Pitavastatin Fails to Lower Serum Lipid Levels or Inhibit Gastric Carcinogenesis in *Helicobacter pylori*–Infected Rodent Models

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Abstract Statins are commonly used lipid-lowering drugs that reduce the risk of cardiovascular morbidity and mortality. Although recent studies have pointed to chemopreventive effects of statins against various cancers, their efficacy for gastric cancer is unclear. Here, we examined the effects of pitavastatin, a lipophilic statin, on *Helicobacter pylori* (*H. pylori*)–associated stomach carcinogenesis and gastritis using Mongolian gerbil and mouse models. The animals were allocated to *H. pylori* + *N*-methyl-*N*-nitrosourea administration (gerbils, 52 weeks) or *H. pylori* infection alone groups (gerbils and mice, 12 weeks). After *H. pylori* infection, they were fed basal diets containing 0 to 10 ppm of pitavastatin. The incidences of *H. pylori*–associated gastric adenocarcinomas and degrees of chronic gastritis were not decreased by pitavastatin compared with those of control values. Expression of interleukin-1β and tumor necrosis factor-α mRNAs in the pyloric mucosa was markedly up-regulated in pitavastatin-treated animals. Furthermore, in the *H. pylori*–infected groups, serum total cholesterol, triglyceride, and low-density lipoprotein levels were significantly increased by pitavastatin treatment, contrary to expectation. In the short-term study, *H. pylori*–infected gerbils and mice also showed significant up-regulation of serum triglyceride levels by pitavastatin, whereas total cholesterol was markedly reduced and low-density lipoprotein exhibited a tendency for decrease in noninfected animals. These findings indicate pitavastatin to be ineffective for suppressing gastritis and chemoprevention of gastric carcinogenesis in *H. pylori*–infected gerbils. Our serologic results also suggest that the *H. pylori* infection and consequent severe chronic gastritis interfere with the cholesterol-lowering effects of pitavastatin.

Statins are widely used drugs for the treatment of hypercholesterolemia, with beneficial effects on cardiovascular disease (1, 2). They are potent inhibitors of 3-hydroxy-3-methylglutaryl CoA reductase, a rate-limiting enzyme in cholesterol biosynthesis, and decrease serum lipid levels, especially low-density lipoprotein (LDL) cholesterol and triglyceride (TG). Recent studies have shown multifunctionality of statins, including anti-inflammatory and antiangiogenic effects, independent of their lipid-lowering influence (3, 4). Epidemiologic research has also suggested chemopreventive properties for various types of cancer, including colorectal tumors (5–7). However, studies of cancer prevention by statins have produced conflicting results (8–12).

Stomach cancer is the fourth most common cancer and second leading cause of cancer-related death worldwide (13). In spite of its importance, no large epidemiologic research into inhibitory effects of statin on stomach carcinogenesis has thus far been conducted. Moreover, there has been no *in vivo* examination of gastric carcinogenesis using animal models, although several rodent studies have shown statins to be preventive agents for colorectal cancer (14, 15). *Helicobacter pylori* (*H. pylori*) is now recognized as a major risk factor for chronic active gastritis and stomach cancer development (16, 17). In addition, it has been suggested to be also associated with coronary heart disease due to the alteration of the serum lipid profile (18, 19). Therefore, there is a possibility that *H. pylori* infection might influence the pharmacologic activity of statins.

The Mongolian gerbil (*Meriones unguiculatus*) provides a useful animal model of *H. pylori*–induced chronic active gastritis, allowing investigation of morbidity-related pathologic epithelial alterations in gastric mucosa and their development into gastric neoplasia (20). The purpose of the present study

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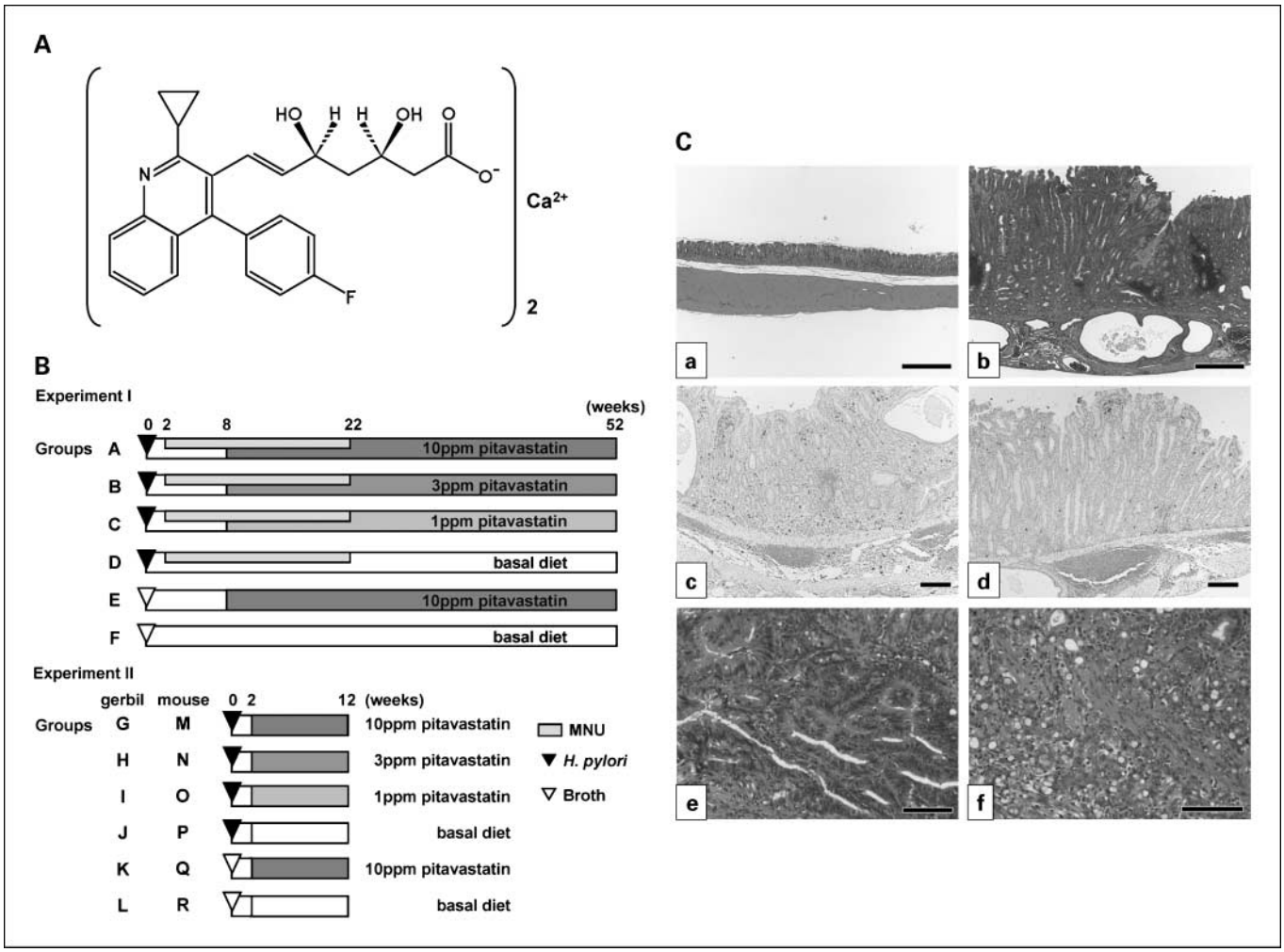

Fig. 1. *A*, chemical structure of pitavastatin. $C_{50}H_{46}CaF_2N_2O_8$ (molecular weight, 880.98). *B*, experimental design. Six-week-old male Mongolian gerbils or C57BL/6J mice were inoculated with *H. pylori* (ATCC43504 strain for gerbils or SS1 strain for mice) or Brucella broth. In the long-term experiment (experiment I), the gerbils (groups A-F) were given 10 ppm MNU in their drinking water for 20 wk and basal diet (CE-2) containing pitavastatin (0, 1, 3, or 10 ppm) from weeks 8 to 52. In the short-term experiment (experiment II), the gerbils (groups G-L) and mice (groups M-R) were given CE-2 diet containing pitavastatin from weeks 2 to 12. *C*, histopathology and immunohistochemistry in experiment I. a, normal gastric mucosa in a group F (untreated control group) gerbil at 52 wk (H&E). Magnification, ×30. *Bar*, 500 μm. b, marked infiltration of inflammatory cells and hyperplasia is evident in a group D (*H. pylori* + MNU) gerbil at 52 wk after infection (H&E). Magnification, ×30. *Bar*, 500 μm. c and d, note that the intensity of iNOS immunoreactivity in a group A (*H. pylori* + MNU + 10 ppm pitavastatin) gerbil is higher than that in a group D gerbil. Magnification, ×50 (*c* and *d*). *Bar*, 200 μm. e, well-differentiated adenocarcinoma in the glandular stomach of a group D gerbil (H&E). Magnification, ×160. *Bar*, 100 μm. f, poorly differentiated adenocarcinoma at 52 wk in a group D gerbil (H&E). Magnification, ×200. *Bar*, 100 μm.

was to evaluate the effect of pitavastatin, a recently developed lipophilic statin (21), on *H. pylori*–associated gastric carcinogenesis, and to clarify the effect of *H. pylori* infection and associated chronic gastritis on cholesterol-lowering effects of pitavastatin, using two rodent models.

Materials and Methods

Chemicals and diets

Pitavastatin (Fig. 1A) was kindly donated by Kowa Pharmaceutical Co. Ltd. CE-2 powder diet was purchased from Clea Japan, Inc. Experimental diets containing pitavastatin were prepared every 8 d in our laboratory and stored in a refrigerator. Food cups were replenished with fresh diet every second day. The gastric carcinogen *N*-methyl-*N*-nitrosourea (MNU) was purchased from Sigma Chemical, dissolved in distilled water at the concentration of 10 ppm, and administered via light-shielded bottles in drinking water *ad libitum*. MNU solutions were freshly prepared thrice per week.

Inoculation of *H. pylori*

H. pylori was prepared by the same method as described previously (22). Briefly, *H. pylori* strain ATCC43504 or Sydney strain 1 (American Type Culture Collection) was grown in Brucella broth (Becton Dickinson), containing 7% (v/v) heat-inactivated fetal bovine serum, at 37°C under microaerophilic conditions using an Anaero Pack Campylo (Mitsubishi Gas Chemical Co., Inc.) at high humidity for 24 h. After 24-h fasting, animals were inoculated via an oral catheter with 1.0 mL (gerbils) or 0.8 mL (mice) of aliquots of *H. pylori* culture containing 1.0×10^8 colony-forming units/mL of the organisms. Before inoculation, the broth cultures of *H. pylori* were checked under a phase-contrast microscope (TMS; Nikon Co.) for bacterial shape and mobility. Four hours later, the animals were again allowed free access to food.

Animals and experimental protocol

Two hundred thirty-three specific pathogen-free male Mongolian gerbils (MGS/Sea; Kyudo Co. Ltd.) and 118 specific pathogen-free male C57BL/6J mice (Clea Japan), 6 wk old, were used in this study.

All animals were housed in plastic cages on hardwood chip bedding in an air-conditioned biohazard room with a 12-h light/12-h dark cycle and allowed free access to food and water. The experimental designs were approved by the Animal Care Committee of the Aichi Cancer Center Research Institute, and the animals were cared for in accordance with institutional guidelines as well as the Guidelines for Proper Conduct of Animal Experiments (Science Council of Japan, June 1, 2006). The experimental design is illustrated in Fig. 1B. The animals were allocated to experiments I and II.

In experiment I, 175 gerbils were divided into six groups (groups A-F). Two weeks after inoculation of *H. pylori*, gerbils of groups A to D were administered MNU for 20 wk, and groups E and F were given broth and autoclaved distilled water. From weeks 8 to 52, the gerbils received CE-2 diets containing pitavastatin at concentrations of 10 (groups A and E), 3 (group B), 1 (group C), or 0 ppm (groups D and F). All surviving animals were sacrificed under deep anesthesia at 52 wk after inoculation and subjected to laparotomy with excision of the stomach.

In experiment II, a total of 58 gerbils and 118 mice were divided into six groups (groups G-L and M-R, respectively). Groups G to J and M to P were inoculated with *H. pylori* as in experiment I. From weeks 2 to 12, the animals received CE-2 diet containing pitavastatin at the concentrations of 10 (groups G, K, M, and Q), 3 (groups H and N), 1 (groups I and O), or 0 ppm (groups J, L, P, and R). Sacrifice was at 12 wk after inoculation.

Histology and immunohistochemistry

For histologic and immunohistochemical examination, the stomachs were fixed in 10% neutral-buffered formalin for 24 h, sliced along the longitudinal axis into strips of equal width, and embedded in paraffin. Serial sections (4 μm thick) were prepared and stained with H&E for morphologic observation and immunohistochemistry for cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase (iNOS). The degree of chronic active gastritis was graded according to criteria modified from the updated Sydney System (23), by scoring the infiltration of neutrophils and mononuclear cells, as well as intestinal metaplasia and heterotopic proliferative glands (HPGs), on a four-point scale (0-3; 0, normal; 1, mild; 2, moderate; 3, marked). Immunohistochemical analysis of COX-2 and iNOS was carried out with a mouse monoclonal anti–COX-2 antibody (diluted 1:200; BD Biosciences) and a rabbit polyclonal anti-iNOS antibody (1:500; Calbiochem) as previously described (24). To quantitate the degree of staining, the grading system used the following criteria: grade 0 (negative), grades 1 to 3 (increasing degrees of intermediate immunoreactivity), and grade 4 (extensive reactivity; ref. 25). The sections were analyzed on BX50 light microscope (Olympus). Images were captured using AxioVision 4.6 software (Carl Zeiss Co. Ltd.) and further processed with Adobe Photoshop software (Adobe Systems).

Serologic examination

Before removal of the stomachs, blood samples were collected from the inferior vena cava after laparotomy. Sera were separated from blood and the total cholesterol (T-Chol), TG, high-density lipoprotein (HDL), and LDL levels were measured by ELISA (SRL, Inc.). The titers of anti–*H. pylori* antibodies were also determined with an ELISA kit (Biomerica) and values were expressed using an arbitrary index (26).

Analysis of mRNA expression of inflammatory factors by real-time quantitative PCR

Total RNA was extracted from the antrum and corpus in the glandular stomach of gerbils using a QuickGene RNA Tissue Kit SII (Fujifilm). After DNase treatment, first-strand cDNAs were synthesized using a SuperScript III First-Strand Synthesis System for reverse transcription-PCR (Invitrogen) according to the manufacturer's instructions. Quantitative PCR of interleukin (IL)-1β, tumor necrosis factor (TNF)-α, and iNOS was done using a StepOne Real-Time PCR System (Applied Biosystems) with the gerbil-specific *glyceraldehyde-3-phosphate dehydrogenase* gene as an internal control. The PCR was done basically following the manufacturer's instructions using a QuantiTect SYBR Green PCR kit (Qiagen). For PCR amplification, the following primers were used: glyceraldehyde-3-phosphate dehydrogenase, 5′-AACGGCACAGTCAAGGCTGAGAACG-3′ and 5′-CAACATACTCGGCACCGGCATCG-3′; IL-1β, 5′-TTGGGCCTCAAGGGAAAGAATCTGT-3′ and 5′-GGTATTGTTTGGGGTCCACGCTCTC-3′; TNF-α, 5′-GCCCCCACCTCGTGCTCCTCAC-3′ and 5′-GGCAGGGGCTCTTGATGGCAGACAG-3′; and iNOS, 5′-GCTTGAGCGAGGAGCAGGTTGAGGA-3′ and 5′-CGCTGGCCTTTTTCACCCCATAGGA-3′. Specificity of the PCR was confirmed using a melt curve program provided with the StepOne software. To further confirm that there was no obvious primer dimer formation or amplification of any extra bands, the samples were electrophoresed in 3% agarose gels and visualized with ethidium bromide after the StepOne reaction. Relative quantification was done as previously established using the internal control without the necessity for external standards (27). The expression levels of mRNAs were expressed relative to 1.0 in the control group.

Table 1. Summary of the general data and incidences of gastric carcinomas in Mongolian gerbils in experiment I

Group (*n*)	Treatment	BW (g)	Anti-Hp IgG titer (AI)	Relative organ weights (%)		Adenocarcinoma		
				Liver	Kidney	Well	Por	Incidence (%)
A (40)	Hp + MNU + 10 ppm PS	96.6 ± 14.9	257.8 ± 225.4	5.09 ± 0.81*	0.85 ± 0.06	15	3	18/40 (45.0)
B (39)	Hp + MNU + 3 ppm PS	97.3 ± 9.4	415.4 ± 452.3	5.08 ± 0.77*	0.84 ± 0.07	22	0	22/39 (56.4)
C (40)	Hp + MNU + 1 ppm PS	89.1 ± 16.6	233.6 ± 218.3	4.76 ± 1.00*	0.85 ± 0.07	19	1	20/40 (50.0)
D (41)	Hp + MNU	91.5 ± 14.9	296.5 ± 197.7	4.73 ± 1.03*	0.85 ± 0.11	15	2	17/41 (41.5)
E (10)	Broth + 10 ppm PS	94.7 ± 9.7	3.8 ± 3.2	3.84 ± 0.11	0.71 ± 0.06*	0	0	0/10 (0)
F (5)	Untreated control	89.7 ± 12.9	ND	3.65 ± 0.31	0.83 ± 0.07	0	0	0/5 (0)

NOTE: Values for results are expressed as mean ± SD.

Abbreviations: BW, body weight; Hp, *Helicobacter pylori*; AI, arbitrary index; Well, well-differentiated adenocarcinoma; Por, poorly differentiated adenocarcinoma; PS, pitavastatin; ND, not done.

*$P < 0.01$ versus group F.

Table 2. Body weights and histopathologic evaluation of gastritis in Mongolian gerbils and mice in experiment II

Animal	Group (*n*)	Treatment	BW (g)	Scores of gastritis			
				Infiltration of neutrophils		Infiltration of mononuclear cells	
				Antrum	Corpus	Antrum	Corpus
Gerbils	G (10)	Hp + 10 ppm PS	76.4 ± 4.5	2.3 ± 0.5	1.6 ± 0.9	2.4 ± 0.4	1.9 ± 0.5
	H (10)	Hp + 3 ppm PS	74.2 ± 10.6	2.2 ± 0.4	1.9 ± 1.1	2.5 ± 0.5	2.0 ± 0.8
	I (10)	Hp + 1 ppm PS	74.3 ± 7.7	2.4 ± 0.4	1.6 ± 0.8	2.5 ± 0.4	2.0 ± 0.8
	J (10)	Hp	70.8 ± 8.2	2.3 ± 0.4	1.2 ± 0.9	2.7 ± 0.2	1.7 ± 0.7
	K (8)	Broth + 10 ppm PS	73.8 ± 4.2	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.2	0.1 ± 0.2
	L (10)	Broth	75.2 ± 6.4	0.1 ± 0.2	0.0 ± 0.0	0.2 ± 0.2	0.0 ± 0.0
Mice	M (19)	Hp + 10 ppm PS	30.2 ± 2.2	1.0 ± 0.3	1.3 ± 0.6	0.9 ± 0.2	1.2 ± 0.4
	N (19)	Hp + 3 ppm PS	30.5 ± 2.0	1.1 ± 0.3	1.4 ± 0.4	1.0 ± 0.2	1.3 ± 0.4
	O (19)	Hp + 1 ppm PS	30.4 ± 1.8	0.8 ± 0.3	1.0 ± 0.5	0.8 ± 0.3	1.1 ± 0.5
	P (20)	Hp	28.9 ± 2.8	1.0 ± 0.5	1.2 ± 0.6	0.8 ± 0.3	1.0 ± 0.5
	Q (21)	Broth + 10 ppm PS	29.2 ± 1.8	0.0 ± 0.1	0.2 ± 0.3	0.0 ± 0.0	0.0 ± 0.1
	R (20)	Broth	28.4 ± 1.0	0.1 ± 0.2	0.4 ± 0.5	0.0 ± 0.0	0.0 ± 0.0

NOTE: Values for results are expressed as mean ± SD.

Statistical analysis

The Fisher's exact test was used to assess incidences of gastric adenocarcinomas. Quantitative values were expressed as mean ± SD or SE, and differences between means were statistically analyzed by the ANOVA or Kruskal-Wallis followed by the multiple comparison test. P values of <0.05 were considered to be statistically significant.

Results

Average body weights, titer of anti–*H. pylori* antibodies, and relative organ weights

Data for average body weights, titer of anti–*H. pylori* antibodies, and relative organ weights in the long-term experiment (experiment I) and average body weights in the short-term experiment (experiment II) are shown in Tables 1 and 2, respectively. There was no significant variation of body weights in experiments I and II. In experiment I, all *H. pylori*–infected groups (groups A-D) showed significantly higher values for anti–*H. pylori* antibody titers than the noninfected group (group E). The relative liver weights in groups A to D were markedly higher than in nontreated control group (group F). The relative kidney weights in group E were statistically decreased compared with group F. In internal organs other than the stomach, including the liver, kidney, spleen, heart, and lung of all groups (groups A-F), no macroscopic or microscopic lesions were observed.

Status of gastritis

All gastric mucosal specimens from uninfected gerbils and mice had normal histomorphology (Fig. 1C, *a*). Histologic findings for chronic gastritis in experiments I (Table 3) and II (Table 2) are summarized. The long-term *H. pylori*–infected gerbils showed severe gastritis with intestinal metaplasia and HPGs (Fig. 1C, *b*). There were no significant differences in inflammatory scores, including infiltration of neutrophils or mononuclear cells, intestinal metaplasia, and HPGs, both in the antrum and corpus, among all infected groups in experiment I. In experiment II, the infiltration of neutrophils and mononuclear cells of short-term *H. pylori*–infected gerbils was greater than that in mice. There were no statistically significant differences in the degree of inflammation among *H. pylori*–infected animals, as in experiment I. In experiment I, the score for iNOS immunohistochemistry in group A (*H. pylori* + MNU + 10 ppm pitavastatin) was markedly higher than that in group D (*H. pylori* + MNU) both in the antrum and corpus (Fig. 1C, *c* and *d*).

Incidences of glandular stomach adenocarcinomas

In experiment I, both well-differentiated and poorly differentiated adenocarcinomas were found in *H. pylori*–infected and MNU-treated groups (groups A-D) at 52 weeks after infection (Fig. 1C, *e* and *f*). However, there were no significant differences in the incidences among groups A to D [group A, 45.0% (18 of 40); group B, 56.4% (22 of 39); group C, 50.0% (20 of 40); group D, 41.5% (17 of 41); Table 1]. In noninfected control groups (groups E and F) and short-term infected groups (experiment II), no tumors developed in the stomach.

Serologic results

On serologic examination, pitavastatin treatment significantly increased serum T-Chol, TG, and LDL levels in *H. pylori*–infected gerbils in a dose-dependent manner (groups A-D) in the long-term experiment (experiment I; Fig. 2). Similarly, in noninfected animals (groups E and F), serum LDL levels were increased by 10 ppm pitavastatin treatment. On the other hand, HDL levels were markedly reduced in both group D (*H. pylori* + MNU) and group E (10 ppm pitavastatin) compared with group F (untreated control).

In experiment II, serum TG and HDL levels showed significant up-regulation by pitavastatin treatment in *H. pylori*–infected gerbils (groups G-J). In contrast, T-Chol and HDL

levels were markedly decreased by 10 ppm pitavastatin in noninfected gerbils (groups K and L). In *H. pylori*–infected mice (groups M-P), serum TG levels were significantly increased by pitavastatin, as in the gerbil case. In noninfected mice (groups Q and R), the serum LDL level showed a tendency for decrease with 10 ppm pitavastatin treatment, although this was not statistically significant ($P = 0.063$).

Administration of pitavastatin and mRNA expression of IL-1β, TNF-α, and iNOS

Gastric IL-1β, TNF-α, and iNOS mRNA were found to be expressed at very low levels in the noninfected control gerbils. However, in the *H. pylori*–infected animals, the levels of these inflammatory factors were markedly elevated in the antrum and corpus (Fig. 3A and B). In the long-term experiment (experiment I), relative expression of IL-1β and TNF-α in the antrum of pitavastatin-treated groups (groups A-C) was significantly up-regulated compared with the untreated group (group D; Fig. 3A).

Discussion

The present study did not provide any evidence of statin protection against gastritis or gastric carcinogenesis in two animal models. The relationship between statin use and cancer incidence has been evaluated in numerous epidemiologic studies. Some reports supported a role in cancer chemoprevention (6, 28), and others refuted the hypothesis (29). Recently, Lubet et al. (30) suggested that atorvastatin and lovastatin fail to inhibit mammary carcinogenesis of rodents. In case of gastrointestinal cancer, clinical studies of statins for preventive effects have also produced conflicting results (31). Statins are the most widely used drugs both in the amounts prescribed and the proceeds of sales (32), so we need to clarify whether they are truly effective for cancer chemoprevention. Here, we showed that *H. pylori*–associated gastric carcinogenesis in Mongolian gerbils is not prevented by oral administration of pitavastatin at 10 ppm in the diet. We selected the pitavastatin as a strong candidate to alleviate gastritis and gastric carcinogenesis as well as to lower the serum lipid levels because pitavastatin has more potent lipid-lowering effects than pravastatin, simvastatin, and atorvastatin (21, 33). Furthermore, pitavastatin has been known to be minimally affected by cytochrome *P*450 3A4 inhibitors unlike simvastatin, lovastatin, and atorvastatin (34). Because cytochrome *P*450 metabolisms in gerbils have not been fully clarified yet, we selected pitavastatin to avoid species difference in the drug metabolism in this experiment. Among mice strains, C57BL/6 mice showed excellent colonization of *H. pylori* in the antrum, whereas BALB/c and CBA mice showed only mild gastritis (35); thus, the former was chosen here. Pitavastatin has been shown to prevent digestive system carcinogenesis, such as colorectal and lingual cancer, in mouse models (14, 36); however, the degree of gastritis in our study was not attenuated by pitavastatin in *H. pylori*–infected gerbils and mice. The major determining factor of stomach carcinogenesis is the severity of *H. pylori*–induced gastritis (37). Therefore, the ineffectiveness of pitavastatin regarding prevention of gastric cancer development in the gerbil model might be due to the lack of suppressive effects on *H. pylori*–induced gastritis.

In the long-term experiment, interestingly, our data suggested that the serum lipid levels (T-Chol, TG, and LDL) of *H. pylori*–infected and MNU-treated gerbils were significantly increased by pitavastatin in a dose-dependent manner. In noninfected gerbils, similarly, values for LDL cholesterol level were markedly elevated by the statin, although the HDL cholesterol level was significantly decreased. It was expected that pitavastatin would lower the LDL without changing HDL levels. Therefore, we did the additional short-term experiment (experiment II) to clarify whether the effect of pitavastatin on serum lipid profile was influenced by *H. pylori* infection, MNU treatment, or biological trait of gerbils. Again, inflammatory

Table 3. Histopathologic evaluation of gastritis in Mongolian gerbils in experiment I

Group (*n*)	Treatment	Infiltration of neutrophils		Infiltration of mononuclear cells		Intestinal metaplasia		HPGs		COX-2 immunostaining		iNOS immunostaining	
		Antrum	Corpus	Antrum	Corpus	Antrum	Corpus	Antrum	Corpus	Antrum	Corpus	Antrum	Corpus
A (40)	Hp + MNU + 10 ppm PS	2.0±0.7	2.3±0.8	2.8±0.4	2.6±0.5	1.2±0.8	1.6±0.8	2.3±0.7	2.2±0.9	1.3±0.7	1.6±0.7	2.0±0.6*	1.8±0.6*
B (39)	Hp + MNU + 3 ppm PS	2.1±0.6	2.4±0.8	2.7±0.4	2.5±0.5	1.3±0.8	1.5±0.6	2.3±0.7	2.3±0.7	1.5±0.8	1.7±0.7	1.7±0.5	1.7±0.6
C (40)	Hp + MNU + 1 ppm PS	2.0±0.7	2.3±0.7	2.7±0.4	2.5±0.6	1.3±0.9	1.6±0.8	2.3±0.6	2.0±0.9	1.5±0.7	1.6±0.7	1.6±0.6	1.4±0.5
D (41)	Hp + MNU	1.8±0.6	2.1±0.8	2.6±0.5	2.4±0.5	1.0±0.7	1.2±0.7	2.1±0.7	1.9±0.8	1.6±0.8	1.5±0.6	1.5±0.6	1.4±0.5
E (10)	Broth + 10 ppm PS	0.0±0.0	0.0±0.0	0.3±0.4	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.1±0.3	0.0±0.0
F (5)	Untreated control	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0

NOTE: Values for results are expressed as mean ± SD.
*$P < 0.01$ versus group D.

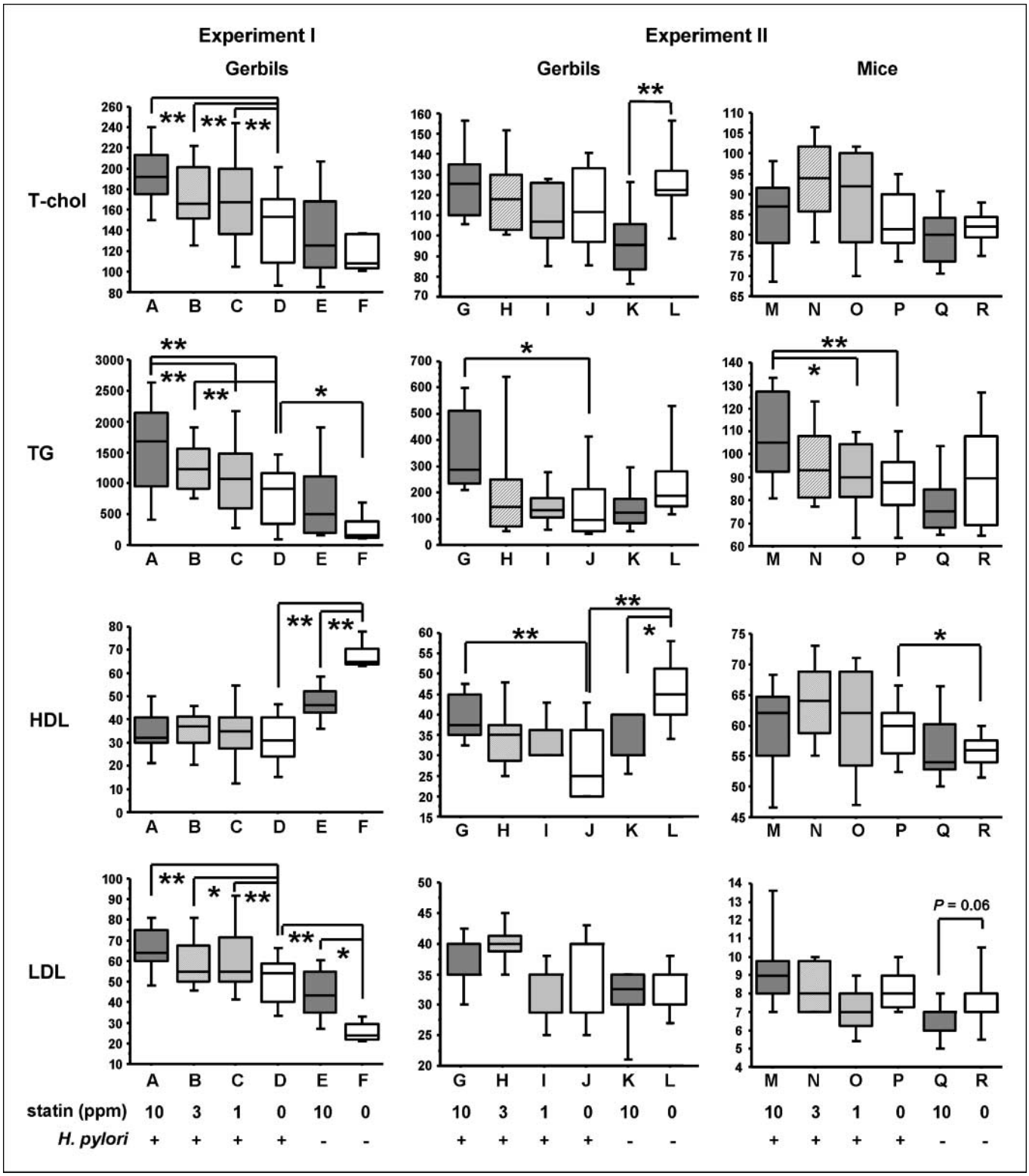

Fig. 2. Serologic results were depicted by box plots. *Line inside each box*, median; *boxes*, 25th and 75th percentiles; *error bars*, 90th and 10th percentiles. *, $P < 0.05$; **, $P < 0.01$.

scores for gastritis in *H. pylori*–infected gerbils and mice were not attenuated by pitavastatin, and serum TG levels were significantly increased. On the other hand, in the noninfected mice, LDL cholesterol showed tendency for decrease. Similarly, in noninfected gerbils, pitavastatin significantly reduced the serum T-Chol and HDL levels. These serologic results suggest that *H. pylori* infection might influence the effects of the statin. Oral administrated pitavastatin is absorbed mainly in the

duodenum and colon with a minimum metabolic change but partly in the stomach. Thus, there is a possibility that the pharmacokinetics of pitavastatin might be modified by *H. pylori*–induced severe chronic gastritis.

Some infectious diseases, such as *Chlamydia pneumoniae* infection, have been considered as risk factors for coronary heart disease (18), and several studies have pointed to an association between *H. pylori* infection and vascular changes due to the alteration of the serum lipid profile (19, 38). Previous studies reported that the serum T-Chol, TG, or LDL concentrations in *H. pylori*–infected persons are significantly elevated over those in noninfected individuals (39, 40). On the other hand, several authors described HDL cholesterol levels to be decreased by long-term infection with *H. pylori* (41–43). In the present study, *H. pylori*–infected animals showed similar lipid dynamics with significant elevation of TG and LDL and depression of HDL, and pitavastatin markedly up-regulated the T-Chol and TG levels in infected groups. Thus, our data support the hypothesis that the conversion of serum lipid dynamics caused by *H. pylori* infection influences the cholesterol-lowering effect of pitavastatin.

The antral mRNA expression levels of inflammatory cytokines (IL-1β and TNF-α) were found to be significantly increased by pitavastatin treatment in *H. pylori*–infected and MNU-treated gerbils, although there were no significant differences in inflammatory scores. In addition, the immunoreactivity scores of iNOS both in the antrum and corpus of these gerbils were higher than those of control (*H. pylori* + MNU) gerbils. Recently, Habara et al. (44) showed that pitavastatin up-regulates iNOS expression in cytokine-stimulated hepatocytes. The findings described here suggest potential enhancing effects of statins on *H. pylori*–induced gastritis through up-regulation of these inflammatory factors, in contrast to the anti-inflammatory effects reported in colon.

Statins are well recognized as relatively safe drugs, although adverse effects include hepatotoxicity and myopathy at low incidence. In the present study, there was no significant variation in body weights with pitavastatin treatment in either *H. pylori*–infected or noninfected animals. No macroscopic lesions in the liver, spleen, kidney, heart, lung, pancreas, testis, and skeletal muscles were observed. In addition, histologic examination revealed no pathologic findings in the liver, spleen, kidney, heart, lung, and skeletal muscles

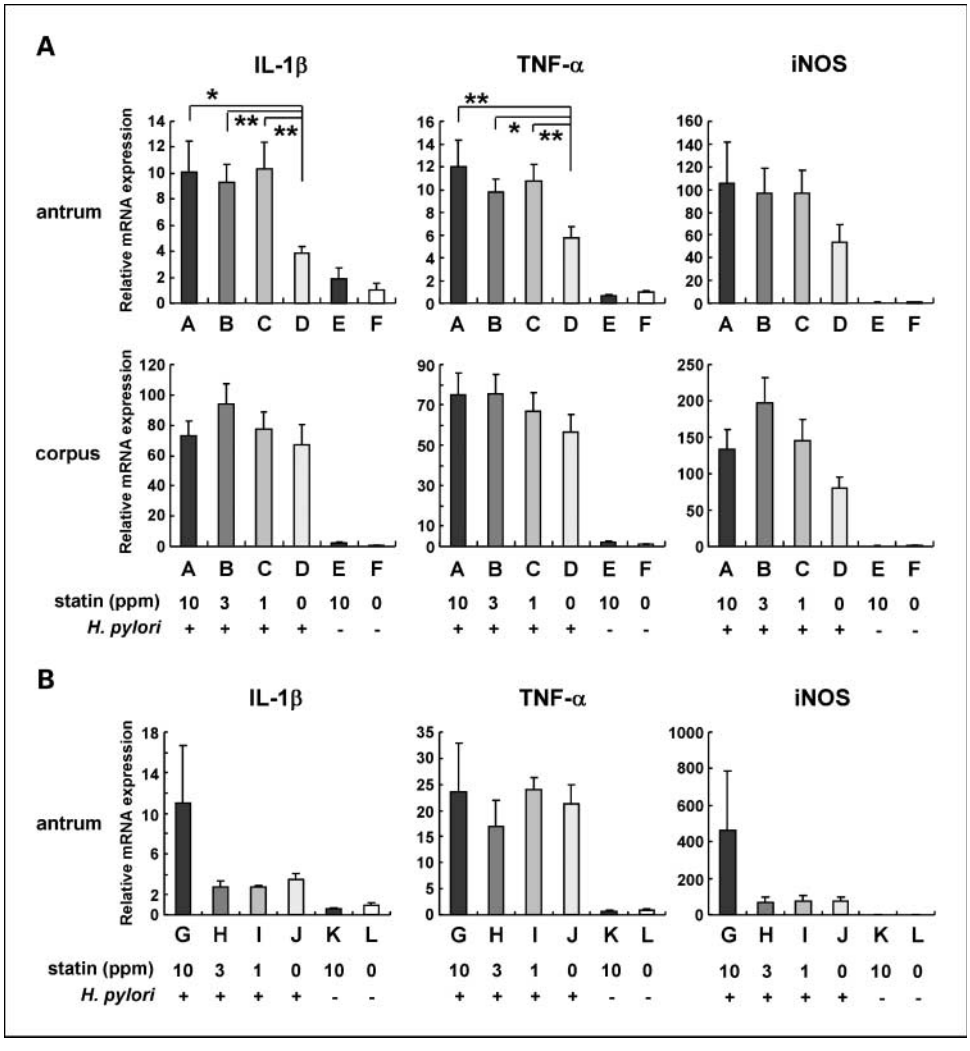

Fig. 3. Relative expression levels of IL-1β, TNF-α, and iNOS mRNAs in the gastric mucosa. *A,* expression in the antrum and corpus of gerbils at 52 wk after infection. Columns, mean arbitrary units relative to 1.0 for controls (group F); bars, SE. Note increase in groups A to C (pitavastatin-treated groups) compared with group D (*H. pylori*–infected control group), especially in the antrum. *, $P < 0.05$; **, $P < 0.01$. *B,* expression levels in the antrum of glandular stomachs of gerbils at 12 wk after infection. Columns, mean arbitrary units relative to 1.0 for controls (group L); bars, SE. Note increase in group G (10 ppm pitavastatin–treated gerbils) compared with group J (*H. pylori*–infected control gerbils), although statistically significant differences are lacking among groups G to J.

of gerbils at 52 weeks. Therefore, it was considered that pitavastatin toxicity was lacking or limited at the dose used in the present study.

In conclusion, pitavastatin does not seem to be associated with reduced risk of stomach carcinogenesis in *H. pylori*–infected Mongolian gerbils. Furthermore, *H. pylori* infection interferes with the serum lipid-lowering effects of pitavastatin in gerbil and mouse models. Our results therefore suggest that care is needed in use of statins for *H. pylori*–infected individuals, especially those with severe chronic gastritis. Large-scale epidemiologic studies should be recommended to determine whether statins have effects on stomach cancer development.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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