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Research Article

Cancer Prevention Research

Inhibition of Intestinal Polyp Formation by Pitavastatin, a HMG-CoA Reductase Inhibitor

Naoya Teraoka[1], Michihiro Mutoh[1], Shinji Takasu[1], Toshiya Ueno[1], Masafumi Yamamoto[1], Takashi Sugimura[1], and Keiji Wakabayashi[1,2]

Abstract

It has been suggested that hyperlipidemia is positively associated with colon carcinogenesis. Statins, 3-hydroxy-3-methyl-glutaryl-coenzyme A reductase inhibitors, reduce serum lipid levels. In this study, we clarified the effects of a novel chemically synthesized statin, pitavastatin, on intestinal polyp formation in Min mice, and further examined serum lipid and adipocytokine levels, and proinflammatory and adipocytokine gene levels in intestinal mucosa of Min mice. Treatment with pitavastatin at doses of 20 and 40 ppm decreased the total number of polyps dose-dependently to 85.2% and 65.8% ($P < 0.05$) of the untreated value, respectively. Serum levels of total cholesterol and triglyceride were slightly reduced and those of IL-6, leptin, and MCP-1 were decreased by 40-ppm pitavastatin treatment. mRNA expression levels of *cyclooxygenase-2*, *IL-6*, *inducible nitric oxide* (*iNOS*), *MCP-1*, and *Pai-1* were significantly reduced in intestinal nonpolyp parts by pitavastatin treatment. Among them, *iNOS* mRNA levels were also reduced in the intestinal polyps. Moreover, oxidative stress represented by 8-nitroguanosine in the small intestinal epithelial cells was reduced by pitavastatin treatment. Related to these proinflammatory genes, PPARγ activity was activated in the intestinal nonpolyp parts and in the liver of Min mice with pitavastatin treatment. These results indicated that pitavastatin has potential benefit for the suppression of intestinal polyp development. *Cancer Prev Res; 4(3); 445–53. ©2011 AACR.*

Introduction

Epidemiological studies have suggested that mortality and morbidity of colon cancer are increasing in developed countries (1, 2). Thus, it is very important to establish effective methods to prevent colon cancer development. Consumption of a high-fat diet is a considerable risk factor of colon cancer with clear link with hyperlipidemia. Hyperlipidemia has also been indicated to be positively associated with colon carcinogenesis (3, 4). We have reported *Apc* deficient, Min and Apc^{1309} mice, which developed a large number of intestinal polyps showed the hyperlipidemic state (5–7). Interestingly, improvement of hyperlipidemic state by peroxisome proliferator-activated receptor (PPAR) α and γ agonists and a selective LPL-inducing agent, NO-1886, which does not possess PPARs agonistic activity, suppressed intestinal polyp formation in Min mice. Thus, it is conceivable that drugs, which effectively improve hyperlipidemia, could also prevent colon cancer development.

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Statins, 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase inhibitors, are commonly used for the treatment of hypercholesterolemia (8, 9). Among statins, pravastatin, lovastatin, and simvastatin are so called the first-generation statins (10, 11), and fluvastatin is the second-generation statin (12). Recently, the third-generation statins, such as atorvastatin and rosuvastatin, were developed (13, 14), which strongly suppress serum LDL-cholesterol levels compared with the former generation statins. The Molecular Epidemiology of Colorectal Cancer (MECC) study has indicated that use of statins for 5 years or longer significantly reduced the risk of colorectal cancer by 47% (15). Meanwhile, some epidemiological studies using the first-generation statins, lovastatin and pravastatin (16, 17), are not fully consistent with above data. One of the reasons might be the suboptimal administration of the drug, in which efficacy of statins could not be shown, and another reason might be the difference of statin generation. Thus, epidemiological and/or experimental data on third-generation statins are desired to evaluate its chemopreventive effects on colon cancer.

Previous animal studies have shown that pravastatin and atorvastatin suppressed 1,2-dimethylhydrazine (DMH) or azoxymethane (AOM)-induced colon cancer development in mice and rats, respectively (18, 19). Recent animal studies have shown that 100-ppm atorvastatin reduced the incidnce of small intestinal polyp (adenoma) in Min mice to about 70% of the control group (20). Meanwhile, 10-ppm pitavastatin, a novel third-generation lipophilic

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statin (21), reduced the incidence of colon adenoma or adenocarcinoma induced by AOM/dextran sodium sulfate treatment in ICR mice to about 78% of the control group (22). These results implied that pitavastatin may have a potent effect against colon tumor formation in rodent.

Pitavastatin may also have several clinical advantages over other statins. Similar serum triglyceride (TG) levels could be achieved by doses of pitavastatin (2 mg/d) lower than those of atorvastatin or rosuvastatin (10 mg/d). Lowering potentiality of pitavastatin on serum LDL-cholesterol is greater than that of pravastatin and is similar to atorvastatin (23, 24). As pitavastatin is hardly metabolized by cytochrome P450 compared with other statins, pitavastatin has advantage of not having unexpected interactions with other drugs.

In addition to the main function of statins, which is inhibition of the synthesis of mevalonate, statin also suppresses inflammation. Statins concomitantly suppress geranylgeranylation of protein, such as the small GTP-binding proteins RhoA, Ras, Cdc42, and Rac (25,26), which activate intracellular signaling molecules. Thus, pleiotropic action of pitavastatin could be involved in the suppression of cancer development.

In this study, we clarified the suppressive effect of pitavastatin on intestinal polyp development in Min mice. The mechanism involved in the suppressive effect of pitavastatin treatment on intestinal polyp formation in Min mice was also examined and further discussed.

Materials and Methods

Animals and chemicals

Male C57BL/6J-*Apc*$^{Min/+}$ mice (Min mice) were purchased from The Jackson Laboratory at 6 weeks of age and genotyped as previously reported (27). Heterozygotes of the Min strain and wild-type (C57BL/6J) mice were acclimated to laboratory conditions for 1 week. Four or five mice were housed per plastic cage with sterilized softwood chips as bedding in a barrier-sustained animal room at 24 ± 2°C and 55% humidity on a 12-hour light/dark cycle. The pitavastatin, (+)-monocalcium bis{(3*R*,5*S*,6*E*)-7-[2-cyclopropyl-4-(4-fluorophenyl)-3-quinolyl]-3,5-dihydroxy-6-heptenoate} ($C_{50}H_{46}CaF_2N_2O_8$, MW 880.98), was kindly provided by Kowa Pharmaceutical Co., Ltd. Pitavastatin was well mixed at the concentrations of 20 and 40 ppm in AIN-76A powdered basal diet (CLEA).

Animal Experimental Schedule

To investigate the effects of pitavastatin on intestinal polyp formation, male Min mice at 6 weeks of age were given 0, 20, 40 ppm of pitavastatin in the diet for 14 weeks. Min mice were divided into groups of 20. With 20 ppm of pitavastatin, 4 mice died during the experiment. Food and water were available *ad libitum*. The animals were observed daily for health appearance and mortality. Body weights and food consumption were measured weekly. Animals were anesthetized with ether and sacrificed, and blood samples were collected from the caudal vena cava. Serum levels of TG and total cholesterol were measured as reported previously (6). The experiments were carried out according to the "Guidelines for Animal Experiments in the National Cancer Center" and were approved by the Institutional Ethics Review Committee for Animal Experimentation in the National Cancer Center.

The intestinal tract was removed and separated into the small intestine, cecum, and colon. The small intestine was divided into the proximal segment (4 cm in length), and then the proximal (middle) and distal halves of the remainder. Polyps in the proximal segments were counted and all polyps were picked up under a stereoscopic microscope and the remaining intestinal mucosa (nonpolyp part) was removed by scraping, and then both stored at −80°C for the further real-time PCR analysis. Other segments were opened longitudinally and fixed flat between sheets of filter paper in 10% buffered formalin. The numbers and sizes of polyps and their distributions in the intestine were assessed with a stereoscopic microscope (6). A part of liver, femoral muscle, visceral fat, and right kidney were kept in 10% buffered formalin, and residues of liver, femoral muscle, visceral fat, and left kidney were frozen by liquid nitrogen and then stored at −80°C.

Determination of Serum Adipocytokine Levels

Serum samples from 20-week-old male Min mice with or without pitavastatin were measured for serum concentrations of adiponectin (R&D Systems), leptin (B-Bridge International, inc.), Pai-1 (Innovative) by an enzyme immunoassay and IL-1β, IL-6, MCP-1, TNFα, VEGF were determined by using Procarta Cytokine Assay mouse (Affymetrix, inc.) according to the manufacturer's protocol.

Immunohistochemical Staining

The middle segments of the small intestines were fixed, embedded, and sectioned as Swiss rolls for further immunohistochemical examination with the avidin–biotin complex immunoperoxidase technique. Polyclonal goat anti-COX-2 and anti-MCP-1 antibody (Santa Cruz Biotechnology), polyclonal rabbit anti-Pai-1 antibody (Santa Cruz Biotechnology), polyclonal rabbit anti-PPARγ antibody (Cell Signaling), monoclonal mouse anti-iNOS antibody, and anti-nitrotyrosin antibody (Santa Cruz Biotechnology) were used at 100x dilution. Polyclonal rabbit anti-8-nitroguanosine antibody (Cosmo Bio Co., Ltd.) and polyclonal goat anti-IL-6 antibody (Santa Cruz Biotechnology) were used at 50x dilution. As the secondary antibody, biotinylated anti-goat, -rabbit, and -mouse IgG (Vector Laboratories) were employed at 200x dilution. Staining was done using avidin biotin reagents (Vectastain ABC reagents; Vector Laboratories), 3,3′-diaminobenzidine and hydrogen peroxide, and the sections were counterstained with hematoxylin to facilitate orientation. As a negative control, consecutive sections were immunostained without exposure to the primary antibody.

Real-time PCR Analysis

Polyps and nonpolyp parts from proximal segments of small intestine of Min mice were rapidly deep-frozen in

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Table 1. Number of intestinal polyps/mouse in Min mice with or without pitavastatin treatment

Pitavastatin (ppm)	No. of mice	Small intestine			Colon	Total
		Proximal	Middle	Distal		
0	20	4.6 ± 1.2	19.2 ± 5.2	46.2 ± 7.2	0.6 ± 0.4	70.5 ± 13.3
20	16	5.4 ± 1.8	13.3 ± 2.2	40.4 ± 6.6	0.9 ± 0.3	60.1 ± 10.1
40	20	5.1 ± 1.5	13.4 ± 3.2	27.4 ± 4.1**	0.5 ± 0.3	46.4 ± 8.0*

Data are mean ± SE

*, ** Significantly different from the pitavastatin untreated group at $P < 0.05$, $P < 0.01$.

liquid nitrogen and stored at −80°C. Total RNA was isolated from tissues by using Isogen (Nippon Gene), treated with DNase (Invitrogen) and 3-μg aliquots in a final volume of 20 μL were used for synthesis of cDNA using an Omniscript RT Kit (Qiagen) and an oligo (dT) primer. Real-time PCR was carried out using a DNA Engine Opticon 2 (MJ Japan) with SYBR Green Real-time PCR Master Mix (Toyobo) according to the manufacturer's instructions. Primers for mouse *adiponectin* (5′primer-AGGATGCTACTGTTGCAAGCTCTC, 3′primer-CAGTCAGTTGGTATCATGGTAGAG), *COX-2* (5′primer- AGAAGGAAATGGCTGCAGAA, 3′primer-GCTCGGCTTCCAGTATTGAG), *iNOS* (5′primer-CCGGCAAACCCAAGGTCTACGTT, 3′primer-CACATCCCGAGCCATGCGCACATCT), *IL-6* (5′primer-ACAACCACGGCCTTCCCTACTT, 3′primer-CACGATTTCCCAGAGAACATGTG), *MCP-1* (5′primer-CCACTCACCTGCTGCTACTCAT, 3′primer- TGGTGATCCTCTTGTAGCTCTCC), *Pai-1* (5′primer-GACACCCTCAGCATGTTCATC, 3′primer- GACTGTACAAATCACGTTGGGA), and *GAPDH* (5′primer-TTGTCTCCTGCGACTTCA, 3′primer-CACCACCCTGTTGCTGTA) were employed (28–31). To assess the specificity of each primer set, amplicons generated from the PCR reaction were analyzed for melting curves and also by electrophoresis in 2% agarose gels. Standard curves for absolute quantification were obtained with plasmids containing the various amplicons. From each plasmid a 10-fold dilution series was measured in duplicate. Quantification and generation of standard curves was carried out using a DNA Engine Opticon 2 (MJ Japan).

PPARγ Activity in Intestinal Mucosa and Liver

Nuclear extracts containing PPARγ from nonpolyp parts of intestinal mucosa and liver (50 mg each) of mice with or without 40-ppm pitavastatin treatment were prepared using NE-PER Nuclear and Cytoplasmic Extraction Reagents (PIERCE Biotechnology). PPARγ activation by pitavastatin treatment was assayed using an ELISA-based transactivation TransAM PPARγ kit (Active Motif) following the manufacturer's protocol.

Statistical Analysis

All the results are expressed as mean ± standard errors (SE) values, with statistical analysis using Dunnett's test and PPARγ activity in intestinal nonpolyp parts and liver were performed with Student's *t*-test. Differences were considered to be statistically significant at $P < 0.05$.

Results

Suppression of intestinal polyp formation in Min mice by pitavastatin treatment

Treatment with pitavastatin at doses of 20 and 40 ppm for 14 weeks did not affect body weights or health appearance of Min mice throughout the experimental period. Average daily food intake did not differ among the groups, being 2.39 ± 0.37 (mean ± SE), 2.49 ± 0.39, and 2.47 ± 0.25 g per mouse per day for the 0, 20, and 40-ppm groups of Min mice, respectively. No changes were observed in the liver, heart, kidney, and thymus weights that might have been attributable to toxicity.

Table 1 summarizes data on the number and distribution of intestinal polyps in the untreated and pitavastatin-treated groups. Almost all polyps developed in the small intestine, with only a few in the colon as reported previously (6). The treatment with pitavastatin at a dose of 40 ppm significantly reduced the total number of polyps to 65.8% ($P < 0.05$) of the value in the untreated group. Strong suppression of intestinal polyp development was observed at the distal parts of small intestine, with 41% reduction ($P < 0.01$) at 40 ppm. The maximum number of polyps was observed in the range of size from 0.5 mm to 2.5 mm in diameter. Treatment with 40-ppm pitavastatin significantly reduced the numbers of polyps ranging from 1.5 mm to 2.0 mm ($P < 0.05$ vs. 0 ppm; Fig. 1A). Small-size polyps (<2.0 mm) were mainly distributed in distal parts of the small intestine (Fig. 1B). The group treated with 40-ppm pitavastatin significantly reduced the number of small-size polyps (21.6 ± 1.9/mouse, $P < 0.01$) in distal part compared with the untreated group (33.9 ± 2.4/mouse; Fig. 1B). The number of large-size polyps (≥2.0 mm) in distal parts of the untreated group was 11.9 ± 3.5/mouse, and the number in 40-ppm pitavastatin–treated group was decreased to 5.4 ± 1.6/mouse ($P < 0.05$; Fig. 1C).

Serum lipid and adipocytokine levels in Min mice with pitavastatin treatment

Consistent with our previous reports (5–7), Min mice fed with the basal diet at 20 weeks of age were in the

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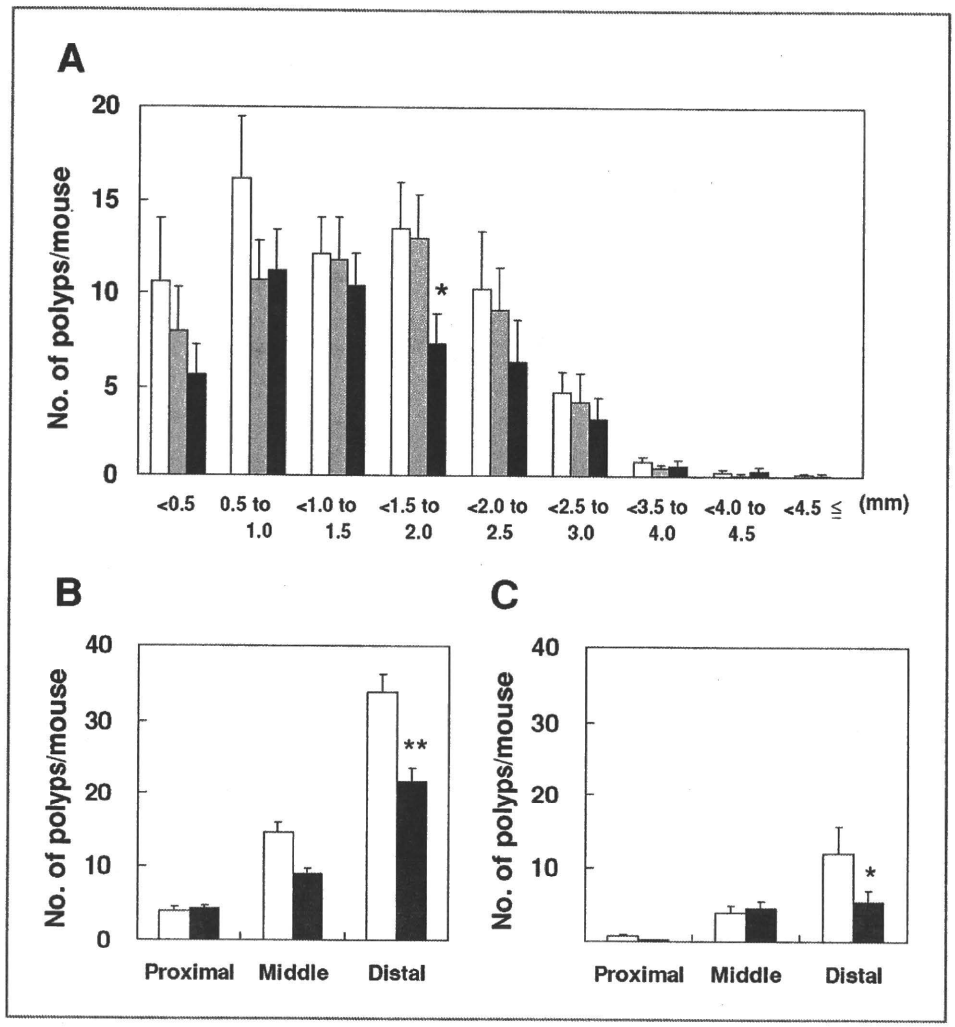

Figure 1. Effect of pitavastatin on the intestinal polyp size distribution in Min mice. Min mice were fed with a basal diet (open box) or a diet containing 20 ppm (gray box), 40-ppm (black box) pitavastatin. All of the intestinal polyps are grouped by size and its number/mouse is shown (A). Small intestinal polyps are sectioned into proximal, middle, and distal part, and the number/mouse having a diameter of less than 2 mm is shown in *B*, and 2 mm or more than 2 mm is shown in C. Date are mean ± SE, $^{*}P < 0.05$ vs. 0 ppm; $^{**}P < 0.01$ vs. 0 ppm.

hypertriglyceridemic state, with a TG level of 285.3 ± 64.3 mg/dL (mean ± SE). On the other hand, the TG level in wild-type mice was 32.7 ± 4.2 mg/dL. Treatment with 20 and 40-ppm pitavastatin slightly decreased serum levels of TG to 277.4 ± 68.1 mg/dL and 242.8 ± 49.9 mg/dL, respectively. However, these changes were not statistically significant. The level of total cholesterol had tendency to decrease to approximately 8%–9% of untreated group level and it did not seem to be influenced by dosage.

Serum concentrations of adiponectin, IL-1β, IL-6, leptin, MCP-1, TNFα, Pai-1, and VEGF were also measured to evaluate systemic effects of pitavastatin. Among the adipocytokines, IL-6, leptin, and MCP-1 were reduced significantly from 4.5 to 1.1 pg/mL, 2.5 to 1.4 ng/mL, and 12.1 to 7.7 pg/mL, respectively (Table 2).

COX-2, *iNOS* and adipocytokine mRNA levels in intestinal polyps and nonpolyp parts of Min mice treated with pitavastatin

To clarify the mechanisms of suppression on the development of intestinal polyps by pitavastatin treatment, mRNA expressions of *COX-2*, *iNOS*, and several adipocytokines in intestinal polyps and nonpolyp parts were investigated. Real-time PCR revealed that treatment with 20 and 40-ppm pitavastatin for 14 weeks effectively suppressed *COX-2*, *iNOS*, *IL-6*, *MCP-1*, and *Pai-1* mRNA levels in intestinal nonpolyp parts of Min mice (Fig. 2A and C). Treatment with 20-ppm pitavastatin reduced *COX-2*, *iNOS*, *IL-6*, *MCP-1*, and *Pai-1* mRNA levels to 15% ($P < 0.05$), 36% ($P < 0.05$), 36% ($P < 0.05$), 27%, and 73% of the untreated value, respectively, and 40 -ppm pitavastatin significantly reduced to 9% ($P < 0.01$), 22% ($P < 0.01$), 23% ($P < 0.01$), 15% ($P < 0.05$), and 18% ($P < 0.05$), respectively. Another adipocytokine, adiponectin, sustained basal mRNA level of wild-type in intestinal nonpolyp parts (data not shown). As shown in Figure 2B, mRNA expression levels of *COX-2* were higher in polyp than in nonpolyp parts. Treatment with 20 and 40-ppm pitavastatin slightly suppressed mRNA levels of *COX-2* in the polyps, but significantly suppressed its mRNA levels in nonpolyp parts.Meanwhile, treatment with 20 ppm of pitavastatin significantly suppressed *iNOS* mRNA levels in nonpolyp parts, and 40-ppm pitavastatin significantly suppressed both polyp and nonpolyp parts (Fig. 2C).

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Table 2. Serum adipocytokine levels in Min mice with 40 ppm or without pitavastatin treatment

Pitavastatin (ppm)	Adiponectin (μg/mL)	IL-1β (pg/mL)	IL-6 (pg/mL)	Leptin (ng/mL)	MCP-1 (pg/mL)	Pai-1 (ng/mL)	TNFα (pg/mL)	VEGF (pg/mL)
0	12.20 ± 0.89	35.80 ± 8.00	4.51 ± 1.18	2.47 ± 1.80*	12.10 ± 1.30*	4.04 ± 2.41	5.90 ± 3.64	11.20 ± 1.51
40	13.90 ± 0.91	20.30 ± 6.20	1.11 ± 0.66*	1.38 ± 0.38*	7.66 ± 1.27*	3.32 ± 0.43	6.03 ± 2.62	8.90 ± 1.20

Data are mean ± SE ($n = 3\sim6$)
* Significantly different from the pitavastatin untreated group at $P < 0.05$.

Evaluation of nitrative stress in the intestinal polyp in Min mice treated with pitavastatin.

To evaluate the effects of iNOS overexpression as a nitrative stress in intestinal polyp in Min mice, localization of iNOS and the resultant nitration reaction were examined by immunohistochemistry using an anti-nitrotyrosin antibody and anti-8-nitroguanosine antibody. Nitrotyrosin was observed mainly in the stroma cells and 8-nitroguanosine was observed mainly in the cytoplasm of epithelial cells. Both nitrotyrosin and 8-nitroguanosine were weakly suppressed by 40-ppm pitavastatin treatment (Fig. 3A–F). In addition, localization and expression of COX-2 (Fig. 3G and H), IL-6, MCP-1, Pai-1, and PPARγ in the intestinal polyps were examined by immunohistochemistry. COX-2 was observed mainly in the stroma cells and IL-6, MCP-1, Pai-1, and PPARγ were observed mainly in the cytoplasm of epithelial cells without being affected by 40 ppm-pitavastatin treatment (Supplemental Fig. 1).

Effect of pitavastatin on PPARγ-DNA binding activity in intestinal nonpolyp parts and liver

Statins are reported to suppress some inflammatory adipocytokines through the PPARγ activation (32). Thus, we further evaluated the effect of pitavastatin on PPARγ activation in nonpolyp parts of the small intestine and liver of Min mice. Treatment with pitavastatin increased

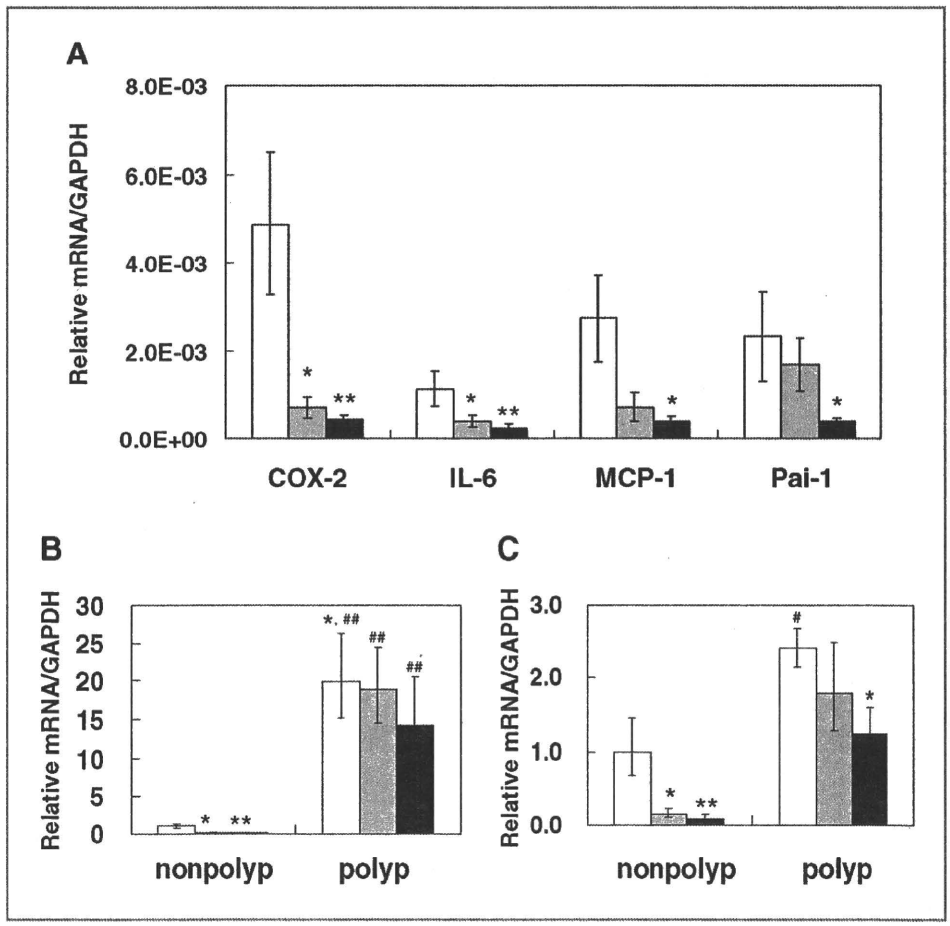

Figure 2. Changes of inflammation-related factors in intestinal nonpolyp parts and/or polyp parts of Min mice. Real-time PCR was conducted to detect *COX-2*, *IL-6*, *iNOS*, *MCP-1*, and *Pai-1* using intestinal tissue of Min mice with 0 (open box), 20 (gray box), and 40-(black box) ppm pitavastatin treatment. The data of molecule copy number are shown on the Y-axis (A). Regarding *COX-2* and *iNOS*, relative expression levels in the intestinal nonpolyp parts and polyp parts are shown in *B*, and *C*, respectively. Data are normalized with GAPDH. Data are mean ± SE, $n = 7$–11 (nonpolyp parts), $n = 5$ (polyps). *$P < 0.05$ vs. 0 ppm; **$P < 0.01$ vs. 0 ppm. #$P < 0.05$ vs. nonpolyp part (0 ppm); ##$P < 0.01$ vs. nonpolyp part (0 ppm).

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0	12.20 ± 0.89	35.80 ± 8.00	4.51 ± 1.18	2.47 ± 1.80*	12.10 ± 1.30*	4.04 ± 2.41	5.90 ± 3.64	11.20 ± 1.51
40	13.90 ± 0.91	20.30 ± 6.20	1.11 ± 0.66*	1.38 ± 0.38*	7.66 ± 1.27*	3.32 ± 0.43	6.03 ± 2.62	8.90 ± 1.20

Data are mean ± SE ($n = 3$~6)

* Significantly different from the pitavastatin untreated group at $P < 0.05$.

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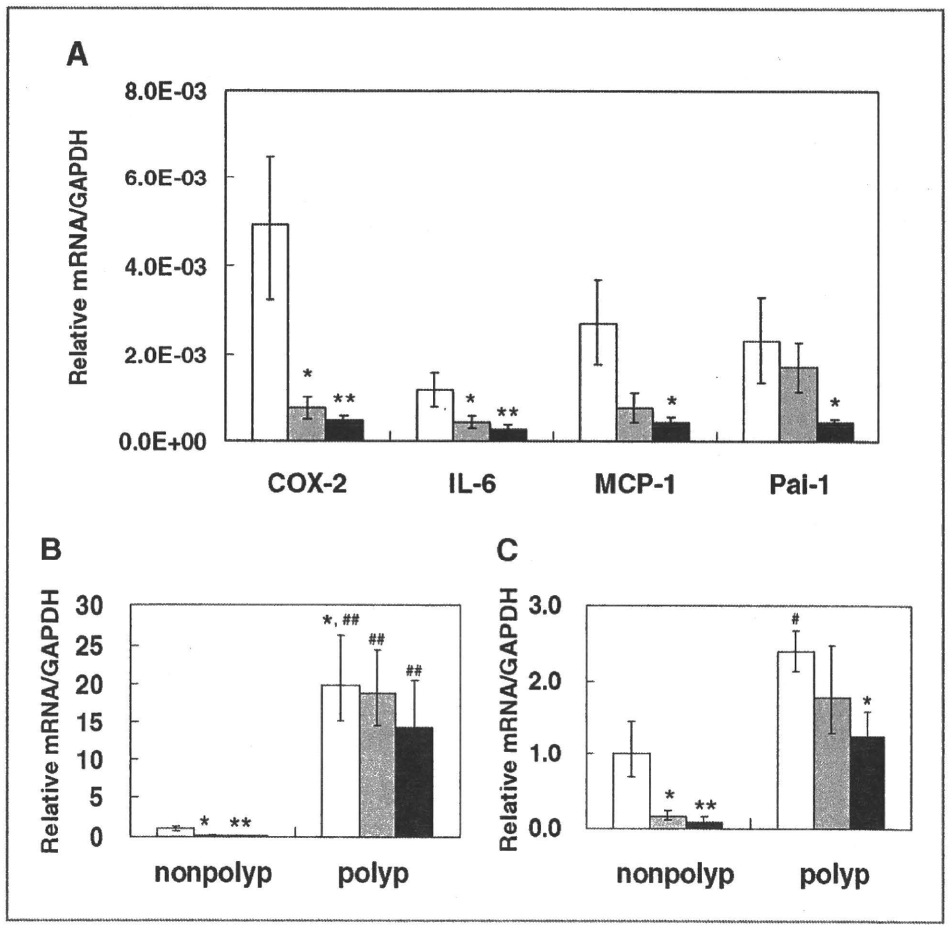

Figure 2. Changes of inflammation-related factors in intestinal nonpolyp parts and/or polyp parts of Min mice. Real-time PCR was conducted to detect *COX-2*, *IL-6*, *iNOS*, *MCP-1*, and *Pai-1* using intestinal tissue of Min mice with 0 (open box), 20 (gray box), and 40-(black box) ppm pitavastatin treatment. The data of molecule copy number are shown on the Y-axis (A). Regarding *COX-2* and *iNOS*, relative expression levels in the intestinal nonpolyp parts and polyp parts are shown in *B*, and *C*, respectively. Data are normalized with GAPDH. Data are mean ± SE, $n = 7$–11 (nonpolyp parts), $n = 5$ (polyps). *$P < 0.05$ vs. 0 ppm; **$P < 0.01$ vs. 0 ppm. #$P < 0.05$ vs. nonpolyp part (0 ppm); ##$P < 0.01$ vs. nonpolyp part (0 ppm).

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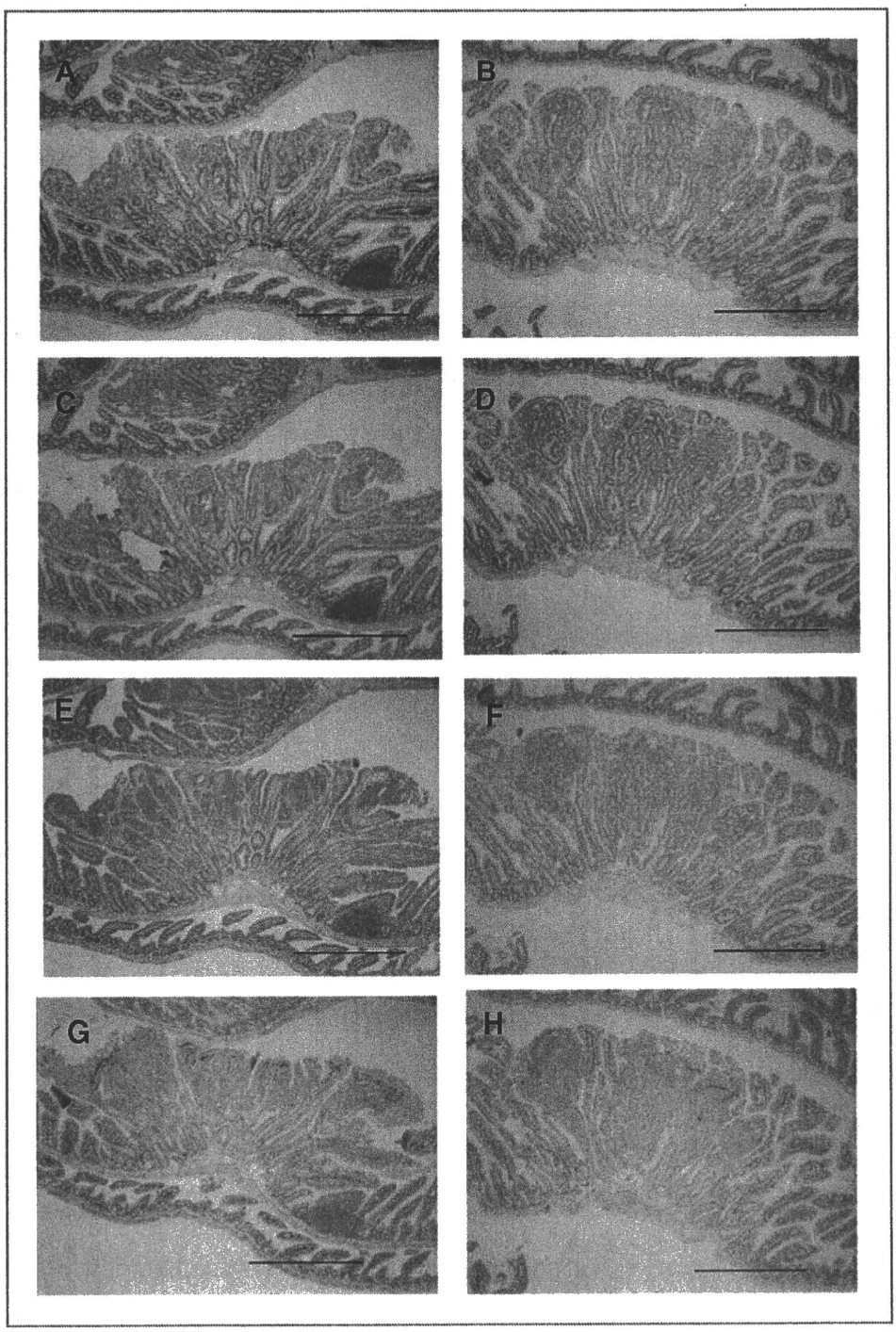

Figure 3. Modulation of mucosal oxidative/nitrosative stress by pitavastatin in Min mice. Immunohistochemical staining of iNOS (A and B), nitrotyrosine (C and D), 8-nitroganosine (E and F) and COX-2 (G and H), protein treated with (B, D, F and H) or without 40-ppm pitavastatin (A, C, E, and G), in Min mice. Bars represent 500 μm.

PPARγ-DNA binding activity in the intestinal nonpolyp parts and the liver at the dose of 40 ppm (Fig. 4A and B).

Discussion

In this study, it was showed that the treatment with pitavastatin suppressed intestinal polyp formation in Min mice with slight reduction of serum levels of total cholesterol and TG. The antiinflammatory effects were also observed in pitavastatin-treated Min mice, such as down regulation of *COX-2*, *iNOS*, and some adipocytokines including proinflammatory cytokines (*IL-6*, *MCP-1*, and *Pai-1*)mRNA levels. Moreover, guanosine nitration induced by reactive nitrogen oxides could be an important

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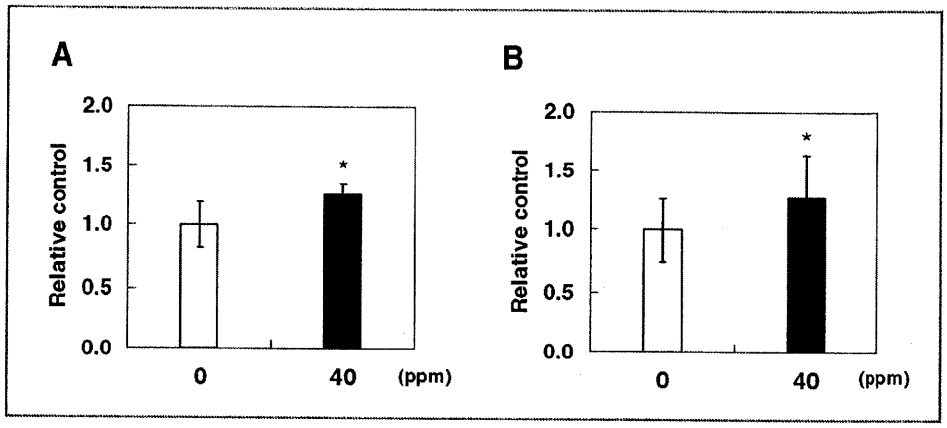

Figure 4. Changes of PPARγ-DNA binding activity by pitavastatin treatment in nonpolyp parts of the small intestinal and liver samples of Min mice. Nuclear fraction of small intestinal mucosa cells (A), and liver cells (B), were isolated and analyzed for PPARγ-DNA binding activity as described in Materials and Methods. Data are mean ± SE, $n = 9, 10$. *$P < 0.05$ vs. 0 ppm.

mediator of nitrative stress in the pathogenesis of intestinal polyp development in Min mice, and was reduced by pitavastatin treatment.

To date, lipid-lowering effects of statins have not been investigated in *Apc*-deficient mice model, which feature a hyperlipidemic state. Thus, we examined the effect of pitavastatin on serum lipid levels in Min mice and obtained a result of slight reduction. This result is similar to those shown in other rodent hyperlipidemic models. It has been shown that cholesterol synthesis enzymes were remarkably induced by feedback regulation in rodents (33), and a *de novo* cholesterol synthesis experiment by injection of ^{14}C-acetic acid showed that pitavastatin potently inhibits *de novo* cholesterol synthesis, without affecting serum lipids levels (34, 35). Taking these into consideration, HMG-CoA reductase activity might be inhibited by pitavastatin treatment in this study.

It has been reported that certain statins are able to exert antiinflammatory activities. Simvastatin inhibits proinflammatory gene expression by blocking nuclear factor kappa B (NFκB) signaling in intestinal epithelial cells, and attenuates dextran sodium sulfate-induced acute murine colitis (36). It has also been reported that pitavastatin inhibits NFκB activation and decreases IL-6 in human mammary carcinoma cells (37). Moreover, pitavastatin suppressed colitis-related colon carcinogenesis through modulation of mucosal inflammation with reduced nitrotyrosine-positivity (22). In this study, we have shown that clear downregulation of mRNA expression levels of *COX-2*, iNOS, and some adipocytokines (*IL-6*, *MCP-1*, and *Pai-1*) in the nonpolyp parts of the intestine by pitavastatin treatment, and significant reduction of *iNOS* mRNA level was observed in the polyp parts. These expression level changes of COX-2, iNOS, and adipocytokines, especially iNOS, could be associated with intestinal polyp development in Min mice. Indeed, it has been reported that iNOS inhibition, Pai-1 inhibition, COX-2 inhibition, and *IL-6* knock out suppressed intestinal polyp development in Min mice (31, 38, 39, 40). It has also been reported that 100-ppm atorvastatin treatment in Min mice slightly, but not significantly, reduced the activity and expression levels of COX-2 in the intestinal polyp (20). Expression of COX-2 was higher in polyp tissue than in nonpolyp parts, which may result in more resistance to pitavastatin's effects. INOS-dependent NO overproduction resulted in a nitration reaction, which takes place not only in tyrosine moieties of proteins but also in the nucleotide base guanosine, including RNA (41), and may account for the NO-induced cytotoxicity.

To further investigate the mechanisms of suppression of these proinflammatory genes by pitavastatin treatment, we focused on the levels of serum adipocytokines, including leptin, and activity of PPARγ, a member of the nuclear receptor superfamily. PPARγ, activated by statins (32), suppresses proinflammation gene expression (42). This study showed that pitavastatin treatment decreased serum leptin levels and increased PPARγ activity in the intestinal mucosa and the liver. It has been shown that simvastatin suppressed leptin expression in 3T3-L1 cells (43). Moreover, leptin induces iNOS and NO production (44), suggesting the interactions between leptin and NO. PPARγ activity induced by 40-ppm pitavastatin treatment might not be adequate to explain the reduction of adipocytokine levels by the same dose of pitavastatin treatment. As mentioned previously, NFκB signaling or other signaling may be additionally playing a role in the suppression of proinflammatory genes by pitavastatin treatment. In our previous study, a PPARγ ligand, pioglitazone, and an antiinflammatory drug, indomethacin, reduced intestinal polyps in Min mice (6, 45). Thus, it is assumed that PPAR activation and antiinflammatory activities of pitavastatin contribute, to some extent, to reduction of the development of intestinal polyps.

To explain the specific effect of pitavastatin on suppression of polyp development of the distal part in the small intestine, we investigated the expression levels of COX-2, IL-6, MCP-1, Pai-1, and PPARγ in the immunohistological study of distal and middle parts of the small intestine. However, the data did not show clear difference between the parts (data not shown). Further investigation is needed to clarify the differences between the distal and middle parts.

In conclusion, pitavastatin has potential benefit for suppression of intestinal polyp development. Thus,

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pitavastatin might be a candidate for chemopreventive agent for human colon cancer.

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Chemoprevention of Colorectal Carcinogenesis by Natural Anti-Inflammatory Agents

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Abstract: Accumulating epidemiological, clinical, and experimental evidence demonstrates that chronic inflammation plays a critical role in multistep oncogenesis. While long-term users of selective cycloxygenase (COX)-2 inhibitors (-coxibs) and non-steroidal anti-inflammatory drugs (NSAIDs) exert a reduced risk of development of certain types of cancer, including colorectal cancer (CRC), the adverse gastrointestinal and cardiovascular side effects associated with these drugs have limited their daily use for cancer chemoprevention. The role of various proinflammatory mediators during carcinogenesis and their promise as potential targets for chemoprevention of inflammation-associated carcinogenesis has been recently highlighted. A variety of chemopreventive phytochemicals and phytonutrients are able to alter or correct undesired cellular functions caused by abnormal pro-inflammatory signal transmissions that are mediated by NF-kappaB, etc. Modulation of cellular signaling involved in chronic inflammatory responses, induced by anti-inflammatory agents, hence provides a rational and practical strategy in molecular target-based cancer chemoprevention. This short review will focus on the role of chronic inflammation in colorectal oncogenesis and introduce dietary cancer chemopreventive agents with anti-inflammatory activity.

Keywords: Colon carcinogenesis, inflammation, chemoprevension, flavonoid, carotenoid.

INTRODUCTION

Colorectal cancer (CRC) is one of the major causes of premature death in the worldwide. Although the progress of surgical and anti-cancer therapies has been remarkable, the death from malignancies including CRC is unavoidable when malignancies in the patients are not detected during the early stage and they do not receive adequate therapies are not done.

The most practical approach to reduce the morbidity and mortality of cancer is to stop and/or delay the process of carcinogenesis. For this, the strategy of cancer chemoprevention using appropriate chemopreventive agents is quite attractive. Since long-term use of cancer chemopreventive agents is necessary, we should examine and determine safer compounds that are from synthetic and naturally occurring as chemopreventive agents.

There are numerous reports regarding chemoprevention of CRC using synthetic (drug) or natural compounds. They are based on multi-step pathogenesis of CRC. The candidate compounds are proposed mechanistic investigations along with their molecular targets and then they are evaluated their efficacy in chemopreventive clinical trials. Compounds and molecules originating from living nature are an especially attractive group of potentially chemopreventive agents against malignancies, including CRC. This is because of their relative harmlessness for humans even at high doses, convenient and often pleasant administration, simple delivery, and direct effects on the target tissues. In addition, many natural chemicals display a wide variety of anti-cancer effects.

Epithelial malignancy (cancer) is an un-controlled and hyper-proliferative disorder that involves morphological cellular transformation, dysregulation of apoptosis, invasion, angiogenesis, and metastasis [1]. Clinical and epidemiologic studies have suggested a strong association between chronic infection, inflammation, and cancer [2,3]. Several lines of evidence are consistent with the view that inflammation plays a role in the process of cancer development: chronic inflammation predisposes to cancer; immune inflammatory cells and inflammatory mediators are present within and surrounding cancer; deletion of inflammatory mediators inhibits development of experimental cancers; and long-term use of non-steroidal anti-inflammatory agents reduces the risk of certain malignancies [4]. Thus, chronic inflammation is involves in the initiation, promotion and progression phages of carcinogenesis [3]. Recent data using mouse models of human cancer have established that inflammation, which orchestrates the tumor microenvironment, is a critical component of tumor evolution [5-7]. Moreover, excessively and chronically produced pro-inflammatory mediators contribute to tumor promotion and progression [3,6]. CRC is thus one of the most serious complications of inflammatory bowel disease (IBD), such as ulcerative colitis (UC) and Crohn's disease [8]. Thus, colonic inflammation is one of the key factors of CRC development.

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This review will focus on the pre-clinical and clinical evidences of certain natural anti-inflammatory agents as chemopreventive compound against CRC. They include (-)-epigallocatechin-3-gallate (EGCG), quercetin, and astaxanthin.

EGCG

EGCG is the major polyphenol present in green tea (Fig. **1**). Green tea is reported to exert remarkable anti-inflammatory and cancer chemopreventive effects in a variety of experimental animal cancer models, cell culture systems, and epidemiological studies [9]. Its biological effects are mediated by tea polyphenols, including EGCG. EGCG also exerts remarkable anti-inflammatory effects *in vivo* and *in vitro* investigations [10,11]. EGCG inhibits several signaling pathways involved in inflammation, including the nuclear factor-kappaB (NF-κB) and activator protein (AP)-1 [12-15]. These transcription factors regulate the expression of genes encoding pro-inflammatory cytokines, chemokines, immune receptors, and adhesion molecules that play a key part in inflammatory related injury [15,16]. Activation of NF-κB is also involved in cancer development and progression. NF-κB is activated in preneoplastic cells and in cells that are recruited to and constitute the tumor microenvironment [17,18]. Thus, EGCG might combat both inflammation and cancer by modulating NF-κB activation.

EGCG inhibits proliferation and induces apoptosis of cancer cells [9,19]. EGCG also inhibits the expression of cyclooxygenase (COX)-2 by inhibiting the activation of epidermal growth factor receptor family of receptor tyrosine kinases (RTK) and their downstream signaling molecules, such as extracellular signal-regulated kinase and Akt proteins, in human CRC cells [20,21]. In the human colon cancer cells (HT-29 and SW837), EGCG is capable of inducing cell cycle arrest in G1 phase and started apoptosis characterized by activation of caspase-3 and caspase-9. Activation of epidermal growth factor receptor (HER)-2 is responsible for cell proliferation. EGCG suppresses this activation of HER-2. Concomitantly, EGCG is able to suppress the downstream effectors of extracellular signal-regulated kinases (ERK) and protein kinase B (AKT) kinases as well as AP-1, c-fos, NF-κB and cyclin D1 promoters [20].

In addition, EGCG inhibits the activation of insulin-like growth factor-1 receptor (IGF-1R), which belongs to a separate family of RTKs, in human CRC and liver cancer cells [22,23]. Expression of IGF-1R and its related molecules is one of the focuses of the experimental study investigating their involvement in a variety of CRC cell lines, those expressing high level of the IGF-1R (Caco-2, HT-29, SW837 and SW480) and those with constitutive activation of receptor (SW837 and SW 480). Treatment with EGCG at a concentration of 20 μg/ml (the IC_{50} concentration for growth inhibition) results in time-dependent alterations in the expression of IGF-R1, IGF-1 and insulin-like growth factor binding protein (IGFBP)-3 proteins and mRNAs. In addition, long time treatment (up to 96 hours) with a low dose of EGCG (1 μg/ml) inhibits the activation of IGF-1R, decreases expression of IGF-1 and increases IGFBP-3 protein expression along with changes in the expression patterns of matrix metalloprotease (MMP)-7, MMP-9, and transforming growth factor (TGF)-β2 [22].

Several studies have also demonstrated that treatment with EGCG in cancer cells results in substantial production of reactive oxygen species (ROS) that can diffuse into the cells and cause stressful or cytotoxic effects [24,25]. However, the active constituents of ROS produced by EGCG and signaling mechanisms leading to apoptosis remain unsolved so far. Furthermore, it has been argued that the oxidative stress produced by the treatment of EGCG in certain cell systems can be harmful, causing extensive DNA damage [26]. EGCG is reported to prevent hyper-proliferation HT-29 colon cancer cell line induced by the addition of low-dose of H_2O_2, and this growth inhibition was significantly blocked by pre-treatment with a ROS scavenger, *N*-acetyl-l-cysteine, indicating that EGCG exerts its cytotoxic effect through ROS-mediated mechanisms [27]. Furthermore, the findings indicates that ROS produced by EGCG plays a significant role in cellular events of AMP-activated kinase or COX-2 signaling and apoptosis.

In vivo study, oral administration with EGCG significantly decreases intestinal tumorigenesis in the APC^{Min+} mice, possibly through the attenuation of the carcinogenic events, including aberrant nuclear β-catenin and activated Akt and ERK signaling, suggesting a direct effect of EGCG on the regulation of proliferation of malignant intestinal cells [28]. On the other hand, recent evidence indicates that obesity and related metabolic abnormalities, such as hyperglycemia, hyper-lipidemia, and hyper-leptinamia, are associated with an increased incidence of CRC [29,30]. Shimizu *et al.* [31] reported that EGCG suppresses the development of azoxymethane (AOM)-induced colonic premalignant lesions in the C57BL/Ksj-*db/db* (*db/db*) mice by decreasing in the expression of IGF/IGF-1R, p-glycogen synthase kinase (GSK)-3β, β-catenin, Cox-2, and cyclin D1 proteins. They also found that treatment with EGCG causes decreases in the serum levels of IGF-1, insulin, triglyceride, cholesterol, and leptin. Taken together, EGCG is a potential chemopreventive agent against obesity-related CRC as well as sporadic CRC.

QUERCETIN

Quercetin (3, 5, 7, 3', 4' pentahydroxyflavone) (Fig. **1**) is the major representive of the flavonol subclass of flavonoids, present in human diets, making it a major contributor to the bioactive food from fruits and vegetable. The average human intake of quercetin is estimated to be 25 mg per day.

A number of studies have repeatedly proven that different flavonoid molecules have anti-inflammatory function. The anti-inflammatory activities of flavonols (quercetin, rutin and morin), and flavanones (hesperetin and hesperidin) are demonstrated using acute and chronic inflammation animal models [32]. Recently, quercetin is demonstrated tto exert its anti-inflammatory ability through down-regulating NF-kB pathway, regulating nitric oxide (NO) release, and alleviation oxdative damage in the tissues [33,34]. Quecetin also inhibits IL-1β, IL-6 and tumor necrosis factor (TNF)-α production in the lipopolysaccharide (LPS)-stimulated RAW 264.7 cells [35]. The findings suggest that quercetin affects pro-inflammatory cytokines and NO/inducible NO synthase (iNOS) by suppressing the activation of ERK and p38 mitogen-activated protein (MAP) kinase, and NF-κB/IκB signal transduction pathways, and thereby exerts its anti-

inflammatory and immunomodulatory properties. Furthermore, pre-treatment with quercetin inhibits LPS-induced delay in spontaneous apoptosis of neutrophils [36]. The results suggest that quercetin decreases the susceptibility of neutrophils to pro-inflammatory factors, which could partially explain the anti-inflammatory mechanisms of quercetin.

Anti-cancer effects of quercetin have also been reported. Quercetin exerted an anti-proliferative effect on human CRC cell lines of diverse lineages [37,38]. The apoptotic effect of quercetin is also observed in HT-29 human colon cancer cells [39]. Quercetin induces cell cycle arrest at the sub-G1 phase, and causes chromatin condensation. In the HT-29 human colon cancer cell line, quercetin exposure activates AMP-activated protein kinase (AMPK) and inhibits COX-2 expression in a dose-dependent manner. These results indicate that quercetin regulates the AMPK-COX-2 signaling pathway in the HT-29 cells and AMPK is a key regulator of cell growth and/or apoptosis in cancer cells when treated with quercetin. There are several reports focusing the effects of quercetin on Wnt/β-catenin pathway. The wnt/β-catenin pathway plays a pivotal role in cellular development and carcinogenesis process, and a central role in early events

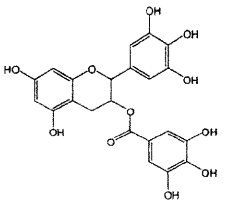
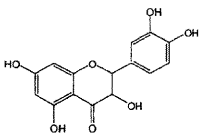
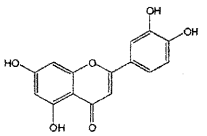
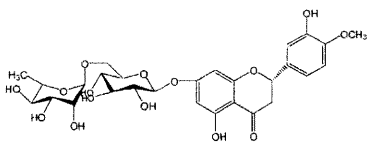
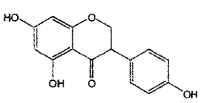
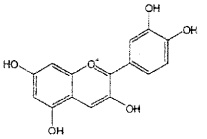
Flavonoid subgroup	Food source	Representative flavonoids	
Flavanols	Green tea, chocolate, beans, red wine, apple, cherry, apricot	EGCG → Epigallocatechin Catechin Epicatechin	
Flavonols	Onion, apple, cherry, broccoli, tomato, berries, tea, red wine, leek	Quercetin → Myricetin Kaempherol Rutin	
Flavones	Capsicum pepper, thyme, celery, parsley	Luteolin → Apigenin Chrysin	
Flavanones	Orange, grapefruit	Hesperidin → Naringenin Eriodictyol	
Isoflavonoide	Soya beans, legumes	Genistein → Daidzein	
Anthocyanidins	Rhubarb, cherry, strawberry, red wone	Cyanidin → Pelargonidin Malvidin	

Fig. (1). Major dietary sources of commonly occurring flavonoids.

during colorectal carcinogenesis. Quercetin is known to be an excellent inhibitor of β-catenin/Tcf signaling in the SW480 human colon cancer cells, and lowering β-catenin/Tcf transcriptional activity by quercetin is due to decreased expression of nuclear β-catenin and Tcf-4 proteins [40]. These results suggest that the molecular mechanism underlying the anti-tumor effects of quercetin includes suppression of β-catenin/Tcf signaling via decrease in the nuclear shift of β-catenin proteins. Quercetin dose-dependently induces apoptosis of the SW480 cells, and down-regulates transcriptional activity of β-catenin/Tcf in SW480 cells transiently transfected with the TCF-4 reporter gene. Cyclin D1 and survivin gene are also down-regulated by quercetin treatment in a dose-dependent manner at both the transcription and protein expression levels [41].

In vivo study, dietary administration with quercetin markedly suppresses the formation of AOM-induced aberrant crypt foci (ACF), putative preneoplastic lesions, in F344 rat [42,43]. Quercetin suppresses proliferation and induces apoptosis. These effects contribute to a significant reduction in the development AOM-induced ACF in Sprague-Dawly rats [44]. Quercetin suppresses mRNA levels of COX-1, COX-2 and iNOS expression in the target tissues. In the rats treated with 1,2-dimethylhydrazine (DMH), quercetin administration causes a significantly reduction in the number of large ACF in the distal colon, suggesting that quercetin can inhibit intestinal cryptal cell proliferation, although the effect diminishes when the dose level of quercetin increases. Apoptosis is not involved in the effects [45]. In addition, quercetin is able to reduce the hyper-proliferation of colonic epithelial cells and the incidence of dysplasia incidence in mice that received AOM [46].

Epidemiologic studies indicate that obesity is an important cofactor for several types of cancer, including CRC. Animal experiments also suggest that obesity enhances tumor development [47]. Dietary administration with quercetin significantly reduces the numbers of AOM-induced ACF and β-catenin accumulated crypts (BCAC), which are microadenomas in conjunction with decrease in the serum leptin level in the *db/db* mice with obesity and diabetic phenotypes [48]. Furthermore, quercetin is reported to decrease the leptin mRNA expression and secretion in differentiated 3T3-L1 mouse adipocytes.

ASTAXANTHIN

Carotenoids are a class of lipophilic compounds with a polyisoprenoid structure (Fig. **2**). Most carotenoids contain a series of conjugated double bonds, which are sensitive to oxidative modification and *cis-trans* isomerization. Based on

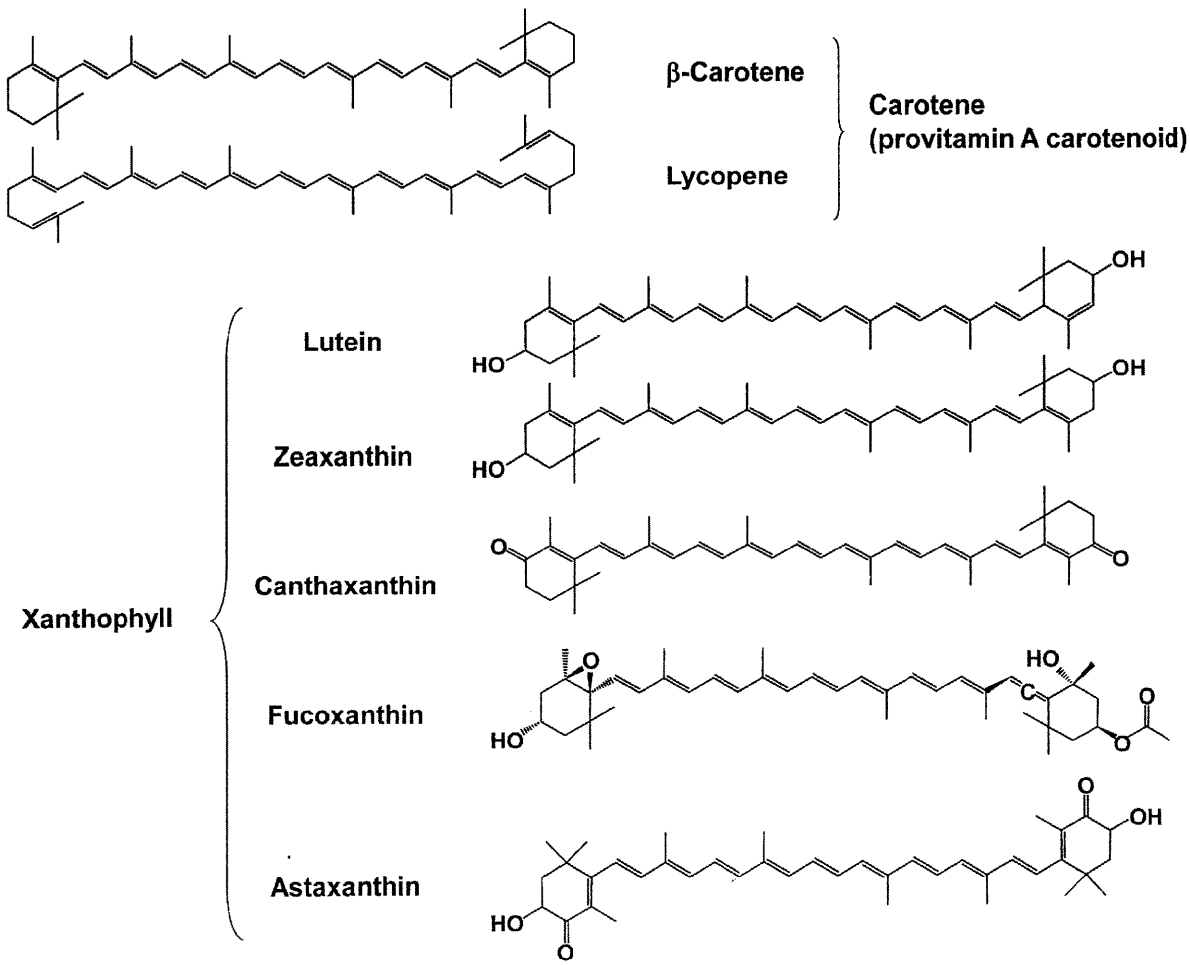


Fig. (2). Structures of carotenoids.

extensive epidemiological observation, fruits, vegetable, and marine products, which are a rich source of carotenoids, are thought to provide health benefits by decreasing the risk of various diseases, particularly certain types of cancer and eye diseases. The carotenoids that have been most studied in this regard are β-carotene, lycopene, lutein, and zeaxanthin. In part, the beneficial effects of carotenoids are thought to be due to their role as antioxidant [49]. In addition, non-provitamin A carotenoids, such as lutein, zeaxanthin, and astaxanthin, have also strong antioxidative effect when compared with provitamin A carotenoids [50].

Astaxanthin is a red-orange carotenoid pigment and is a xanthophyll carotenoid with powerful antioxidant properties that exists naturally in salmonid, algae, and crustacean aquaculture to provide the pink color characteristic of that species. Furthermore, *Haematococcus pluvialis* is the richest source of natural astaxanthin and was able to now cultivate at industrial scale. The potent antioxidant property of astaxanthin has been implicated in its various biological activities, such as anti-inflammatory therapeutic effect on cardiovascular disease, demonstrated in both experimental animals and clinical studies [51-53]. Astaxanthin inhibits the expression of formation production of pro-inflammatory mediators and cytokines, such as NO, prostaglandin E_2 (PGE_2), iNOS, COX-2, TNF-α, and interleukin (IL)-1β, in both LPS-stimulated RAW264.7 cells and primary macrophages. Astaxanthin also suppresses the serum levels of NO, PGE_2, TNF-α, and IL-1β in the LPS-treated mice, and inhibits NF-κB activation as well as iNOS promoter activity in RAW264.7 cells stimulated by LPS. Also, astaxanthin directly inhibits the intracellular accumulation of ROS in the LPS-stimulated RAW264.7 cells [51]. The similar findings are observed *in vitro* and *in vivo*.exapeiments using rats [52].

The role of carotenoids in reducing the risk of certain cancers has been postulated for several decades. In the 1980s, several observational studies appeared the literature in which a potential role of the carotenoids, particularly β-carotene, in reducing lung cancer risk was suggested. Since then, data from large observational and human intervention studies have been published on the role of the carotenoids in cancer risk reduction, mostly with conflicting results.

Increasing evidence suggests that astaxanthin is a potent anti-tumor agent in experimental animal models. The carotenoid protects mice from carcinogenesis of the urinary bladder by reducing the incidence of chemically induced bladder carcinoma [54]. Dietary astaxanthin also exerts anti-tumor activity in the post-initiation phase of carcinogen-induced colon [55] and oral [56] cancer models. Suppressive effects of dietary astaxanthin are also found in transplantable tumor cells, including methylcholanthrene-induced fibrosarcoma cells [57] and murine mammary tumor cells [58,59]. Astaxanthin-rich *Haematpcoccus pluvialis* acts as a potent inhibitor of cell growth in several colon cancer cell lines [60], suggesting that astaxanthin mediates its protective effects through a decrease in the expression of cyclin D1 and an increase in the expression of p53 and some cyclin kinase inhibitors including $p21^{WAF-1/CIP1}$ and p27, which arrest cell cycle progression. Moreover, astaxanthin promotes apoptosis through a down-regulation of the phosphorylation of AKT, alterations in apoptosis-related proteins, including Bax, Bcl-2 and Bcl-XL, and in MAP kinases signaling. Recent studies have demonstrated that dietary administration with astaxanthin suppresses the development of early phase of DMH-induced colon carcinogenesis in rat model [61,62]. Treatment with astaxanthin before DMH exposure (pre-treatment) results in fewer development of ACF when compared with the post-treatment in rats. Treatment with astaxanthin causes decreases in the activities of colonic enzymes, such as superoxide dismutase and catalase, and non-enzymic antioxidants, such as vitamin C and E, and increases in levels of lipid peroxidation markers in the rats that received DMH [61]. Astaxanthin is reported to exert anti-inflammatory and anti-cancer effects by inducing apoptosis in the DMH-induced rat colon carcinogenesis by modulating the expressions of NF-κB, COX-2, MMPs-2/9, Akt, and ERK-2 [62]. In our recent study using a inflammation-associated mouse colon carcinogenesis model, AOM/dextran sodium sulfate (DSS) model, feeding with astaxanthin significantly suppressed the occurrence of colonic ulcer and dysplastic crypts, and colonic adenocarcinoma. Further, astaxanrthin feeding suppressed the expression of TNF-α NF-κB, IL-1β, PCNA, and survivin in the colonic epithelial malignancies. This suggest possible application of astaxanthin as a chemopreventive agent in the inflammation-associated colon carcinogenesis in human.

Astaxanthin has important metabolic functions in animals and humans ranging from protection against oxidation of essential polyunsaturated fatty acids, protection against UV light effects, pro-vitamin A activity and vision, immune response, pigmentation and communication to reproductive behaviour and improved reproduction. Oxidative stress and inflammation are implicated in several different manifestations of cardiovascular disease [63-65]. Important activators of NF-κB and oxidative stress are closely associated with various risk factors of atherosclerosis. In contrast to other antioxidants, such as vitamin E and lutein, ataxanthin reduces measurements of low-density lipoprotein (LDL) oxidation in human [66]. This suggests that consumption of astaxanthin inhibits LDL oxidation and thereby contributes to the prevention of atherosclearosis. Astaxanthin is also useful for prevention and treatment of neural damage associated with age-related macular degeneration and effective at treating Alzheimer's disease, Parkinson's disease, spinal cord injuries and other central nervous system injuries.

CONCLUSION

Chemoprevention by modulation of diet and eating habits in people will no doubt become a crucial strategy in future management of cancer of the intestines but also of many other malignant or non-malignant chronic diseases. Flavonoids, including EGCG and quercetin, are potent bioactive molecules that influence the multi-step carcinogenesis. Both EGCG and quercetin have strong anti-inflammatory activities. Carotenoids, including astaxanthin, lutein and zeaxanthin, possess potent cancer chemopreventive properties in conjunction with their antioxidant and anti-inflammatory activity. Long-term intake of vegetables, cereals, fruits, marine products, containing a large proportion of these phytochemicals will stop and/or delay the process of carcinogenesis.

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ABBREVIATIONS

ACF	=	Aberrant crypt foci
AKT	=	Protein kinase B
AMPK	=	AMP-activated protein kinase
AOM	=	Azoxymethane
AP-1	=	Activator protein-1
BCAC	=	β-Catenin accumulated crypts
COX	=	Cyclooxygenase
CRC	=	Colorectal cancer
DMH	=	1,2-Dimethylhydrazine
DSS	=	Dextran sodium sulfate
EGCG	=	(-) - Epigallocatechin-3-gallate
ERK	=	Extracellular signal-regulated kinases
GSK	=	Glycogen synthase kinase
HER	=	Epidermal growth factor receptor
IBD	=	Inflammatory bowel disease
IGF-1R	=	Insulin-like growth factor -1 receptor
IGFBP	=	Insulin-like growth factor binding protein
IL	=	Interleukin
iNOS	=	Inducible NO synthase
LDL	=	Low-density lipoprotein
LPS	=	Lipopolysaccharide
MAP	=	Mitogen-activated protein
MMP	=	Matrix metalloprotease
NF-κB	=	Nuclear factor-kappaB
NO	=	Nitric oxide
PGE_2	=	Prostaglandin E_2
ROS	=	Reactive oxygen spesies
RTK	=	Receptor tyrosine kinases
TGF	=	Transforming growth factor
TNF	=	Tumor necrosis factor
UC	=	Ulcerative colitis

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Review

Biomarkers for Colorectal Cancer

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Abstract: Colorectal cancer (CRC) is the third most common epithelial malignancy in the world. Since CRC develops slowly from removable precancerous lesions, detection of the lesion at an early stage by regular health examinations can reduce the incidence and mortality of this malignancy. Colonoscopy significantly improves the detection rate of CRC, but the examination is expensive and inconvenient. Therefore, we need novel biomarkers that are non-invasive to enable us to detect CRC quite early. A number of validation studies have been conducted to evaluate genetic, epigenetic or protein markers for identification in the stool and/or serum. Currently, the fecal occult blood test is the most widely used method of screening for CRC. However, advances in genomics and proteomics will lead to the discovery of novel non-invasive biomarkers.

Keywords: biomarkers; colorectal cancer; fecal biomarkers; genomic and epigenetic biomarkers; serum biomarkers; microRNA

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1. Introduction

Various types of cancer biomarkers are listed in Table 1.

Table 1. Cancer biomarkers.

Type of biomarkers	Analysis
Genetic	Gene mutations
	Tumor suppressor gene status
DNA	Gene amplification
	Microsatellite instability
	Mitochondrial DNA
Epigenetic	DNA methylation
RNA	microRNAs
Protein	-
Metabolic	-
Immunological	T-cell and cytokine responses

Any measurable specific molecular alteration of a cancer cell either at the DNA, RNA, protein, or metabolite level can be referred to as a cancer biomarker. The expression of a distinct gene can enable its identification in a tissue in which none of the surrounding non-cancerous cells express the specific marker. It is difficult to distinguish related disease subtypes that have different clinical outcomes. There is therefore a need for more exact molecular biomarkers for use in clinical practice. Recently, the discovery of cancer biomarkers has become a major focus of cancer research and there are thousands of publications on cancer biomarkers. The ideal biomarkers for cancer have applications in determining predisposition, early detection, assessment of prognosis, and drug response. The biomarker that serves as a target for drug development would have an additional advantage. Desirable characteristics of molecular markers for cancer are postulated, but no biomarker meets these ideal characteristics. Hence, there is an urgent need for cancer biomarkers with more accurate diagnostic capability, particularly for early-stage cancer.

Colorectal cancer (CRC) is the third most common malignancy in the world. In addition, there are approximately 1,000,000 new cases of CRC and 500,000 deaths associated with CRC each year. Indeed, CRC represents one of the primary causes of cancer deaths in Europe and the United States [1]. In Asia, including Japan, CRC is the fourth leading cause of mortality by cancer, and its incidence is increasing [2]. CRC develops slowly via a progressive accumulation of genetic mutations. Therefore, the risk of recurrence and subsequent death due to CRC is closely related to the stage of the disease at the time of the first diagnosis. Recent studies have shown that shifting the detection of the disease to an earlier stage via mass screening and intervening at early stage can reduce the risk of death from CRC [3,4]. These findings thus suggest the clinical need for biomarkers for early detection of CRC.

Biomarkers are used as indicators of a biological state of tissues. Therefore, biomarkers have characteristics that enable them to be objectively measured and evaluated as indicators of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention. One of the key requirements of biomarkers for detecting CRC is that it must allow detection of the