expression are possibly due to the differences of positional and geometrical isomers between CLA and PGO. In the current study, CLA contained 30.9% *c*9,*t*11-CLA and 33.5% *t*10,*c*12-CLA. Feeding of CLA enhanced the accumulation of *c*9,*t*11-CLA and *t*10,*c*12-CLA in colonic mucosa and liver. Recently, Brown *et al*.[55] suggested that *c*9,*t*11-CLA is an agonist, and *t*10,*c*12-CLA an antagonist of PPARγ. Therefore, it is likely that, when given as an isomeric mixture, they may largely negate each other's effects.

A possible contribution of antioxidant activity to the inhibition of colon carcinogenesis by PGO cannot be justified without evidence indicating that oxidative stress is involved in AOM-induced colon carcinogenesis. Ha *et al*.[15] suggested that the inhibitory effects of CLA intubation on benzo(*a*)pyrene-induced mouse forestomach tumorigenesis might be due to the antioxidative property of CLA. Interestingly, the *c*9,*t*11-CLA isomer was incorporated into forestomach phospholipids after CLA intubation in their study. In addition, Dhar *et al*.[56] reported that *c*9,*t*11,*t*13-18:3 from bitter melon acts as an antioxidant. These compounds have more than two conjugated double bonds, and conjugation is known to increase the rate of oxidation. Conjugated trienoic fatty acids may be more rapidly oxidized than LA. After oxidation, conjugated trienoic fatty acids may form more hydroperoxides than LA. Similarly, docosahexaenoic acid, eicosapentaenoic acid and α-LA are more readily oxidized to hydroperoxides than LA.[57] Although we did not determine these parameters, it is possible that, in this study, PGO reduces the formation of hydroperoxides by diminishing the generation of free radicals and the peroxidation of PUFAs in cell membranes of cryptal cells initiated with AOM. Additional in-depth studies of the mechanisms of action of PGO as a colon tumor inhibitor are warranted to provide a clearer understanding of its effects.

In the present study, the estimated daily CLN intakes in rats given diets containing 0.01%, 0.1%, and 1% PGO were 2.63, 27.9, and 273 mg/kg body weight respectively. Though the CLA used in animal feeding studies contains a mixture of positional and geometrical isomers, *c*9,*t*11-18:2 is considered to be the most active constituent.[13, 15, 58] In this current study, only the *c*9,*t*11-18:2 isomer accumulated in the colonic mucosa and liver when rats were fed PGO. The finding that PGO exerted cancer chemopreventive activity even at the 0.1% dose level suggests that PGO has promise as a naturally occurring preventive agent against colon cancer development.

In conclusion, this study has explored for the first time whether PGO, a food component, exhibits chemopreventive efficacy against experimental colon carcinogenesis. Our findings suggest that dietary PGO rich in CLN has a preventive effect on chemically induced rat colon carcinogenesis. The efficacy of a chemopreventive agent may depend on the timing of administration. Thus, further experiments on the chemopreventive ability of PGO are needed, to establish whether pre-initiation or post-initiation administration is preferable to inhibit tumor development. Such a study is under way in our laboratory.

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