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A lipophilic statin, pitavastatin, suppresses inflammation-associated mouse colon carcinogenesis

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3-Hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors are known to modulate carcinogenesis. In this study, we investigated whether a lipophilic HMG-CoA reductase inhibitor pitavastatin suppresses inflammation-related mouse colon carcinogenesis. Male CD-1 (ICR) mice were initiated with a single intraperitoneal injection of azoxymethane (AOM, 10 mg/kg body weight) and promoted by 2% (w/v) dextran sodium sulfate (DSS) in drinking water for 7 days. The experimental diets containing pitavastatin at 2 dose levels (1 and 10 ppm) were fed to male CD-1 (ICR) mice for 17 weeks, staring 1 week after the cessation of DSS exposure. The effects of dietary pitavastatin on colonic tumor development were assessed at Weeks 5, 10 and 20. Feeding with pitavastatin at both doses significantly inhibited the multiplicity of colonic adenocarcinoma at Week 20. Furthermore, the treatment significantly lowered the positive rates of proliferating cell nuclear antigen and increased the apoptotic index in the colonic epithelial malignancies. The treatment also reduced nitrotyrosine-positivity in the colonic mucosa. Our findings thus show that pitavastatin is effective in inhibiting colitis-related colon carcinogenesis through modulation of mucosal inflammation, oxidative/nitrosative stress, and cell proliferation.

Key words: statin; chemoprevention; inflammation; colon carcinogenesis; mouse

Statins, which are 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors, are commonly-used drugs for the treatment of hypercholesterolemia.[1,2] They are able to decrease low-density lipoprotein (LDL) cholesterol levels by inhibiting HMG-CoA reductase. Furthermore, a triglyceride (TG)-lowering effect and a high-density lipoprotein (HDL) cholesterol-raising effect were observed in patients with hyperlipidemia, who take statins.[3,4] Statins have multibiological effects other than antilipidemia. Recently, it has been highlighted that statins are linked with several beneficial effects beyond their effect on cardiovascular disease. They include reduction in the risk of dementia,[5,6] fracture[7] and cancer.[8–10] Several recent preclinical studies indicated that statins may have chemopreventive potential against cancer at various sites,[10–12] including colon.[13–16] In addition, there is growing evidence that statins exert anti-inflammatory and antioxidative actions that are independent of their serum lipid lowering effects.[17]

Association between inflammation and cancer has long been suspected.[18,19] An example is that inflamed colon is a high risk for colorectal cancer (CRC) development.[20] CRC is thus one of the most serious complications of inflammatory bowel disease (IBD), including ulcerative colitis (UC)[20] and Crohn's disease (CD).[21] For understanding the pathogenesis of IBD and IBD-related CRC, we have developed a novel colitis-related and two-stage mouse CRC model, using a colon carcinogen azoxymethane (AOM) and a colitis-inducing agent dextran sodium sulfate (DSS).[22] In this animal model, numerous large bowel adenocarcinomas occur within a short-term period, and their histology and biological alterations resemble those found in human.[22] The model can be used for investigating and determining cancer chemopreventive agents against CRC[23] as well as initiating or modulating agents for CRC.[24]

A lipophilic statin 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, Fig. 1), that has been developed in Japan is highly effective for lowering serum cholesterol and TG levels.[25] The lowering effect of pitavastatin on serum LDL-cholesterol is more potent than that of pravastatin, simbastatin and atorvastatin.[26–28] The drug possessing a high oral bioavailability is only slightly metabolized, suggesting a longer duration of action and is less potent for drug interactions.[28] Therefore, the agent is currently undergoing Phase III trials in Europe, US and Japan.[25] Since pitavastatin possesses pleiotrophic biological effects, including anti-inflammatory actions,[29,30] we in the present study investigated the potential chemopreventive ability of colitis-related colon cancer development using our mouse model[22] to find desirable cancer chemopreventers against IBD-related CRC.[31] Since numerous evidence demonstrates that a high-fat diet is associated with the risk of CRC development and serum levels of TG and cholesterol are positively associated with colon carcinogenesis,[32] we monitored serum levels of TG and cholesterol during the study.

Material and methods

Animals, chemicals and diets

Male Crj: CD-1 (ICR) mice (Charles River Japan, Tokyo, Japan) aged 5 weeks were used in this study. They were maintained at Kanazawa Medical University Animal Facility according to the Institutional Animal Care Guideline. All animals were housed in plastic cages (4 or 5 mice/cages) with free access to drinking water and pelleted basal diet, CRF-1 (Oriental Yeast, Tokyo, Japan), under controlled conditions of humidity (50 ± 10%), light (12/12 hr light/dark cycle) and temperature (23 ± 2°C). After arrived, animals were quarantined for the first 7 days, and then randomized by their body weights into experimental and control groups. A colonic carcinogen AOM was purchased from Sigma Chemical (St. Louis, MO). DSS with a molecular weight of 36000–50000 (Cat. No. 160110) was purchased from MP Biomed-

Abbreviations: AOM, azoxymethane; CD, Crohn's disease; CRC, colorectal cancer; DSS, dextran sodium sulfate; H&E, hematoxylin and eosin; HMG-CoA, 3-hydroxy-3-methylglutaryl coenzyme A; IBD, inflammatory bowel disease; iNOS, inducible bitric oxide synthase; LDL, low-density lipoprotein; NF-κB, nuclear factor-kappa B; NO, nitric oxide; PCNA, proliferating cell nuclear antigen; PSC, primary sclerosing cholangitis; ssDNA, single-stranded DNA; TG, triglycerides; UC, ulcerative colitis; UDCA, ursodeoxycholic acid.

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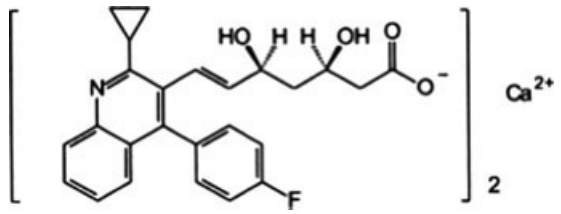

FIGURE 1 – Chemical structure of pitavastatin. (+)-Monocalcium bis{(3*R*.5*S*,6*E*)-7-[2-cyclopropyl-4-(4-fluorophenyl)-3-quinolyl]-3,5-dihydroxy-6-heptenoate}, $C_{50}H_{46}CaF_2N_2O_8$, MW 880.98.

icals, LLC (Aurora, OH). DSS for induction of colitis was dissolved in water at a concentration of 2% (w/v).

Experimental procedures

A total of 132 male ICR mice were divided into 7 experimental and control groups (Fig. 2). Mice in Groups 1–3 were given a single intraperitoneal injection of AOM (10 mg/kg body weight). Starting 1 week after the injection, animals received 2% DSS in the drinking water for 7 days. Subsequently, they were fed the diets containing 0, 1 and 10 ppm pitavastatin for 17 weeks, respectively, starting 1 week after the cessation of DSS exposure. Group 4 was fed the diet containing 10 ppm pitavastatin, and received no further treatments. Groups 5 and 6 were given AOM alone and DSS alone, respectively. Group 7 was an untreated control. Animals are sequentially sacrificed at Weeks 5, 10 and 20 by ether overdose to determine the effects of pitavastatin on colon tumorigenesis and biochemical profiles, including serum lipids measurements. Prior to sacrifice, animals were starved overnight for clinical chemistry. At sacrifice, the large bowels were flushed with saline, and excised. After measuring their length (from the ileocecal junction to the anal verge), large bowels were cut open longitudinally along the main axis, and gently washed with saline. The whole large bowel was macroscopically inspected for the presence of tumors, cut along a vertical axis and fixed in 10% buffered formalin for a least 24 hr. Histopathological examination was performed on paraffin-embedded sections after hematoxylin and eosin (H&E) staining. On H&E-stained sections, pathological lesions, such as mucosal ulceration, dysplasia and colonic tumors, were determined.

Clinical chemistry

At autopsy, whole blood anticoagulated with heparin lithium was taken from the inferior vena cava with a sterile syringe (Terumo, Tokyo, Japan) at each time point. The serum was obtained by centrifugation (3,000 rpm for 10 min), and stored at −80°C until measurement. Serum cholesterol was determined enzymatically using cholesterol esterase and cholesterol oxidase. The serum TG was assayed by enzymatic hydrolysis with lipase. These measurements were expressed as mg/dL.

Scoring of inflammation in the large bowel

Inflammation in the large bowel was scored on the H&E-stained sections. For scoring, large intestinal inflammation was graded according to the following morphological criteria described by Cooper *et al.*[33]: Grade 0, normal appearance; Grade 1, shortening and loss of the basal 1/3 of the actual crypts with mild inflammation in the mucosa; Grade 2, loss of the basal 2/3 of the crypts with moderate inflammation in the mucosa; Grade 3, loss of the entire crypts with severe inflammation in the mucosa and submucosa, but with retainment of the surface epithelium; Grade 4, presence of mucosal ulcer with severe inflammation (infiltration of neutrophils, lymphocytes, and plasma cells) in the mucosa, submucosa, muscularis propria and/or subserosa. The scoring was made on the entire colon with or without proliferative lesions and expressed as a mean average score/mouse.

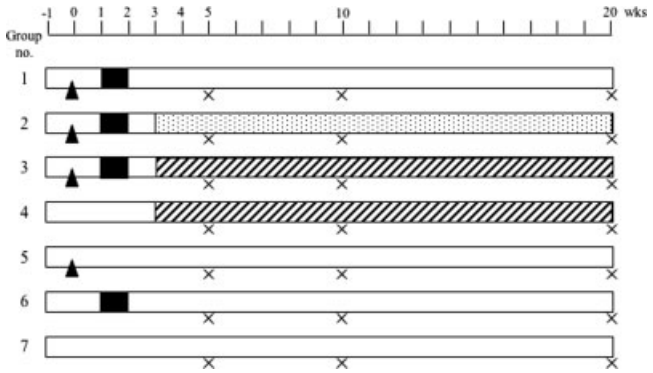

FIGURE 2 – Experimental protocol. ▲: AOM (10 mg/kg i.p.); ■: 2% DSS in drinking water; □: Basal diet and tap water; ⬚: 1 ppm pitavastatin in diet; ▨: 10 ppm pitavastatin in diet; ×: Sacrifice.

Immunohistochemistry

Immunohistochemistry for proliferating cell nuclear antigen (PCNA)-positive nuclei, apoptotic nuclei, and nitrotyrosine-positive cells was performed on 4-μm-thick paraffin-embedded sections, from the colons of mice in each group by the labeled strepravidin biotin method, using a LSAB KIT (DAKO Japan, Kyoto, Japan), with microwave accentuation. The paraffin-embedded sections were heated for 30 min at 65°C, deparaffinized in xylene and rehydrate through grade ethanols at room temperature. A 0.05 M Tris HCl buffer (pH 7.6) was used to prepare solutions and for washes between various steps. Incubations were performed in a humidified chamber. For the determination of PCNA-incorporated nuclei, the PCNA-immunohistochemistry was performed. Apoptotic index was also evaluated by immunohistochemistry for single-stranded DNA (ssDNA). Sections were treated for 40 min at room temperature, with 2% BSA, and incubated overnight at 4°C with primary antibodies, anti-PCNA mouse monoclonal antibody (PC10, 1:50 dilution, DAKO Japan), anti-ssDNA rabbit polyclonal antibody (1:300 dilution, DAKO Japan) and anti-nitrotyrosine rabbit polyclonal antibody (1:500 dilution, Update Biotechnology, Lake Placid, NY). To reduce the nonspecific staining of mouse tissue by a mouse antibody (anti-PCNA), a Mouse On Mouse IgG blocking reagent (Vector Laboratories, Burlingame, CA) was applied for 1 hr. House-radish peroxidase activity was visualized by treatment with H_2O_2 and 3,3′-diaminobenzidine for 5 min. At the last step, the sections were weakly counterstained with Mayer's hematoxylin (Merck, Tokyo, Japan). For each case, negative controls were performed on serial sections. On the control sections, incubation with the primary antibodies was omitted.

Intensity and localization of immunoreactivity against all primary antibodies used were assessed using a microscope (Olympus BX41, Olympus Optical, Tokyo, Japan). The indices for PCNA and apoptosis were determined by counting the number of positive nuclei among at least 200 cells in 5 adenocarcinomas developed at Week 20 from each of Groups 1–3, and were indicated as percentages. The nitrotyrosine-positive cells were evaluated for their intensity of immunoreactivity on a 0 or 4+ scale. The overall intensity of the staining reaction was scored with 0 indicating no immunoreactivity and no positive cells, 1+ weak immunoreactivity and <10% of positive cells, 2+ mild immunoreactivity and 10–30% of positive cells, 3+ moderate immunoreactivity and 31–60% of positive cells and 4+ strong immunoreactivity and 61–100% of positive cells. This evaluation was done on the colonic mucosa with or without tumors from all the mice of each sacrifice time point (4 mice each from all groups at Week 5; 4 mice each from all groups at Week 10; and 9 mice each of Groups 1 and 3, 10 mice each of Groups 2 and 6, and 5 mice each of Groups 4, 5 and 7 at Week 20).

TABLE I – BODY, LIVER WEIGHT AND LENGTH OF LARGE BOWEL OF MICE AT WEEK 20

Group no.	Treatment (no. of mice examined)	Body weight (g)	Liver weight (g)	Relative liver weight (g/100 g body weight)	Length of colon (cm)
1	AOM/2% DSS (9)	44.02 ± 3.44[a]	2.45 ± 0.34	5.56 ± 0.44	11.63 ± 0.41
2	AOM/2% DSS/1 ppm pitavastatin (7)	43.09 ± 6.79	2.21 ± 0.26	5.15 ± 0.28	11.66 ± 0.61
3	AOM/2% DSS/10 ppm pitavastatin (9)	38.40 ± 2.61	2.28 ± 0.32	5.94 ± 0.64[b]	11.29 ± 0.86
4	10 ppm pitavastatin (5)	42.47 ± 4.17	2.28 ± 0.23	5.39 ± 0.27	11.70 ± 1.54
5	AOM (5)	53.26 ± 6.63[c]	2.50 ± 0.40	4.68 ± 0.38[d]	12.18 ± 0.47
6	2% DSS (7)	44.16 ± 5.12	2.45 ± 0.30	5.59 ± 0.76	11.13 ± 0.28
7	None (4)	42.84 ± 4.23	2.40 ± 0.32	5.58 ± 0.23	12.78 ± 0.17

[a]Mean ± SD.–[b]Significantly different from Group 2 by Tukey–Kramer multiple comparison post test ($p < 0.05$).–[c]Significantly different from Groups 1, 6, and 7 by Tukey–Kramer multiple comparison post test ($p < 0.05$).–[d]Significantly different from Group 1 by Tukey–Kramer multiple comparison post test ($p < 0.05$).

TABLE II – INCIDENCE OF COLONIC LESIONS AT WEEKS 5, 10 AND 20

Group no.	Treatment (no. of mice examined at wk 5/wk 10/wk 20)	Mucosal ulcer			Dysplasia		
		Wk 5	Wk 10	Wk 20	Wk 5	Wk 10	Wk 20
1	AOM/2% DSS (4/4/9)	4/4, 100%	4/4, 100%	6/9, 67%	4/4, 100%	4/4, 100%	9/9, 100%
2	AOM/2% DSS/1 ppm pitavastatin (4/4/10)	4/4, 100%	3/4, 75%	3/10, 30%	4/4, 100%	4/4, 100%	8/10, 80%
3	AOM/2% DSS/10 ppm pitavastatin (4/4/9)	2/4, 50%	3/4, 75%	0/9, 0%	3/4, 75%	4/4, 100%	9/9, 100%
4	10 ppm pitavastatin (4/4/5)	0/4, 0%	0/4, 0%	0/5, 0%	0/4, 0%	0/4, 0%	0/5, 0%
5	AOM (4/4/5)	0/4, 0%	1/4, 25%	0/5, 0%	1/4, 25%	0/4, 0%	0/5, 0%
6	2% DSS (4/4/10)	4/4, 100%	4/4, 100%	0/10, 0%	1/4, 25%	0/4, 0%	0/10, 0%
7	None (4/4/5)	0/4, 0%	0/4, 0%	0/5, 0%	0/4, 0%	0/4, 0%	0/5, 0%

Data were from histopathological analysis.

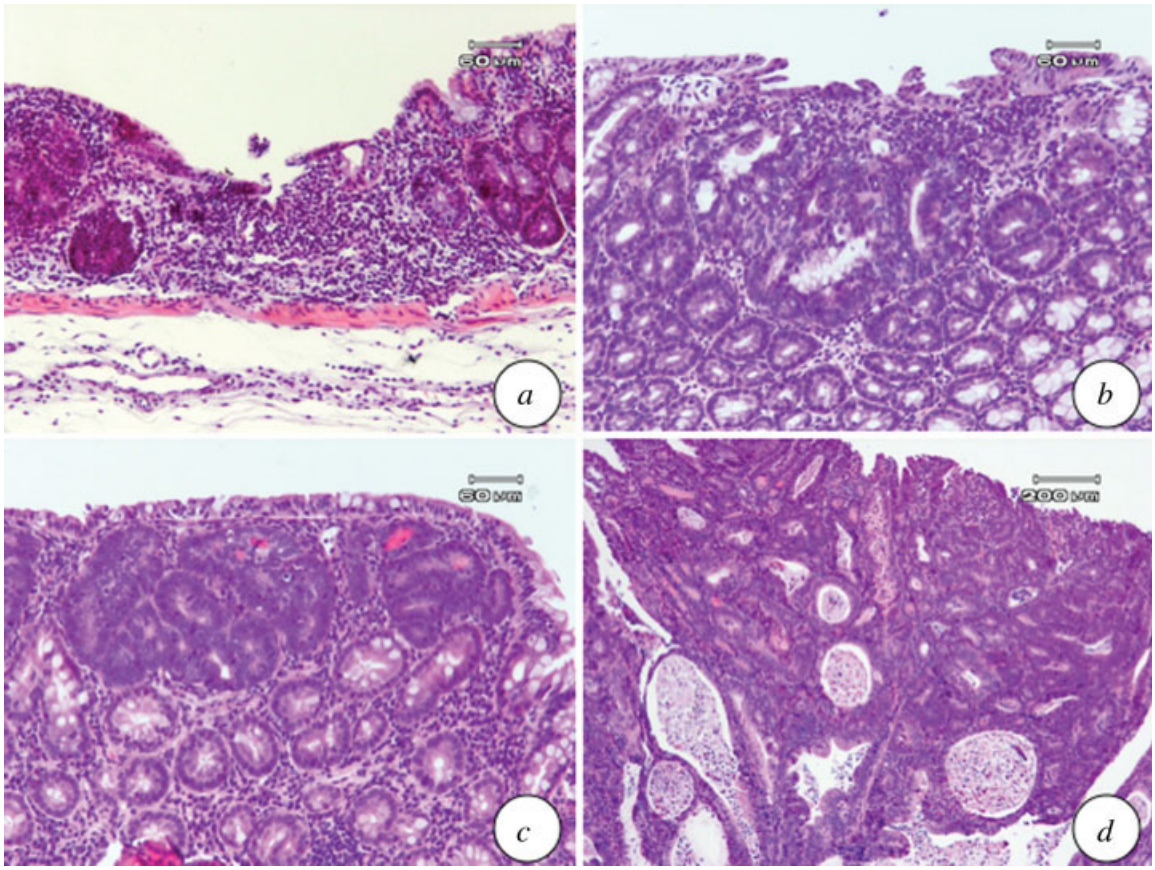

FIGURE 3 – Colonic lesions induced by AOM and 2% DSS. (*a*) A mucosal ulcer, (*b*) dysplastic crypts, (*c*) a tubular adenomas and (*d*) a tubular adenocarcinoma that developed in a mouse that received AOM and 2% DSS (Group 1). Bars inserted are (a) 60 μm, (b) 60 μm, (c) 60 μm and (d) 200 μm.

TABLE III – MULTIPLICITIES OF COLONIC LESIONS AT WEEKS 5, 10 AND 20

Group no.	Treatment (no. of mice examined at wk 5/wk 10/wk 20)	Mucosal ulcer			Dysplasia		
		Wk 5	Wk 10	Wk 20	Wk 5	Wk 10	Wk 20
1	AOM/2% DSS (4/4/9)	3.25 ± 1.71[a]	2.75 ± 0.96	0.82 ± 0.98	4.50 ± 1.30	4.00 ± 1.41	3.18 ± 1.66
2	AOM/2% DSS/1 ppm pitavastatin (4/4/10)	2.00 ± 0.82	1.25 ± 0.96	1.50 ± 2.46	3.25 ± 0.50	3.30 ± 1.30	2.20 ± 1.99
3	AOM/2% DSS/10 ppm pitavastatin (4/4/9)	1.25 ± 1.50	0.75 ± 0.50[b]	0	2.00 ± 1.83	3.75 ± 1.89	1.89 ± 0.93
4	10 ppm pitavastatin (4/4/5)	0	0	0	0	0	0
5	AOM (4/4/5)	0	0.25 ± 0.50	0	0.25 ± 0.50	0	0
6	2% DSS (4/4/10)	6.00 ± 2.16	3.75 ± 1.71	0	0.25 ± 0.50	0	0
7	None (4/4/5)	0	0	0	0	0	0

Data were from histopathological analysis.
[a]Mean ± SD.–[b]Significantly different from Group 1 by Tukey–Kramer multiple comparison post test ($p < 0.05$).

TABLE IV – INCIDENCE OF COLONIC TUMOR AT WEEKS 5, 10 AND 20

Group no.	Treatment	Adenoma			Adenocarcinoma			Total		
		Wk 5	Wk 10	Wk 20	Wk 5	Wk 10	Wk 20	Wk 5	Wk 10	Wk 20
1	AOM/2% DSS (4/4/9)	4/4, 100%	3/4, 75%	9/9, 100%	4/4, 100%	3/4, 75%	9/9, 100%	4/4, 100%	4/4, 100%	9/9, 100%
2	AOM/2% DSS/1 ppm pitavastatin (4/4/10)	4/4, 100%	4/4, 100%	9/10, 90%	3/4, 75%	3/4, 75%	9/10, 90%	4/4, 100%	4/4, 100%	9/10, 90%
3	AOM/2% DSS/10 ppm pitavastatin (4/4/9)	2/4, 50%	4/4, 100%	7/9, 78%	2/4, 50%	4/4, 100%	7/9, 78%	2/4, 50%	4/4, 100%	8/9, 89%
4	10 ppm pitavastatin (4/4/5)	0/4, 0%	0/4, 0%	0/5, 0%	0/4, 0%	0/4, 0%	0/5, 0%	0/4, 0%	0/4, 0%	0/5, 0%
5	AOM (4/4/5)	0/4, 0%	0/4, 0%	0/5, 0%	0/4, 0%	0/4, 0%	0/5, 0%	0/4, 0%	0/4, 0%	0/5, 0%
6	2% DSS (4/4/10)	0/4, 0%	0/4, 0%	0/10, 0%	0/4, 0%	0/4, 0%	0/10, 0%	0/4, 0%	0/4, 0%	0/10, 0%
7	None (4/4/5)	0/4, 0%	0/4, 0%	0/5, 0%	0/4, 0%	0/4, 0%	0/5, 0%	0/4, 0%	0/4, 0%	0/5, 0%

Data were from histopathological analysis.

Statistical analysis

The incidences among the groups were compared using χ^2 test or Fisher's extract probability test (GraphPad Instat version 3.05, GraphPad Software, San Diego, USA). Other measurements expressing mean ± SD were statistically analyzed using Tukey–Kramer multiple comparison post test (GraphPad Instat version 3.05, GraphPad Software). Differences were considered statistically significant at $p < 0.05$.

Results

General observation

The animals that received DSS in their drinking water (Groups 1, 2, 3 and 6) had bloody stool between Weeks 1–3. Also, some mice treated with AOM/DSS with or without pitavastatin (Groups 1, 2 and 3) had bloody stool, and tumors developed in their colon. However, other groups including Group 4 (the pitavastatin alone group) did not have such symptom. Body weights, liver weights, and relative liver weights in all groups at Week 20 are shown in Table I. With regard to the mean body weights, Group 5 (the AOM alone group, 53.3 ± 6.6 g) significantly increased when compared with all other groups. However, the mean liver weight did not significantly differ among the groups, whereas the mean relative liver weight (g liver weight/100 g body weight) of Group 3 (the AOM/DSS/10 ppm pitavastatin group, 5.94 ± 0.64) was significantly greater than that of Group 2 (the AOM/DSS/1 ppm pitavastatin group, 5.15 ± 0.28, $p < 0.05$), and the value of Group 5 (4.68 ± 0.38) was significantly lower than that of Groups 1 (the AOM/DSS group, 5.56 ± 0.44, $p < 0.05$) and 3 (5.94 ± 0.64, $p < 0.01$). As shown in Table I, the mean length of the colon did not significantly differ among the groups.

Incidence and multiplicity of colonic mucosal ulcer and dysplasia

Table II summarizes the incidence of colonic mucosal ulcer (Fig. 3*a*) and colonic dysplasia (Fig. 3*b*) at each time point. The incidence of mucosal ulcer gradually decreased as administration of pitavastatins doses increased at each time-point. On the other hand, the incidence of dysplasia were unaffected. As given in Table III, the multiplicity of mucosal ulcer in Groups 1, 2, 3 and 6 was the highest at Week 5, and then they gradually decreased. At Weeks 5 and 10, the value was decreased by administration of pitavastatin dose-dependently. The multiplicity of mucosal ulcer of Group 3 ($p < 0.05$) was significantly decreased when compared with Group 1. At Week 20, mucosal ulcer was not found in mice of Group 3. Dysplastic crypts were also present in mice given AOM and DSS with or without pitavastatin treatment at Week 5. Colonic dysplasia tended to decrease during the experiment, as did mucosal ulcer. The multiplicities of dysplasia in the mice of Groups 2 and 3 were lower than that of Group 1, but the differences among the groups did not reach statistical significance.

Incidence and multiplicity of large bowel neoplasms

Table IV shows the incidence of colonic tumor at each time-point. It was observed that adenoma (Fig. 3*c*) and adenocarcinoma (Fig. 3*d*) located in the middle and distal colon at each time point. However, treatment with pitavastatin unaffected the incidence of colonic tumor at Weeks 10 and 20. The multiplicities of colonic neoplasms at Weeks 5, 10 and 20 are given in Table V. Colonic adenoma and adenocarcinoma were observed even at Week 5. The multiplicities of adenoma in Groups 2 and 3 were smaller than that of Group 1 at weeks 5 and 20, but the differences were not statistically significant among the groups. As for the

TABLE V – MULTIPLICITIES OF COLONIC TUMOR AT WEEKS 5, 10 AND 20

Group no.	Treatment (no. of mice examined at wk 5/wk 10/wk 20)	Adenoma			Adenocarcinoma			Total		
		Wk 5	Wk 10	Wk 20	Wk 5	Wk 10	Wk 20	Wk 5	Wk 10	Wk 20
1	AOM/2% DSS (4/4/9)	4.00 ± 1.15[a]	2.25 ± 1.71	3.82 ± 1.78	3.00 ± 1.63	5.30 ± 1.30	5.27 ± 3.13	7.00 ± 1.15	7.50 ± 2.38	9.09 ± 3.86
2	AOM/2% DSS/1 ppm pitavastatin (4/4/10)	2.00 ± 0.82	2.25 ± 1.89	2.70 ± 1.42	2.30 ± 1.70	1.50 ± 1.29	1.50 ± 0.97[b]	4.25 ± 2.06	3.75 ± 2.75	4.20 ± 2.10[c]
3	AOM/2% DSS/10 ppm pitavastatin (4/4/9)	1.25 ± 1.50	3.75 ± 0.96	3.00 ± 1.87	1.50 ± 1.90	1.50 ± 1.00	2.00 ± 1.00[c]	2.75 ± 3.20	5.25 ± 1.89	5.00 ± 2.50[d]
4	10 ppm pitavastatin (4/4/5)	0	0	0	0	0	0	0	0	0
5	AOM (4/4/5)	0	0	0	0	0	0	0	0	0
6	2% DSS (4/4/10)	0	0	0	0	0	0	0	0	0
7	None (4/4/5)	0	0	0	0	0	0	0	0	0

All data were from histopathological analysis.
[a]Mean ± SD.–[b,c,d]Significantly different from Group 1 by Tukey–Kramer multiple comparison post test ([b]$p < 0.001$, [c]$p < 0.01$, and [d]$p < 0.05$).

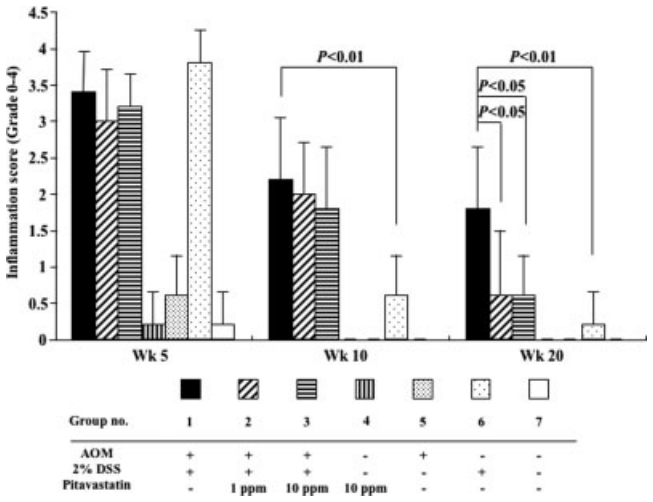

FIGURE 4 – Inflammatory scores in the large bowel of mice in all groups.

multiplicity of adenocarcinoma, the values of Groups 2 and 3 were low without statistical significance when compared to Group 1 at Weeks 5 and 10. However, the values of Groups 2 ($p < 0.001$) and 3 ($p < 0.01$) at Week 20 were significantly smaller than that of Group 1, although the inhibition was not dose-dependent.

Inflammation score in the large bowel

Figure 4 illustrates data on colonic inflammation scores at Weeks 5, 10 and 20. The inflammation scores of Groups 1, 2, 3 and 6 were the greatest at Week 5, and they gradually lowered with time. Colonic inflammation in the mice of Groups 4, 5 and 7, which were not given 2% DSS, were not observed at Weeks 10 and 20, while they had slight colitis at Week 5. At Weeks 5 and 10, the scores in Groups 2 and 3 that were given pitavastatin-containing diets were smaller than that of Group 1, but the differences did not reach the statistical significance. However, their scores were significantly lower than Group 1 at Week 20 (*vs.* Group 2, $p < 0.05$; Group 3, $p < 0.05$; and Group 6, $p < 0.01$).

Immunohistochemical scores for PCNA-, ssDNA- and nitrotyrosine-positive cells in the colonic adenocarcinomas

Scoring data on PCNA- (Fig. 5*a*) and ssDNA- (Fig. 5*b*) in adenocarcinoma cells and nitrotyrosine-positivity (Fig. 5*c*) in colonic mucosa with or without tumors are illustrated in Figure 6. As shown in Figure 6*a*, the mean PCNA-labeling indices of colonic adenocarcinomas developed in Groups 2 ($p < 0.001$) and 3 ($p < 0.001$) were significantly lower than that of Group 1. The mean apoptosis indices of Groups 2 ($p < 0.05$) and 3 ($p < 0.001$), which were measured by ssDNA immunohistochemistry, were significantly greater than that of Group 1, as shown in Figure 6*b*. Immunoreactivity of nitrotyrosine was noted in the adenocarcinoma cells (Fig. 5*c*). The reaction was also observed in the cryptal cells with or without disruption, infiltrated mononuclear inflammatory cells and endothelial cells of the small vessels in the mucosa and submucosa (Fig. 5*c*). The positive reaction was not detected in the colon of mice in Groups 4, 5 and 7. As illustrated in Figure 7, the scores of nitrotyrosine-positivity in Groups 1, 2, 3 and 6 were the greatest at Week 5, and decreased with time. At Week 5, the scores of Groups 2 ($p < 0.001$), 3 ($p < 0.001$) and 6 ($p < 0.05$) were significantly lower than that of Group 1. At Week 10, the scores of Groups 2 ($p < 0.01$), 3 ($p < 0.001$) and 6 ($p < 0.001$) were significantly lower than that of Group 1. Also, the scores of Groups 3 ($p < 0.05$) and 6 ($p < 0.01$) were significantly lower than that of Group 1 at Week 20.

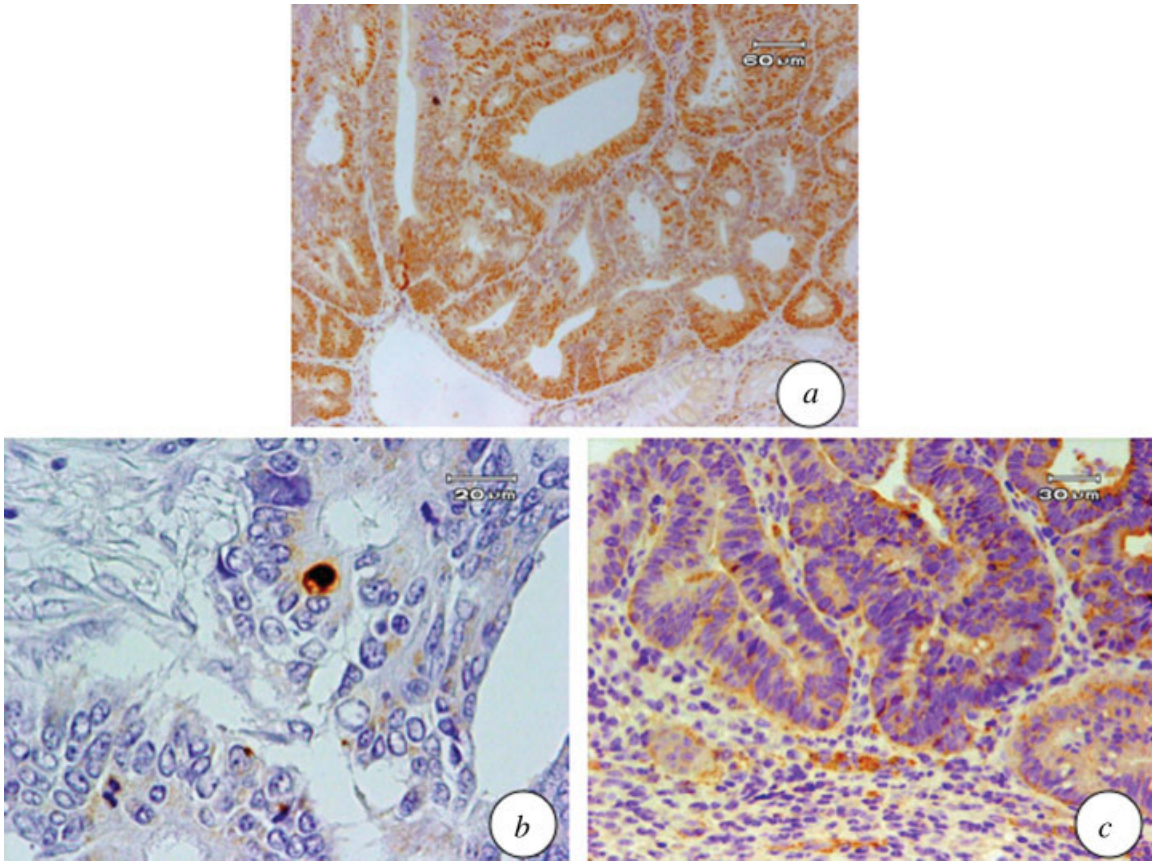

FIGURE 5 – Immunohistochemistry of (*a*) PCNA-labeled nuclei, (*b*) ssDNA-positive nuclei and (*c*) nitrotyrosine-positive cells in adenocarcinomas developed in the colon of a mouse from Group 1. Bars inserted are (a) 60 μm, (b) 20 μm and (c) 30 μm.

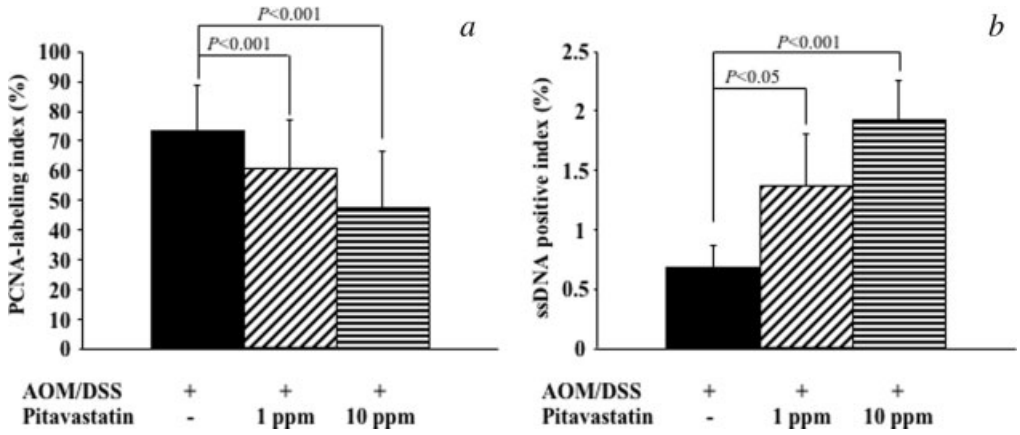

FIGURE 6 – Indices of (*a*) PCNA-labeled nuclei and (*b*) ssDNA-positive nuclei in 5 colonic adenocarcinomas each from Groups 1–3 at Week 20.

Serum levels of TG and total cholesterol

Table VI summarizes data on serum levels of TG and total cholesterol at each time point. The serum TG level of Group 1 (AOM/DSS group) was the greatest among the group at 3 time points. At Week 20, but not at Weeks 5 and 10, the values of Groups 2 ($p < 0.001$) and 3 ($p < 0.001$) were significantly lower than that of Group 1. Similarly, the serum level of total cholesterol of Group 3 ($p < 0.05$) was significantly smaller than that of Group 1, as listed in Table VII.

Discussion

In the current study, we first demonstrated cancer chemopreventive effects of pitavastatin on colitis-related mouse colon carcinogenesis induced by AOM/DSS. Suppressing effects of colitis-related colon carcinogenesis by pitavastatin may be due to reduction of cell proliferation, induction of apoptosis, inhibition of inflammation and suppression of oxidative/nitrosative stress in the colonic malignancy. In the current study, treatment with pitavastatin alone (Group 4) did not affect colonic morphology, including

induction of ulcer and neoplasms. This is important, since a recent case report described the development of UC in a patient who took simvastatin and was fatal.[34]

In the current study, we observed that dietary pitavastatin inhibits the multiplicity, but not the incidence, of colonic adenocarcinomas induced by AOM/DSS. This may be related to weak chemopreventive effects of a low dose of pitavastatin. Also, there was no dose-response of the inhibition, although data on the indices of PCNA and ssDNA may suggest that pitavastatin affects dose-dependently proliferation and apoptosis in adenocarcinoma cells. Since only 2 doses (1 and 10 ppm in diet) of pitavastatin were used for assessing chemopreventive ability of the drug against AOM/DSS-induced mouse colon carcinogenesis in this study, additional doses (>10 ppm in diet) must be investigated to determine the dose-dependent efficacy of pitavastatin in suppressing AOM/DSS-induced colon carcinogenesis. As for colonic adenoma, the incidence did not significantly alter at 3 time points (Weeks 5, 10 and 20). The multiplicity of Group 2 was increased with time, but the increase was insignificant. The findings may suggest that a high dose (10 ppm) of pitavastatin is able to inhibit progress from adenoma to adenocarcinoma.

While statins are primarily known as drugs for the treatment of hypercholesterolemia because of their potency of reduction in LDL-cholesterol level by competitively inhibiting HMG-CoA reductase that is a rate-limiting enzyme in the synthesis of mevalonate, they have pleiotropic distinct effects on process such as angiogenesis[35] and inflammation.[36,37] Thus, statins affect a number of novel molecular targets and complex signaling pathways. Certain statins (simvastatin and rosuvastatin) are able to exert anti-inflammatory action in DSS-induced acute or chronic murine colitis model.[38,39] A lipophilic statin pitavastain also possesses multiple biological function[25] and anti-inflammatory action.[29,30] Pitavastatin is recently reported to down-regulate chemokines[40] that are involved in IBD pathogenesis.[41] Also, a low dose of pitavastatin can affect PI3K-AKT pathway,[42] which plays a critical role in the balance between cell survival and apoptosis, the inflammatory response by activating chemokine receptors and promoting inflammatory cell migration and the human cancer development,[43–45] including colon cancer.[46] In the current study, the treatment with pitavastatin in diet significantly lowered colonic inflammation induced by DSS, as revealed by histopathology (number of mucosal ulcer and inflammation scores). As observed in the colonic mucosa of UC patients, where colonic mucosal damage is associated with increased production of nitric oxide (NO) through the inducible nitric oxide synthase (iNOS) pathway,[47] the numbers of cryptal, infiltrated inflammatory, endothelial and tumor cells positive for nitrotyrosine, being a good biomarker for nitrosative stress,[48] were increased in the inflamed colon induced by DSS in this study. Pitavastatin treatment significantly lowered the nitrotyrosine-positive immunohistochemical score in conjunction with reduction in the number of mucosal ulcer and inflammatory score. iNOS is reported to be over-expressed in colonic tumors of humans[49] and chemically induced colonic tumors.[50] Although there are no reports that pitavastatin affects iNOs expression in inflamed tissues and neoplasms, our findings may suggest the possible effects of pitavastatin on iNOS expression. Activated nuclear factor-kappa B (NF-κB), which is a key player in inflammatory processes in the tissues,[51,52] is observed in different cancer cell lines and primary malignant tissue samples.[53] Recently, Lee *et al.*[38] demonstrated that simvastatin inhibits proinflammatory gene expression by blocking NF-κB signaling in intestinal epithelial cells, and attenuates DSS-induced acute murine colitis. Wang *et al.*[54] have also found that pitavastatin inhibits NF-κB activation and decreases IL-6 production induced by tumor necrosis factor-α in human hepatocellular carcinoma cells. NF-κB activation also plays an important role in enhancing IL-6 and IL-8 in human colon cancer cells.[55] Although we did not examine mRNA expression of NF-κB and cytokines in this study, it is possible that paitavastatin affects the expression in the inflamed mouse colon. The anti-inflammatory and antioxidative/nitrosative potential of pitavastatin is possibly related to prenylation of certain proteins that are involved in inflammatory processes,[56,57] but not its effect on HMG-CoA enzyme, as is the case of other statins.[17,58] The findings reported by others and those described here, thus, may suggest the potential use of statins, including pitavastatin as an anti-inflammatory drug for the treatment of IBD.

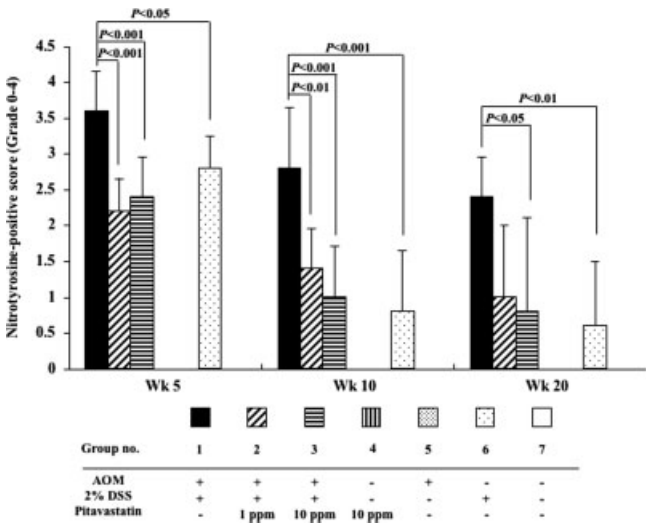

FIGURE 7 – Nitrotyrosine-positive indices in the colonic mucosa with or without tumors from all the mice of each sacrifice time point (4 mice each from all groups at Week 5; 4 mice each from all groups at Week 10; and 9 mice each of Groups 1 and 3, 10 mice each of Groups 2 and 6, and 5 mice each of Groups 4, 5 and 7 at Week 20).

Other interesting findings in the current study are that administration of pitavastatin induced apoptosis in the colonic epithelial malignancies. There are no reports describing apoptosis-inducing effects of pitavastatin on tumor cells, although certain statins possess proapoptotic properties in a variety of tumor cell lines.[59–62]

TABLE VI – SERUM TRIGLYCERIDE (MG/DL) AT WEEKS 5, 10 AND 20

Group no.	Treatment	Wk 5	Wk 10	Wk 20
1	AOM/2% DSS	134.8 ± 63.5[a] (5)	174.6 ± 96.7 (5)	159.0 ± 59.7 (9)
2	AOM/2% DSS/1 ppm pitavastatin	79.4 ± 27.5 (5)	117.4 ± 21.7 (5)	64.4 ± 16.8[b] (7)
3	AOM/2% DSS/10 ppm pitavastatin	67.2 ± 26.8 (5)	84.2 ± 28.0 (5)	61.0 ± 27.5[b] (7)
4	10 ppm pitavastatin	77.8 ± 36.8 (5)	67.2 ± 13.3 (5)	59.0 ± 23.4 (5)
5	AOM	126.0 ± 51.2 (5)	92.0 ± 35.9 (5)	94.8 ± 34.0 (5)
6	2% DSS	70.4 ± 33.4 (5)	105.2 ± 24.8 (5)	79.3 ± 37.9 (7)
7	None	105.2 ± 38.1 (5)	54.0 ± 15.3 (5)	54.5 ± 16.0 (4)

Numbers of parentheses are numbers of mice examined.
[a]Mean ± SD.–[b]Significantly different from Group 1 by Tukey–Kramer multiple comparison post test ($p < 0.001$).

TABLE VII – SERUM TOTAL CHOLESTEROL (MG/DL) AT WEEKS 5, 10 AND 20

Group no.	Treatment	Wk 5	Wk 10	Wk 20
1	AOM/2% DSS	137.2 ± 10.0[a] (5)	137.4 ± 22.7 (5)	152.8 ± 43.7 (9)
2	AOM/2% DSS/1 ppm pitavastatin	127.6 ± 14.8 (5)	119.1 ± 20.9 (5)	114.9 ± 18.2 (7)
3	AOM/2% DSS/10 ppm pitavastatin	156.4 ± 26.2 (5)	105.2 ± 10.5 (5)	105.1 ± 23.5[b] (7)
4	10 ppm pitavastatin	117.2 ± 19.1 (5)	109.0 ± 10.7 (5)	106.6 ± 7.6 (5)
5	AOM	134.8 ± 20.6 (5)	146.4 ± 29.2 (5)	161.4 ± 33.3 (5)
6	2% DSS	151.2 ± 28.2 (5)	137.6 ± 35.4 (5)	119.1 ± 20.3 (7)
7	None	151.8 ± 14.6 (5)	140.6 ± 18.4 (5)	137.5 ± 25.1 (4)

Numbers of parentheses are numbers of mice examined.
[a]Mean ± SD.–[b]Significantly different from Group 1 by Tukey–Kramer multiple comparison post test ($p < 0.05$).

Lipophilic statins are reported to induce apoptosis in malignant cells. For example, Agarwal *et al*.[59] reported that lovastatin induces apoptosis with differing sensitivity in a variety of colon cancer cell lines (SW480, HCT 116, LoVo and HT29). They also found that lovastatin treatment results in decreased expression of the antiapoptotic protein Bcl-2 and increased the expression of the proapoptotic protein Bax. There are some reports describing the comparison of apoptosis inducing ability between lipophilic and hydrophilic statins in tumor[60] and nontumor cells.[63,64] These reports suggested that lipophilic statins are more effective for inducing apoptosis when compared to hydrophilic statins. As to antiproliferative action of statins, the effect of lovastatin on prostate cancer cells is stronger than that of a hydrophilic statin, pravastatin.[65] Thus, the lipophilic property of pitavastatin may be related to the apoptosis induction and inhibition of proliferation in adenocarcinomas observed in this study.

Statins, including pitavastatin, are drugs that primarily affect LDL-cholesterol levels in plasma through the induction of the hepatic LDL receptor.[66] In this experiment, pitavastatin treatment effectively lowered serum total cholesterol level at Week 20. In addition, administration of pitavastatin significantly decreased serum TG level that was 3-fold increased by AOM/DSS exposure at Week 20. Hypertriglyceridemia is a risk for human CRC development.[67,68] Also, hyperlipidemia is a relatively frequent complication in patients with familial adenomatous polyposis patients.[69] In this context, a recent report[70] that lipoprotein lipase gene polymorphism influences lipid metabolism in UC patients and age of onset of UC is of interest.

A growing body of literature has emerged on the prevention of CRC in patients with long-standing CD and UC.[71,72] However, the data are not definitive and consist almost exclusively of retrospective case–control and cohort studies rather than the more rigorous prospective multiple randomized controlled trials.[31] Although the data on statins use are still too limited to endorse its use for the prevention of colitis-related CRC, further studies with statins need to be performed to develop an optimal strategy for the reduction of cancer risk in IBD patients. While most statins are metabolized in part by one or more hepatic cytochrome P450 enzymes (mainly CYP3A4), leading to an increased potential for drug interactions and problems with certain foods, such as grapefruit juice, pitavastatin appears to be metabolized by a substrate of CYP2C9.[25] This property may prove beneficial for the long-term use of the drug in clinic.

In conclusion, our current findings that a lipophilic statin pitavastatin was effective for inhibiting colitis-related mouse colon carcinogenesis through modulating the cell proliferation, mucosal inflammation and oxidative/nitrosative stress in the target tissue suggest possible application of pitavastatin in suppressing colon carcinogenesis in the inflamed colon of patients with IBD. Further studies on detailed mechanisms of the action involved are underway in our laboratory using microarray and proteomics techniques.

References

1. Shepherd J, Cobbe SM, Ford I, Isles CG, Lorimer AR, MacFarlane PW, McKillop JH, Packard CJ, for the West of Scotland Coronary Prevention Study Group. Prevention of coronary heart disease with pravastatin in men with hypercholesterolemia. N Engl J Med 1995;333:1301–7.
2. Collins R, Armitage J, Parish S, Sleigh P, Peto R. MRC/BHF Heart Protection Study of cholesterol-lowering with simvastatin in 5963 people with diabetes: a randomised placebo-controlled trial. Lancet 2003;361:2005–16.
3. Saito Y, Yamada N, Teramoto T, Itakura H, Hata Y, Nakaya N, Mabuchi H, Tushima M, Sasaki J, Goto Y, Ogawa N. Clinical efficacy of pitavastatin, a new 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitor, in patients with hyperlipidemia. Dose-finding study using the double-blind, three-group parallel comparison. Arzneimittelforschung 2002;52:251–5.
4. Streja L, Packard CJ, Shepherd J, Cobbe S, Ford I. Factors affecting low-density lipoprotein and high-density lipoprotein cholesterol response to pravastatin in the West of Scotland Coronary Prevention Study (WOSCOPS). Am J Cardiol 2002;90:731–6.
5. Jick H, Zornberg GL, Jick SS, Seshadri S, Drachman DA. Statins and the risk of dementia. Lancet 2000;356:1627–31.
6. Crisby M. The role of pleiotropic effects of statins in dementia. Acta Neurol Scand Suppl 2006;185:115–18.
7. Meier CR, Schlienger RG, Kraenzlin ME, Schlegel B, Jick H. HMG-CoA reductase inhibitors and the risk of fractures. JAMA 2000;283:3205–10.
8. Graaf MR, Richel DJ, van Noorden CJ, Guchelaar HJ. Effects of statins and farnesyltransferase inhibitors on the development and progression of cancer. Cancer Treat Rev 2004;30:609–41.
9. Chan KK, Oza AM, Siu LL. The statins as anticancer agents. Clin Cancer Res 2003;9:10–19.
10. Jakobisiak M, Golab J. Potential antitumor effects of statins. Int J Oncol 2003;23:1055–69.
11. Campbell MJ, Esserman LJ, Zhou Y, Shoemaker M, Lobo M, Borman E, Baehner F, Kumar AS, Adduci K, Marx C, Petricoin EF, Liotta LA, et al. Breast cancer growth prevention by statins. Cancer Res 2006;66:8707–14.
12. Demierre MF, Higgins PD, Gruber SB, Hawk E, Lippman SM. Statins and cancer prevention. Nat Rev Cancer 2005;5:930–42.
13. Narisawa T, Fukaura Y, Terada K, Umezawa A, Tanida N, Yazawa K, Ishikawa C. Prevention of 1,2-dimethylhydrazine-induced colon tumorigenesis by HMG-CoA reductase inhibitors, pravastatin and simvastatin, in ICR mice. Carcinogenesis 1994;15:2045–8.
14. Narisawa T, Morotomi M, Fukaura Y, Hasebe M, Ito M, Aizawa R. Chemoprevention by pravastatin, a 3-hydroxy-3-methylglutaryl-coenzyme A reductase inhibitor, of *N*-methyl-*N*-nitrosourea-induced colon carcinogenesis in F344 rats. Jpn J Cancer Res 1996;87:798–804.
15. Reddy BS, Wang CX, Kong AN, Khor TO, Zheng X, Steele VE, Kopelovich L, Rao CV. Prevention of azoxymethane-induced colon cancer by combination of low doses of atorvastatin, aspirin, and celecoxib in F 344 rats. Cancer Res 2006;66:4542–6.
16. Swamy MV, Patlolla JM, Steele VE, Kopelovich L, Reddy BS, Rao CV. Chemoprevention of familial adenomatous polyposis by low doses of atorvastatin and celecoxib given individually and in combination to APCMin mice. Cancer Res 2006;66:7370–7.
17. Pleiner J, Schaller G, Mittermayer F, Zorn S, Marsik C, Polterauer S, Kapiotis S, Wolzt M. Simvastatin prevents vascular hyporeactivity during inflammation. Circulation 2004;110:3349–54.
18. Balkwill F, Mantovani A. Inflammation and cancer: back to Virchow? Lancet 2001;357:539–45.
19. Coussens LM, Werb Z. Inflammation and cancer. Nature 2002;420:860–7.

20. Rutter M, Saunders B, Wilkinson K, Rumbles S, Schofield G, Kamm M, Williams C, Price A, Talbot I, Forbes A. Severity of inflammation is a risk factor for colorectal neoplasia in ulcerative colitis. Gastroenterology 2004;126:451–9.
21. Eaden JA, Abrams KR, Mayberry JF. The risk of colorectal cancer in ulcerative colitis: a meta-analysis. Gut 2001;48:526–35.
22. Tanaka T, Kohno H, Suzuki R, Yamada Y, Sugie S, Mori H. A novel inflammation-related mouse colon carcinogenesis model induced by azoxymethane and dextran sodium sulfate. Cancer Sci 2003;94:965–73.
23. Kohno H, Suzuki R, Curini M, Epifano F, Maltese F, Gonzales SP, Tanaka T. Dietary administration with prenyloxycoumarins, auraptene and collinin, inhibits colitis-related colon carcinogenesis in mice. Int J Cancer 2006;118:2936–42.
24. Hata K, Tanaka T, Kohno H, Suzuki R, Qiang SH, Kuno T, Hirose Y, Hara A, Mori H. Lack of enhancing effects of degraded λ-carrageenan on the development of β-catenin-accumulated crypts in male DBA/2J mice initiated with azoxymethane. Cancer Lett 2006;238:69–75.
25. Mukhtar RY, Reid J, Reckless JP. Pitavastatin. Int J Clin Pract 2005; 59:239–4.
26. Flores NA. Pitavastatin Nissan/Kowa Yakuhin/Novartis/Sankyo. Curr Opin Investig Drugs 2002;3:1334–41.
27. Saito Y, Yamada N, Teramoto T, Itakura H, Hata Y, Nakaya N, Mabuchi H, Tushima M, Sasaki J, Ogawa N, Goto Y. A randomized, double-blind trial comparing the efficacy and safety of pitavastatin versus pravastatin in patients with primary hypercholesterolemia. Atherosclerosis 2002;162:373–9.
28. Iglesias P, Diez JJ. New drugs for the treatment of hypercholesterolaemia. Expert Opin Investig Drugs 2003;12:1777–89.
29. Kajinami K, Takekoshi N, Saito Y. Pitavastatin: efficacy and safety profiles of a novel synthetic HMG-CoA reductase inhibitor. Cardiovasc Drug Rev 2003;21:199–215.
30. Morikawa S, Takabe W, Mataki C, Kanke T, Itoh T, Wada Y, Izumi A, Saito Y, Hamakubo T, Kodama T. The effect of statins on mRNA levels of genes related to inflammation, coagulation, and vascular constriction in HUVEC. Human umbilical vein endothelial cells. J Atheroscler Thromb 2002;9:178–3.
31. Chan EP, Lichtenstein GR. Chemoprevention: risk reduction with medical therapy of inflammatory bowel disease. Gastroenterol Clin North Am 2006;35:675–712.
32. Mutoh M, Niho N, Wakabayashi K. Concomitant suppression of hyperlipidemia and intestinal polyp formation by increasing lipoprotein lipase activity in Apc-deficient mice. Biol Chem 2006;387:381–5.
33. Cooper HS, Murthy SN, Shah RS, Sedergran DJ. Clinicopathologic study of dextran sulfate sodium experimental murine colitis. Lab Invest 1993;69:238–49.
34. Rea WE, Durrant DC, Boldy DA. Ulcerative colitis after statin treatment. Postgrad Med J 2002;78:286–7.
35. Skaletz-Rorowski A, Walsh K. Statin therapy and angiogenesis. Curr Opin Lipidol 2003;14:599–603.
36. Crisby M. Modulation of the inflammatory process by statins. Drugs Today (Barc) 2003;39:137–43.
37. Devaraj S, Rogers J, Jialal I. Statins and biomarkers of inflammation. Curr Atheroscler Rep 2007;9:33–41.
38. Lee JY, Kim JS, Kim JM, Kim N, Jung HC, Song IS. Simvastatin inhibits NF-κB signaling in intestinal epithelial cells and ameliorates acute murine colitis. Int Immunopharmacol 2007;7:241–8.
39. Naito Y, Katada K, Takagi T, Tsuboi H, Isozaki Y, Handa O, Kokura S, Yoshida N, Ichikawa H, Yoshikawa T. Rosuvastatin, a new HMG-CoA reductase inhibitor, reduces the colonic inflammatory response in dextran sulfate sodium-induced colitis in mice. Int J Mol Med 2006;17:997–1004.
40. Fujino M, Miura S, Matsuo Y, Tanigawa H, Kawamura A, Saku K. Pitavastatin-induced down-regulation of CCR2 and CCR5 in monocytes is associated with the arrest of cell-cycle in S phase. Atherosclerosis 2006;187:301–8.
41. Papadakis KA. Chemokines in inflammatory bowel disease. Curr Allergy Asthma Rep 2004;4:83–9.
42. Wang J, Tokoro T, Matsui K, Higa S, Kitajima I. Pitavastatin at low dose activates endothelial nitric oxide synthase through PI3K-AKT pathway in endothelial cells. Life Sci 2005;76:2257–68.
43. Procko E, McColl SR. Leukocytes on the move with phosphoinositide 3-kinase and its downstream effectors. Bioessays 2005;27:153–63.
44. Blume-Jensen P, Hunter T. Oncogenic kinase signalling. Nature 2001;411:355–65.
45. Luo J, Manning BD, Cantley LC. Targeting the PI3K-Akt pathway in human cancer: rationale and promise. Cancer Cell 2003;4:257–62.
46. Rychahou PG, Jackson LN, Silva SR, Rajaraman S, Evers BM. Targeted molecular therapy of the PI3K pathway: therapeutic significance of PI3K subunit targeting in colorectal carcinoma. Ann Surg 2006; 243:833–42.
47. Kankuri E, Hamalainen M, Hukkanen M, Salmenpera P, Kivilaakso E, Vapaatalo H, Moilanen E. Suppression of pro-inflammatory cytokine release by selective inhibition of inducible nitric oxide synthase in mucosal explants from patients with ulcerative colitis. Scand J Gastroenterol 2003;38:186–92.
48. Halliwell B. What nitrates tyrosine? Is nitrotyrosine specific as a biomarker of peroxynitrite formation in vivo? FEBS Lett 1997;411:157–60.
49. Ambs S, Merriam WG, Bennett WP, Felley-Bosco E, Ogunfusika MO, Oser SM, Klein S, Shields PG, Billiar TR, Harris CC. Frequent nitric oxide synthase-2 expression in human colon adenomas: implication for tumor angiogenesis and colon cancer progression. Cancer Res 1998;58:334–41.
50. Takahashi M, Mutoh M, Kawamori T, Sugimura T, Wakabayashi K. Altered expression of β-catenin, inducible nitric oxide synthase and cyclooxygenase-2 in azoxymethane-induced rat colon carcinogenesis. Carcinogenesis 2000;21:1319–27.
51. Barnes PJ, Karin M. Nuclear factor-κB: a pivotal transcription factor in chronic inflammatory diseases. N Engl J Med 1997;336:1066–71.
52. Chen C, Edelstein LC, Gelinas C. The Rel/NF-κB family directly activates expression of the apoptosis inhibitor Bcl-x(L). Mol Cell Biol 2000;20:2687–95.
53. Barkett M, Gilmore TD. Control of apoptosis by Rel/NF-κB transcription factors. Oncogene 1999;18:6910–24.
54. Wang J, Tokoro T, Higa S, Kitajima I. Anti-inflammatory effect of pitavastatin on NF-κB activated by TNF-α in hepatocellular carcinoma cells. Biol Pharm Bull 2006;29:634–9.
55. Garat C, Arend WP. Intracellular IL-1Ra type 1 inhibits IL-1-induced IL-6 and IL-8 production in Caco-2 intestinal epithelial cells through inhibition of p38 mitogen-activated protein kinase and NF-κB pathways. Cytokine 2003;23:31–40.
56. Kibayashi E, Urakaze M, Kobashi C, Kishida M, Takata M, Sato A, Yamazaki K, Kobayashi M. Inhibitory effect of pitavastatin (NK-104) on the C-reactive-protein-induced interleukin-8 production in human aortic endothelial cells. Clin Sci (Lond) 2005;108:515–21.
57. Masamura K, Oida K, Kanehara H, Suzuki J, Horie S, Ishii H, Miyamori I. Pitavastatin-induced thrombomodulin expression by endothelial cells acts via inhibition of small G proteins of the Rho family. Arterioscler Thromb Vasc Biol 2003;23:512–17.
58. Davignon J, Mabile L. Mechanisms of action of statins and their pleiotropic effects (in French). Ann Endocrinol (Paris) 2001;62 (Part 2):101–12.
59. Agarwal B, Bhendwal S, Halmos B, Moss SF, Ramey WG, Holt PR. Lovastatin augments apoptosis induced by chemotherapeutic agents in colon cancer cells. Clin Cancer Res 1999;5:2223–9.
60. Cafforio P, Dammacco F, Gernone A, Silvestris F. Statins activate the mitochondrial pathway of apoptosis in human lymphoblasts and myeloma cells. Carcinogenesis 2005;26:883–91.
61. Marcelli M, Cunningham GR, Haidacher SJ, Padayatty SJ, Sturgis L, Kagan C, Denner L. Caspase-7 is activated during lovastatin-induced apoptosis of the prostate cancer cell line LNCaP. Cancer Res 1998;58:76–83.
62. Wang IK, Lin-Shiau SY, Lin JK. Induction of apoptosis by lovastatin through activation of caspase-3 and DNase II in leukaemia HL-60 cells. Pharmacol Toxicol 2000;86:83–91.
63. Nagashima T, Okazaki H, Yudoh K, Matsuno H, Minota S. Apoptosis of rheumatoid synovial cells by statins through the blocking of protein geranylgeranylation: a potential therapeutic approach to rheumatoid arthritis. Arthritis Rheum 2006;54:579–86.
64. Tsujimoto A, Takemura G, Mikami A, Aoyama T, Ohno T, Maruyama R, Nakagawa M, Minatoguchi S, Fujiwara H. A therapeutic dose of the lipophilic statin pitavastatin enhances oxidant-induced apoptosis in human vascular smooth muscle cells. J Cardiovasc Pharmacol 2006;48:160–5.
65. Sivaprasad U, Abbas T, Dutta A. Differential efficacy of 3-hydroxy-3-methylglutaryl CoA reductase inhibitors on the cell cycle of prostate cancer cells. Mol Cancer Ther 2006;5:2310–16.
66. Maron DJ, Fazio S, Linton MF. Current perspectives on statins. Circulation 2000;101:207–13.
67. Tabuchi M, Kitayama J, Nagawa H. Hypertriglyceridemia is positively correlated with the development of colorectal tubular adenoma in Japanese men. World J Gastroenterol 2006;12:1261–4.
68. Yamada K, Araki S, Tamura M, Sakai I, Takahashi Y, Kashihara H, Kono S. Relation of serum total cholesterol, serum triglycerides and fasting plasma glucose to colorectal carcinoma in situ. Int J Epidemiol 1998;27:794–8.
69. Mutoh M, Akasu T, Takahashi M, Niho N, Yoshida T, Sugimura T, Wakabayashi K. Possible involvement of hyperlipidemia in increasing risk of colorectal tumor development in human familial adenomatous polyposis. Jpn J Clin Oncol 2006;36:166–71.
70. Kosaka T, Yoshino J, Inui K, Wakabayashi T, Okushima K, Kobayashi T, Miyoshi H, Nakamura Y, Hayashi S, Shiraishi T, Watanabe M, Yamamoto T et al. Impact of lipoprotein lipase gene polymorphisms on ulcerative colitis. World J Gastroenterol 2006;12:6325–30.
71. Itzkowitz SH. Cancer prevention in patients with inflammatory bowel disease. Gastroenterol Clin North Am 2002;31:1133–44.
72. Rubin DT, Parekh N. Colorectal cancer in inflammatory bowel disease: molecular and clinical considerations. Curr Treat Options Gastroenterol 2006;9:211–20.