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Research Article

Inhibition of Intestinal Polyp Formation by Pitavastatin, a HMG-CoA Reductase InhibitorNaoya Teraoka¹, Michihiro Mutoh¹, Shinji Takasu¹, Toshiya Ueno¹, Masafumi Yamamoto¹, Takashi Sugimura¹, 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, PPAR γ 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.

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 incidence 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{(3R,5S,6E)-7-[2-cyclopropyl-4-(4-fluorophenyl)-3-quinolyl]-3,5-dihydroxy-6-heptenoate} (C₅₀H₄₆CaF₂N₂O₈, 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

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

Pitavastatin (ppm)	No. of mice	Small intestine									
		Proximal			Middle			Distal			Colon
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-AGGATGCTACTGTGCAAGCTCTC, 3'primer-CAGTCAGTTGGTATCATGGTAGAG), *COX-2* (5'primer-AGAAGGAAATGGCTGCA-GAA, 3'primer-GCTCGGCTTCCAGTATTGAG), *iNOS* (5'primer-CCGGCAAACCCAAGGTCTACGTT, 3'primer-CACATCCCAGCCATGCGCACATCT), *IL-6* (5'primer-ACAACCACGGCCTTCCCTACTT, 3'primer-CACGATTC-CCAGAGAACATGTG), *MCP-1* (5'primer-CCACTCACCT-GCTGCTACTCAT, 3'primer-TGGTGATCCTCTTGTAGCT-CTCC), *Pai-1* (5'primer-GACACCCTCAGCATGTTATC, 3'primer-GACTGTACAAATCACGTTGGGA), and *GAPDH* (5'primer-TTGTCTCCTGCGACTTCA, 3'primer-CACCACC-CTGTTGCTGTA) 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).

PPAR γ Activity in Intestinal Mucosa and Liver

Nuclear extracts containing PPAR γ 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). PPAR γ activation by pitavastatin treatment was assayed using an ELISA-based transactivation TransAM PPAR γ kit (Active Motif) following the manufacturer's protocol.

Statistical Analysis

All the results are expressed as mean \pm standard errors (SE) values, with statistical analysis using Dunnett's test and PPAR γ 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 \pm 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

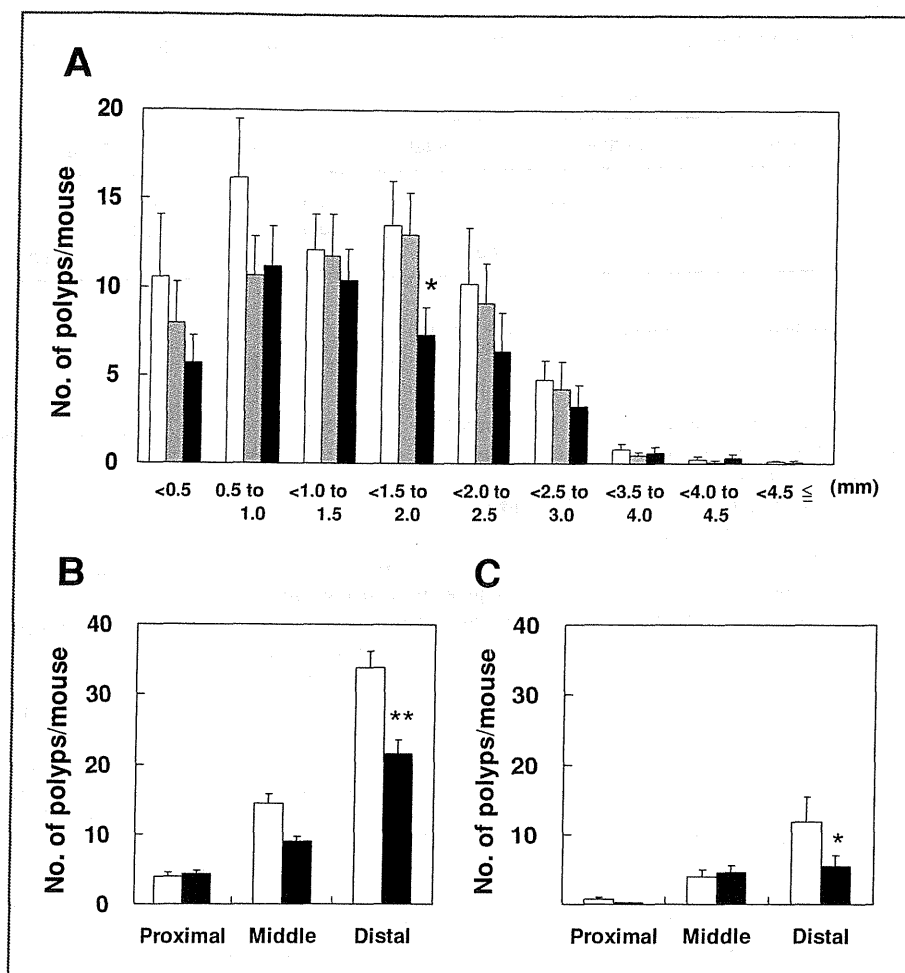


Figure 1. Effect of pitavastatin on the intestinal polyp size distribution in Min mice. Min mice were fed with a basal diet (open box) or a diet containing 20 ppm (gray box), 40-ppm (black box) pitavastatin. All of the intestinal polyps are grouped by size and its number/mouse is shown (A). Small intestinal 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. Data are mean \pm 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 \pm 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-1 β , IL-6, leptin, MCP-1, TNF α , 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 adipocy-

tokines 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).

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

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

Data are mean \pm SE ($n = 3\sim 6$)* Significantly different from the pitavastatin untreated group at $P < 0.05$.**Evaluation of nitrate stress in the intestinal polyp in Min mice treated with pitavastatin.**

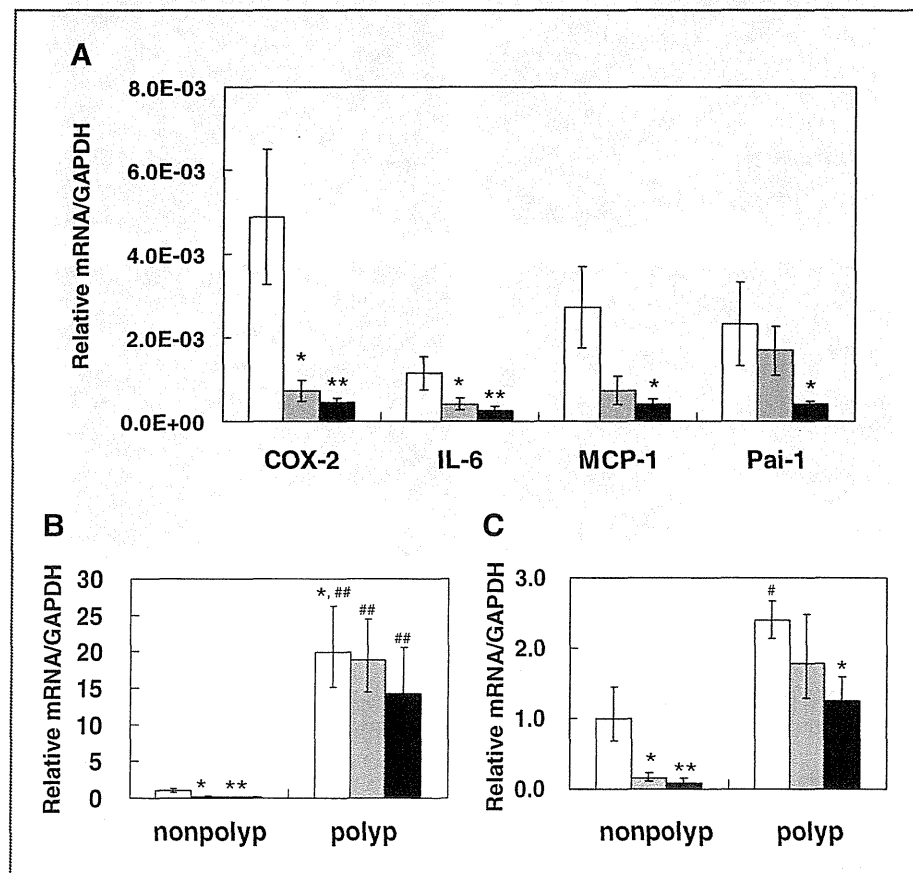
To evaluate the effects of iNOS overexpression as a nitrate stress in intestinal polyp in Min mice, localization of iNOS and the resultant nitration reaction were examined by immunohistochemistry using an anti-nitrotyrosin antibody and anti-8-nitroguanosine antibody. Nitrotyrosin was observed mainly 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 PPAR γ 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 PPAR γ were observed mainly in the cytoplasm of epithelial cells without being affected by 40 ppm-pitavastatin treatment (Supplemental Fig. 1).

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

Statins are reported to suppress some inflammatory adipocytokines through the PPAR γ activation (32). Thus, we further evaluated the effect of pitavastatin on PPAR γ 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 \pm SE, $n = 7\sim 11$ (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).



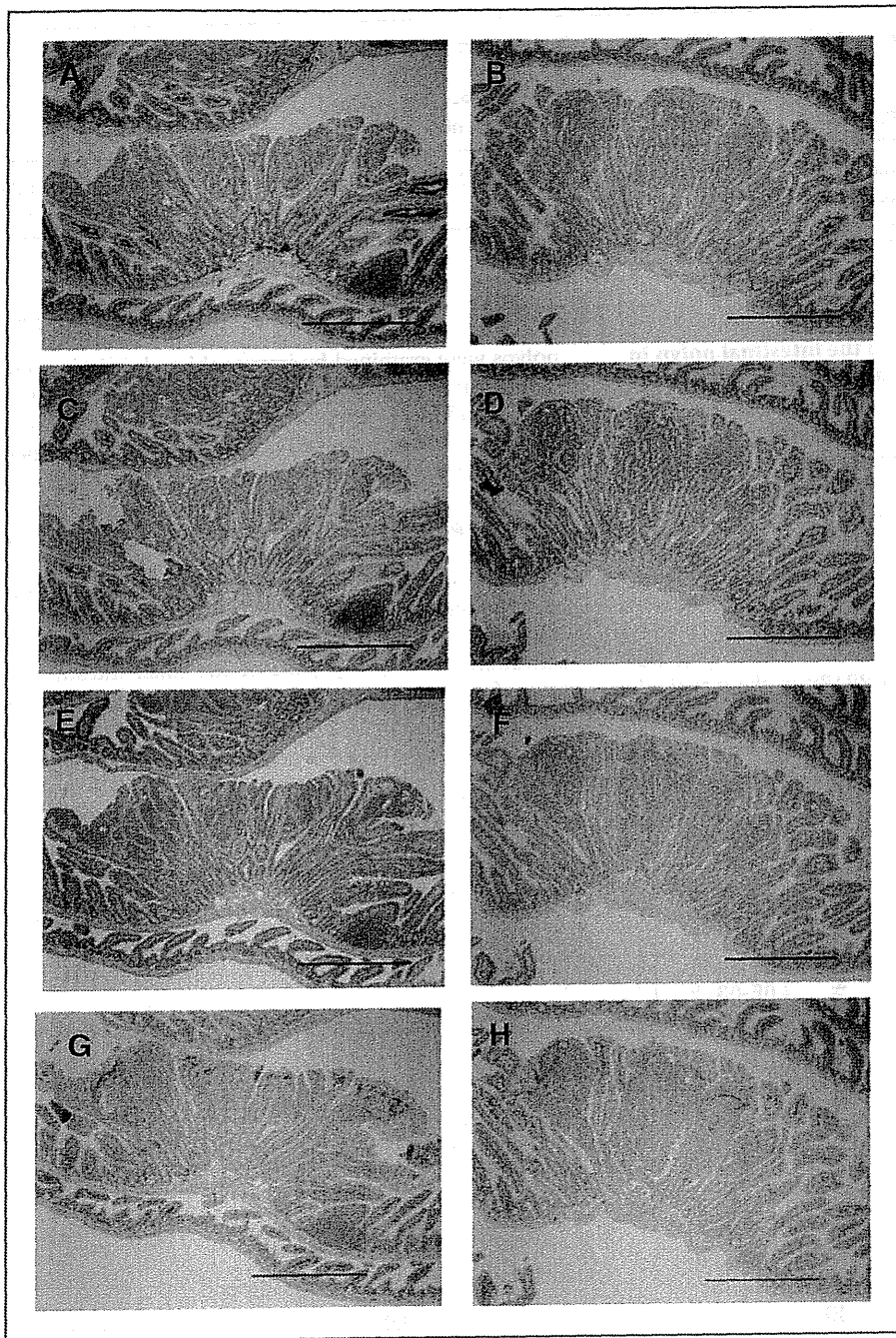


Figure 3. Modulation of mucosal oxidative/nitrosative stress by pitavastatin in Min mice. Immunohistochemical staining of iNOS (A and B), nitrotyrosine (C and D), 8-nitroguanosine (E and F) and COX-2 (G and H), protein treated with (B, D, F and H) or without 40-ppm pitavastatin (A, C, E, and G), in Min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

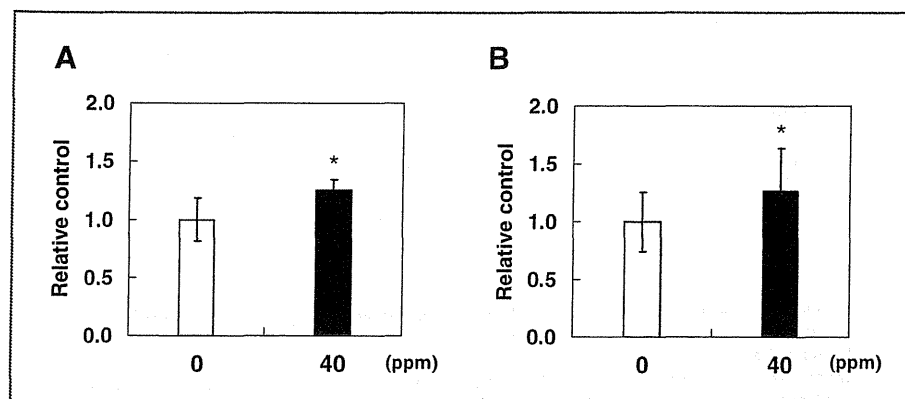
PPAR γ -DNA binding activity in the intestinal nonpolyp parts and the liver at the dose of 40 ppm (Fig. 4A and B).

Discussion

In this study, it was shown 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

Figure 4. Changes of PPAR γ -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 PPAR γ -DNA binding activity as described in Materials and Methods. Data are mean \pm SE, $n = 9$, 10. * $P < 0.05$ vs. 0 ppm.



mediator of nitrate 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 lipid 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 (NF κ B) signaling in intestinal epithelial cells, and attenuates dextran sodium sulfate-induced acute murine colitis (36). It has also been reported that pitavastatin inhibits NF κ B 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 PPAR γ , a member of the nuclear receptor superfamily. PPAR γ , activated by statins (32), suppresses proinflammation gene expression (42). This study showed that pitavastatin treatment decreased serum leptin levels and increased PPAR γ 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. PPAR γ 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, NF κ B signaling or other signaling may be additionally playing a role in the suppression of proinflammatory genes by pitavastatin treatment. In our previous study, a PPAR γ 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 PPAR γ 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,

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

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