Table 2. Association between *Helicobacter pylori* infection or chronic atrophic gastritis and colorectal adenoma

	Controls (n = 239)	Total adenoma cases (n = 239)	Proximal (n = 38)	Bilateral (n = 78)	Distal (n = 123)
***H. pylori* infection**					
(−)	72	38	5	16	17
(+)	167	201	33	62	106
Crude OR (95% CI)	1	2.26 (1.44–3.55)	2.85 (1.07–7.58)	1.67 (0.90–3.10)	2.69 (1.50–4.81)
Adjusted OR[1] (95% CI)	1	2.52 (1.57–4.05)	2.84 (1.07–7.58)	1.72 (0.92–3.22)	2.88 (1.60–5.21)
CAG					
(−)	176	162	22	56	84
(+)	63	77	16	22	39
Crude OR (95% CI)	1	1.31 (0.89–1.93)	2.03 (1.004–4.11)	1.10 (0.62–1.94)	1.30 (0.81–2.09)
Adjusted OR[1] (95% CI)	1	1.45 (0.97–2.17)	2.03 (0.99–4.14)	1.17 (0.65–2.10)	1.38 (0.85–2.24)

[1]Adjusted for current smoking status and total cholesterol by conditional logistic regression analysis.
Abbreviations: OR, odds ratio; CI, confidence interval; CAG, chronic atrophic gastritis.

and controls are summarized in Table 1. No significant differences in mean age, body mass index (BMI), alcohol consumption or serum triglyceride levels (TG) were apparent between cases and controls. Smoking and hypercholesterolemia tended to be more frequent among cases ($p < 0.1$), reflecting those of bilateral and distal adenoma cases, respectively. Therefore, smoking habits and total serum cholesterol (TC) levels were included in the model to control for confounding effects in the following analyses. The serum PG II level was significantly higher in cases than in controls, especially among distal adenoma cases, while the PG I/II ratio was significantly lower among adenoma cases irrespective of their location. The clinicopathologic features of the adenoma cases are also shown in Table 1. The proportion of cases with a lesion $\geq$10 mm in size and the proportion of cases with two or more lesions were larger in bilateral, distal and proximal adenoma cases, in that order. A significant difference was observed between bilateral and proximal cases. There was no significant difference in the histopathology or the grade of dysplasia according to the location of the polyps.

Since PG II and the PG I/II ratio are believed to reflect the activity of inflammation and extent of atrophy, respectively, during the course of *H. pylori*-related chronic gastritis,[17–19] further analyses were performed with special reference to the infection. Table 2 shows that *H. pylori* infection was significantly more prevalent among cases (84.1%) than among controls (69.9%), with a crude OR of 2.26 (95% CI: 1.44–3.55) (Table 2). The risk of adenoma was significantly elevated by *H. pylori* infection regardless of its location, both in the proximal and distal colon, although there was no increase in risk in bilateral adenoma cases. The percentage of subjects with CAG, as determined by serum PG levels using the criteria of PG $\leq$ 70 ng/ml and PG I/II $\leq$ 3.0, was 32.2% in cases and 26.4% in controls, indicating that the presence of CAG did not lead to a significant increase in the risk of colorectal adenoma (crude OR: 1.31; 95% CI: 0.89–1.93) (Table 2). Although adenoma risk was marginally significantly elevated by the presence of CAG in a subgroup of subjects with proximal adenoma, the adjusted OR showed no significantly increased risk.

H. pylori-related chronic gastritis can be classified into three stages based on the results of the two serologic tests: *H. pylori* antibody titer and PG (3). The classification reflects each stage of a serial change in stomach mucosa induced by chronic *H. pylori* infection. The three groups were: Group A: *H. pylori*-negative and PG test-negative; Group B: *H. pylori*-positive and PG test-negative; and Group C: PG test-positive. Group A corresponds to an *H. pylori*-free healthy stomach, Group B corresponds to *H. pylori*-related nonatrophic gastritis and Group C corresponds to the presence of extensive CAG. Table 3 shows the correlations between these three stages of *H. pylori*-related chronic gastritis and risk of colorectal adenoma. The presence of *H. pylori*-related chronic gastritis significantly increased the risk for colorectal adenoma as a whole (Group B: crude OR: 2.61, 95% CI: 1.54–4.41), but the progression of chronic gastritis and resulting CAG development did not show any further increase in the risk of adenoma (Group C: crude OR: 2.30, 95% CI: 1.38–3.83). There was no significant difference in risk between Groups B and C (crude OR: 1.01, 95% CI: 0.66–1.54). Cases were further stratified into three groups based on location of the tumor (proximal, distal or bilateral). The risk of distal adenoma, a major subgroup of colorectal adenoma, was significantly increased with *H. pylori* infection (Group B: crude OR: 2.87, 95% CI: 1.54–5.35), but there was no further increase in risk with the presence of CAG. In contrast, analysis of proximal adenoma cases showed that the adenoma risk increased in a stepwise manner with the presence and progression of *H. pylori*-related chronic gastritis, and it showed a maximal and significant increase in the presence of *H. pylori*-related CAG (Group C: crude OR: 4.51, 95% CI: 1.43–14.2). Bilateral adenoma cases showed no significant risk elevation in the presence of either *H. pylori* infection or CAG.

Stricter criteria for positive PG I ($\leq$30 ng/ml) and PG I/II ratio ($\leq$2.0) are used to detect subjects with more extensive and

Table 3. Association between development of colorectal adenoma and stage of *Helicobacter pylori–related* chronic gastritis

	H. pylori	CAG	Controls (n = 239)	Total adenoma cases (n = 239)	Proximal (n = 38)	Bilateral (n = 78)	Distal (n = 123)
Group A	(–)	(–)	71	35	4	15	16
Group B	(+)	(–)	105	127	18	41	68
Group C		(+)	63	77	16	22	39
Crude OR							
A:B (95% CI)			1	2.61 (1.54–4.41)	3.04 (0.99–9.37)	1.85 (0.95–3.59)	2.87 (1.54–5.35)
A:C (95% CI)			1	2.30 (1.38–3.83)	4.51 (1.43–14.2)	1.65 (0.79–3.46)	2.75 (1.40–5.39)
B:C (95% CI)			1	1.01 (0.66–1.54)	1.48 (0.71–3.11)	0.89 (0.49–1.64)	0.96 (0.58–1.58)
Adjusted OR[1]							
A:B (95% CI)			1	2.81 (1.64–4.81)	3.06 (0.99–9.42)	1.85 (0.94–3.62)	3.05 (1.62–5.73)
A:C (95% CI)			1	2.70 (1.58–4.62)	4.51 (1.43–14.2)	1.76 (0.83–3.74)	3.05 (1.54–6.07)
B:C (95% CI)			1	1.04 (0.68–1.60)	1.54 (0.73–3.27)	0.94 (0.51–1.75)	1.00 (0.60–1.66)
Trend (p value)				0.002	0.009	0.188	0.007

[1]Adjusted for current smoking status and total cholesterol by conditional logistic regression analysis.
Abbreviations: CAG, chronic atrophic gastritis; OR, odds ratio; CI, confidence interval.

Table 4. Association between development of colorectal adenoma and stage of *Helicobacter pylori*-related chronic gastritis (PG test-positive criteria : PGI $\leq$ 30 ng/ml, PG I/II $\leq$ 2)

	H. pylori	CAG	Controls (n = 239)	Total adenoma cases (n = 239)	Proximal (n = 38)	Bilateral (n = 78)	Distal (n = 123)
Group A	(–)	(–)	71	35	4	15	16
Group B	(+)	(–)	155	180	29	56	95
Group C		(+)	13	24	5	7	12
Crude OR							
A:B (95% CI)			1	2.36 (1.49–3.73)	3.32 (1.12–9.80)	1.71 (0.91–3.23)	2.72 (1.49–4.95)
A:C (95% CI)			1	3.75 (1.70–8.23)	6.83 (1.61–28.9)	2.55 (0.87–7.46)	4.10 (1.58–10.6)
B:C (95% CI)			1	1.59 (0.78–3.23)	2.06 (0.68–6.21)	1.49 (0.57–3.93)	1.51 (0.66–3.44)
Adjusted OR[1]							
A:B (95% CI)			1	2.45 (1.54–3.90)	3.33 (1.13–9.83)	1.75 (0.92–3.33)	2.91 (1.58–5.36)
A:C (95% CI)			1	4.20 (1.88–9.40)	7.00 (1.64–29.9)	2.67 (0.88–8.12)	5.16 (1.92–13.9)
B:C (95% CI)			1	1.73 (0.84–3.58)	2.31 (0.75–7.11)	1.48 (0.55–4.04)	1.76 (0.75–4.12)
Trend (p value)				<0.001	0.005	0.049	<0.001

[1]Adjusted for current smoking status and total cholesterol by logistic regression analysis.
Abbreviations: PG, pepsinogen; CAG, chronic atrophic gastritis; OR, odds ratio; CI, confidence interval.

severe CAG.[15] Using these criteria in the study subjects, 31.2% (24/77) who were diagnosed as CAG positive by the less strict criteria (PG I $\leq$ 70 ng/ml and PG I/II $\leq$ 3.0) were considered to be in a more advanced stage of CAG. Table 4 shows that these advanced-stage CAG subjects were at even higher risk for proximal adenoma (crude OR: 6.83, 95% CI: 1.61–28.9), and they were also at higher risk for distal adenoma (crude OR: 4.10, 95% CI: 1.58–10.6) compared to CAG-positive subjects diagnosed by the less strict criteria (PG I $\leq$ 70 ng/ml and PG I/II $\leq$ 3.0). In contrast, the adenoma risk of *H. pylori*-infected CAG-free subjects detected by the stricter criteria was at a comparable level to the subjects diagnosed by the less strict criteria.

Discussion

Our study investigated correlations between *H. pylori* infection and risk of colorectal adenoma. Once established in the stomach mucosa, *H. pylori*-related chronic gastritis is generally believed to trigger a series of events involved in stomach carcinogenesis, as the gastritis-atrophy-metaplasia-dysplasia-cancer sequence.[2] We therefore stratified study subjects based on the stage of *H. pylori*-related chronic gastritis as determined by two serum tests (*H. pylori* antibody titer and PG) and then evaluated colorectal adenoma risk in each stage. As a result, our study clearly indicated that *H. pylori* infection was a risk for colorectal adenoma, which is consistent with

the results from a limited number of previous hospital-based case-control studies[20,21] and comparative studies[22–26] that reported an increased risk of colorectal neoplasia with *H. pylori* infection. However, most of these studies were confounded by uncontrolled factors, so the relationship between colorectal cancer/adenoma and *H. pylori* infection remained unclear. The present population-based case-control study of middle-aged male factory workers was adjusted by potentially confounding factors and clearly demonstrated an increase in the risk of colorectal adenoma in the presence of *H. pylori* infection, although there remains a possibility of uncontrolled confounding factors remaining.

As for the correlation between colorectal neoplasia and CAG, to the best of our knowledge, a single report by Machida *et al.* showed an insignificant increase in the prevalence of CAG among colorectal cancer patients in a hospital-based case-control study.[27] Likewise, in our study, the presence of CAG, as determined by serum PG level, did not contribute to an increase in colorectal adenoma risk as a whole. Accumulating evidence suggests that the risk of colorectal neoplasia associated with various environmental and genetic factors differs for proximal and distal neoplasms,[28] probably reflecting two recently proposed and different tumorigenic pathways based on the molecular features of CpG Island methylator phenotype (CIMP+) and microsatellite instability (MSI+) predominantly occurring in the proximal colon, and chromosomal instability (CIN) occurring in the distal colon.[28] Adenoma cases were thus stratified into proximal and distal groups, and the adenoma risk of each group was analyzed. As a result, a subgroup of subjects with proximal adenoma showed a stepwise increase in adenoma risk with the presence and progression of *H. pylori*-related chronic gastritis, and it reached a maximal and significantly high risk level with the development of CAG, whereas the adenoma risk of the major subgroup with distal adenoma showed no further increase with the development of CAG after *H. pylori* infection. Furthermore, the adenoma risk for both proximal and distal cases appeared to be still higher in about a third of the subjects with CAG, who were in a more advanced stage. Given all these findings, *H. pylori* infection is likely to be involved in the development of colorectal adenoma, and the resultant CAG and its progression appears to further increase the risk, particularly for proximal adenoma.

Various interpretations have been suggested for the mechanism by which *H. pylori* is involved in an increased risk of colorectal neoplasia. First, *H. pylori* infection increases gastrin secretion, which could contribute to colorectal carcinogenesis by inducing mucosal cell proliferation in the colon.[10] As for the correlation between colorectal neoplasia and gastrin, a limited number of epidemiological studies have been done with inconsistent results, some indicating positive correlations[29,30] and others, including a recent large nested case-control study, finding no correlation.[23,31] The differences in these results might be attributable to gastrin precursors such as progastrin or glycine-extended gastrin acting as more important promoters of colorectal carcinogenesis than the fully amidated form of the hormone measured by most commercially available assays.[10,32] Second, *H. pylori* infection might also affect the normal gastrointestinal flora, which contributes to colorectal carcinogenesis,[33–35] as a result of the reduced gastric acid secretion caused by *H. pylori*-related chronic gastritis.

Previous studies have indicated that the presence of an enteric infection and bacterial overgrowth, including intestinal bacteria, are considered to be directly related to a reduction in gastric acid secretion.[36–38] Indeed, our previous study revealed that CAG-positive asymptomatic middle-aged subjects, as determined by serum PG levels of PG I $\leq$ 70 ng/ml and PG I/II ratio of $\leq$ 3.0, were found to have more colonic microflora than CAG-negative subjects.[11] Bacterial overgrowth is reported to lead to an increase in unabsorbed nutrients in the lower intestine due to impaired gastric protein digestion,[39] so some metabolites derived from bacterial fermentation of malabsorbed proteins probably play a role in the etiopathogenesis of colonic disorders, including epithelial neoplasia.[40,41]

In the present results, the association between CAG and adenoma appeared to be particularly high in the proximal colon, but the reason for this is currently unclear. As described above, altered DNA methylation is proposed to be involved in the carcinogenic process of the proximal colon, and it is also known that chronic inflammation induces aberrant DNA methylation in normal tissues.[42] From this viewpoint, it is interesting that interleukin-6, a pro-inflammatory cytokine, whose polymorphisms are involved in the susceptibility to various cancers, is reported to induce expression and activity of DNA methyltransferase.[43] Thus, it is possible that CAG-induced colonic bacterial overgrowth can generate methylation changes to which the proximal colon is more susceptible. In addition, colonic bacterial overgrowth is also known to lead to an enhanced production of secondary bile acids, which are reported to increase the risk for proximal colon cancer.[44] Also, bile acids are presumed to cause DNA damage and activation of the carcinogenic pathway involving DNA methylation particularly in proximal colonic mucosa, and finally lead to the development of cancer.[45,46] Third, *H. pylori* urease could turn gastric juice urea into ammonia and carbon dioxide,[47] which might also affect the normal gastrointestinal flora and contribute to colorectal carcinogenesis. Some studies have correlated high concentrations of luminal ammonia with colon carcinogenesis.[48] Fourth, subjects with *H. pylori* infection might have lifestyles that increase susceptibility to carcinogenesis of the stomach and the rest of the gastrointestinal tract.

This study had some limitations. First, the subjects were asymptomatic men who were susceptible to colon cancer because of their age and who were self-referred for colonoscopy. As such, these subjects may have had a different overall prevalence of colorectal adenoma and risk profile for colorectal cancer compared to the general working population. It is also possible that the subjects were more health-conscious or had undisclosed reasons for suspecting they had colorectal

disease. Although we do not claim a complete absence of selection bias, the prevalence of colorectal adenoma (23.5%) in our study is in a range similar to the recently reported value of 26.5%, based on colonoscopy, of asymptomatic subjects in Japan.[49] Second, patients with hypergastrinemia and hyperchlorhydria secondary to Zollinger-Ellison syndrome show increased proliferation of rectal mucosa,[50] and Machida *et al.* reported that atrophic gastritis with gastric acid reduction (presence of CAG) might increase the risk of rectal cancer.[27] However, we failed to detect a significant association with rectal adenoma, as we did not have a sufficient sample size for tumors located only in the rectum. Third, with respect to the misclassification of exposures, the diagnosis of *H. pylori* infection and atrophic gastritis were based on serological tests. However, misclassification was likely to have occurred equally among cases and controls, and the risk of developing adenomas following infection might have been underestimated.

In conclusion, it is probable that *H. pylori* infection is involved in an increased risk of colorectal adenoma, and the risk of adenoma, particularly in the proximal colon, appears to be further enhanced by the presence and progression of CAG. The stage of *H. pylori*-related chronic gastritis, as determined by the two serologic markers *H. pylori* antibody and PG, will probably be useful for the evaluation of risk of colorectal neoplasia, and may contribute to the selection of high-risk individuals who warrant surveillance by colonoscopy. Further investigation into the role of *H. pylori* infection in the carcinogenesis of the colorectum is necessary. In addition, whether eradication therapy for *H. pylori*-infected subjects reduces the risk of colorectal neoplasia is a problem for future study.

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Preventive effects of etodolac, a selective cyclooxygenase-2 inhibitor, on cancer development in extensive metaplastic gastritis, a *Helicobacter pylori*-negative precancerous lesion

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The present study investigated the preventive effects of etodolac, a selective cyclo-oxygenase (COX)-2 inhibitor, on metachronous cancer development after endoscopic resection of early gastric cancer. Among 267 early gastric cancer patients who underwent endoscopic resection, 47 patients with extensive metaplastic gastritis were selected based on endoscopic findings and our previously described criteria of serum pepsinogen (PG) test-positive and *Helicobacter pylori* antibody-negative conditions. Nonrandomized etodolac treatment (300 mg/day) was administered to 26 patients (Group A), while the remaining 21 patients were untreated (Group B). No significant differences in age, sex distribution, lifestyle factors or extent of metaplastic gastritis at baseline were identified between groups. Patients were followed for metachronous cancer development with endoscopy every 6–12 months for up to 5 years. Mean (standard deviation) follow-up period was 4.2 (0.9) years. In Group B, 5 cancers developed (incidence rate = 6,266/100,000 person-years), significantly more than the 1 cancer in Group A (incidence rate = 898/100,000 person-years; $p < 0.05$). Long-term etodolac treatment did not influence the extent of metaplastic gastritis as revealed by endoscopic findings or by serum PG levels, but effectively reduced metachronous cancer development in patients with extensive metaplastic gastritis. These results strongly suggest that chemoprevention of cancer in the metaplastic stomach is possible by controlling COX-2 expression.

Key words: gastric cancer, pepsinogen, *Helicobacter pylori*, chronic atrophic gastritis, cancer prevention, chemoprevention, COX-2 inhibitor, intestinal metaplasia

Abbreviations: *H. pylori*: *Helicobacter pylori*; CAG: chronic atrophic gastritis; COX: cyclo-oxygenase; ESD: endoscopic submucosal dissection; ELISA: enzyme-linked immunosorbent assay; PG: pepsinogen; NSAIDs: nonsteroidal anti-inflammatory drugs

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Helicobacter pylori triggers chronic inflammation of the infected stomach mucosa and is considered a major risk factor for gastric cancer and associated precursor lesions.[1] As postulated in the multistep model of gastric cancer development by Correa, long-lasting inflammation in the stomach mucosa leads to a cascade of molecular and morphological changes of stomach carcinogenesis, representing the gastritis-atrophy-metaplasia-dysplasia-cancer sequence.[2] This sequence of stomach carcinogenesis is now widely accepted to be strongly promoted by *H. pylori* and is affected by a variety of genetic and environmental factors that may act synergistically.[3] *H. pylori* eradication thus appears to be the most promising approach for the control of gastric cancer development, and the results of animal experiments have revealed that eradication of *H. pylori*, especially in the early stage, is effective for preventing stomach carcinogenesis.[4–6] However, current data indicate that *H. pylori* eradication does not lead to complete eradication of gastric cancer[7–12] and might be effective only in subjects without chronic atrophic gastritis (CAG) together with intestinal metaplasia.[7,10] Moreover, patients with extensive intestinal metaplasia—that is, metaplastic gastritis—should not be treated with eradication

therapy, as bacterial load decreases with the progression of intestinal metaplasia, eventually resulting in spontaneous eradication.[13,14] Alternative chemopreventive measures are thus needed for the prevention of stomach cancer in subjects with metaplastic gastritis.

Cyclo-oxygenase (COX)-2 is an inducible enzyme overexpressed in sites of inflammation and neoplastic tissues. This overexpression leads to enhancement of cell proliferation and migration, suppression of apoptosis, stimulation of neovascularization and alteration of intercellular adhesion, all of which are involved in carcinogenesis.[15] In the stomach, *H. pylori* infection triggers mucosal COX-2 upregulation, and this enhanced expression level is maintained throughout the progression of the aforementioned premalignant lesions to cancer.[16,17] Furthermore, selective inhibition of COX-2 has been shown to prevent the progression of premalignant gastric lesions[18] and the development of gastric cancer in *H. pylori*-infected Mongolian gerbils.[19,20] Treatment with COX-2 inhibitors thus appears to have beneficial preventive effects on *H. pylori*-associated stomach carcinogenesis. We conducted a prospective follow-up study in a group of patients with metaplastic gastritis who underwent endoscopic resection for early gastric cancer to determine whether administration of etodolac, a selective COX-2 inhibitor, prevents metachronous gastric cancer development.

Material and Methods

Study subjects

Between February 2003 and January 2005, a total of 267 patients with early gastric cancer underwent curative endoscopic resection such as endoscopic mucosal resection or endoscopic submucosal dissection (ESD) in Wakayama Medical University Hospital.[21–26] All patients were inhabitants from around the Wakayama area. In Japan, where the incidence of gastric cancer is high, treatment of mucosal gastric cancer without lymph node metastasis is usually achieved with endoscopic resection, preserving the stomach. Although institutional differences in indications for endoscopic resection exist, lesions with preoperative endoscopic diagnoses of intestinal-type intramucosal cancer without ulcer findings, intestinal-type intramucosal cancer $\leq$3 cm in diameter with ulcer findings and intestinal-type minute invasive submucosal (<500 μm below muscularis mucosa) cancer $\leq$3 cm in diameter are considered to be indicated for endoscopic resection.[25,26] In these 267 patients, the extent of coexisting CAG was evaluated endoscopically and by the results of 2 serum tests, pepsinogen (PG) and *H. pylori* antibody level, as described in the following section.

Serologic diagnosis of metaplastic gastritis, etodolac treatment and follow-up

Sera for analyses were obtained from fasting blood samples collected from the 267 patients before endoscopy, stored at −20°C and used for the measurement of serum PG levels and *H. pylori* antibody titers. Serum PG (PGI and PGII) levels were measured using a modification (RIAbeads Kit; Dainabott, Tokyo, Japan) of our previously reported radio-immunoassay.[27] Patients with extensive CAG were diagnosed using the previously described "PG test-positive" criteria: PGI $\leq$ 70 ng/mL and PGI/II ratio $\leq$ 3.0.[28,29] These criteria have a sensitivity of 70.5% and a specificity of 97% for the diagnosis of extensive CAG using pathological diagnosis as the gold standard.[28] Serum anti-*H. pylori* immunoglobulin G titers were measured using enzyme-linked immunosorbent assay (ELISA) (MBL, Nagoya, Japan).[30] Subjects with antibody titers >50 U/mL were classified as positive (*H. pylori*-infected) and those with antibody titers $\leq$50 U/mL were regarded as negative. The sensitivity and specificity of the ELISA test used in the present study were 93.5 and 92.5%, respectively.[30]

As described previously,[31,32] the natural course of *H. pylori* infection can be classified into the following 4 groups based on the results of the 2 serum tests for PG and *H. pylori* antibody: (*i*) healthy subjects without *H. pylori* infection are PG test-negative and *H. pylori* antibody-negative; (*ii*) with the establishment of *H. pylori* infection, the antibody test becomes positive; (*iii*) as infection persists, gastric atrophy advances and the PG test also becomes positive; and (*iv*) as gastric atrophy together with intestinal metaplasia becomes extensive, this leads to a reduction in *H. pylori* load and eventually to spontaneous eradication, so the antibody test again becomes negative. Subjects with metaplastic gastritis can thus be diagnosed serologically based on a PG test-positive, *H. pylori* antibody-negative condition.

Among the 267 patients, those with a previous history of gastric cancer or adenoma; severe liver, kidney or cardiopulmonary disease; past history of gastrointestinal bleeding or peptic ulcer disease; or long-term use of adrenocortical steroids or nonsteroidal anti-inflammatory drugs (NSAIDs) were excluded from the study. In addition, patients who had a previous history of *H. pylori* eradication or renal failure and those who had been prescribed proton pump inhibitors were also excluded from the study. The remaining subjects comprised 47 patients with metaplastic gastritis diagnosed both endoscopically and serologically.

All patients received a full explanation of etodolac treatment after endoscopic mucosal resection and were given the option to undergo this treatment. Patients who consented to treatment received etodolac at 300 mg/day. Patients who rejected this option but provided consent to participate in the study were followed as controls. Patients with and without etodolac treatment were endoscopically followed for metachronous cancer development at 1 week, 4 weeks, 8 weeks and 6 months after resection. Thereafter, patients underwent regular follow-up endoscopy every 6 months. Other than endoscopic follow-up, all patients were reviewed regularly every 1–2 months by clinicians for general health condition, and patients receiving etodolac treatment were monitored for adverse events by interview and clinical laboratory evaluations. Compliance was monitored by pill counts at the time

Table 1. Profiles of subjects in groups A and B

	Group A with etodolac treatment	Group B without etodolac treatment
Number of subjects (male:female)	26 (22:4)	19 (17:2)
Follow-up, years [mean (SD)]	4.3 (1.1)	4.2 (0.7)
Person-years	111.4	79.8
Age, years [mean (SD)]	71.3 (10.2)	70.6 (7.4)
Alcohol drinking, *n* (%)	10 (38.4)	7 (36.8)
Smoker, *n* (%)	9 (34.6)	7 (36.8)
Serum PG levels at the start of the study		
PGI, mg/ml [mean (SD)]	21.4 (18.4)	20.1 (18.2)
PG I/II [mean (SD)]	1.7 (0.8)	1.5 (0.7)
Serum PG levels at the end of the study		
PGI, mg/ml [mean (SD)]	18.2 (14.1)	18.0 (12.4)
PG I/II [mean (SD)]	1.5 (0.8)	1.6 (0.7)
Total gastric cancer developed		
Case/incidence rate[1]	1/898	5/6266[2]
Details of the resected cancers		
Size, mm [mean (SD)]	31.5 (13.6)	32.4 (17.4)
Location [upper/middle/lower (%)][3]	14/10/2 (54/38/8)	11/7/1 (58/37/5)
Macroscopic type [IIa/IIb/IIc (%)]	18/3/5 (69/12/19)	12/2/5 (63/11/26)
Depth of invasion, *n* of mucosal cancer (%)	26 (100)	19 (100)
Histopathology type, *n* of intestinal type (%)	26 (100)	19 (100)
Synchronous multiple cancer cases, *n* (%)	2 (8)	1 (5)
Method of endoscopic resection, *n* of ESD (%)	26 (100)	19 (100)

[1]Per 100,000 person-years. [2]$p < 0.05$ (*vs.* Group A with etodolac treatment). [3]Location and macroscopic type of the cancer were determined according to the Japanese Classification of Gastric Carcinoma (Ref. 34).

of each review. Written informed consent was obtained from all participating patients. The Committee on Ethics at Wakayama Medical University approved all study protocols.

Evaluation of cancer histopathology

Resected specimens of gastric cancer obtained by endoscopy were assessed histopathologically and classified according to Lauren's classification into intestinal or diffuse type.[33] Location and macroscopic type of the cancer in the stomach were classified based on clinical and histopathological records according to the classifications of the Japanese Gastric Cancer Association.[34]

Statistical analysis

Data were analyzed using SPSS 11.0 software (SPSS, Chicago, IL) and STATA software (STATA, College Station, TX). Differences were tested for significance using the Mann–Whitney *U*-test for comparisons between 2 groups. The chi-square test and Fisher's exact test were used to compare categorical variables. Long-term effects of etodolac on gastric cancer development were analyzed by the Kaplan–Meier method, and statistical differences between curves were tested by the log-rank test. For all comparisons, p values less than 5% ($p < 0.05$) were considered statistically significant.

Results

Among the 47 patients with endoscopically and serologically diagnosed metaplastic gastritis who underwent endoscopic resection for early gastric cancer, 26 received etodolac treatment (Group A) and the remaining 21 did not receive any treatment (Group B). These 2 groups of patients were followed and development of gastric cancer was investigated. During the first year of the study, 2 patients in Group B developed cancer. One cancer case was detected 8 weeks after resection and the other 6 months later. In both cases, the cancerous lesions were able to be retrospectively identified on endoscopic images from before resection. These cancers were considered to be synchronous cancers and were thus excluded from the study, and the remaining 45 patients were analyzed. Table 1 shows baseline characteristics for the 2 groups. No significant differences in age, sex distribution, or lifestyle factors at baseline were apparent between groups. In addition, the extent of CAG together with intestinal metaplasia at the time of mucosal resection as evaluated by endoscopic findings was similar between groups, as were serum PG levels. Furthermore, comparison of clinicopathological features (size, location, macroscopic type, depth of invasion, histopathological type, *etc.*) of the resected cancers revealed

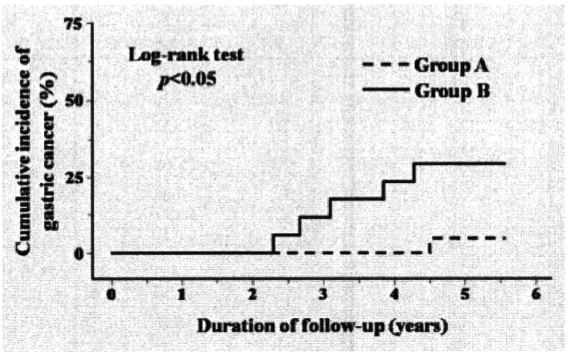

Figure 1. Kaplan–Meier analysis of metachronous cancer development in patients with early gastric cancer resected endoscopically. Group A received etodolac treatment (300 mg/day), while Group B did not receive any treatment. Both groups were followed for up to 5 years. Cancer incidence rates were 898/100,000 person-years for Group A and 6,266/100,000 person-years for Group B, showing a significant difference in cancer development rates between groups ($p = 0.05$; log-rank test).

no significant differences between groups. All cancers in these 47 patients were resected by ESD.

Patients were followed for up to 5 years. Mean (standard deviation) follow-up period was 4.2 (0.9) years. By the end of the study period, cancer development was observed in 1 Group A patient and 5 Group B patients. As shown by Kaplan–Meier analysis, cancer development in the Group A patient was observed 5 years after the start of the study. In contrast, cancer development occurred steadily throughout the study period in Group B patients (Fig. 1). Cancer incidence rates in Groups A and B were 898/100,000 person-years and 6,266/100,000 person-years, respectively, representing a significant difference ($p < 0.05$, log-rank test). Cancers that developed in these patients were all intestinal-type mucosal cancers on histopathology, and sizes were <10 mm in diameter. All these lesions were thus resected endoscopically.

The extent of CAG together with intestinal metaplasia as revealed by endoscopic findings did not change significantly in Group B patients during the study period. In addition, the difference between serum PG levels for each patient at the start compared to the end of the study period was not significantly different and was within the range of interassay variation. Etodolac treatment did not exert any influence on the extent of metaplastic gastritis in Group A patients and did not induce any other specific change in endoscopic findings except for a single case of gastric erosion observed in the prepyloric antrum of 1 patient. Serum PG levels of Group A patients were also unaltered by etodolac treatment. This medication was well tolerated by all patients during the study period.

Discussion

In the present study, long-term treatment with a selective COX-2 inhibitor, etodolac, effectively inhibited metachronous cancer development in curatively treated, early gastric cancer patients with metaplastic gastritis. These results are in line with the results of our previous animal experiment using *H. pylori*-infected Mongolian gerbils,[19] indicating that etodolac can prevent stomach carcinogenesis involving the CAG-metaplasia-dysplasia-cancer sequence. Essentially, the same results have also been reported with the use of another selective COX-2 inhibitor, celecoxib.[20] Furthermore, previous epidemiologic studies have demonstrated that long-term nonselective inhibition of COXs (COX-1 and COX-2) by NSAID treatment is effective for preventing gastric cancer.[35,36] However, evaluation of the preventive effects of selective COX-2 inhibition on gastric cancer by 2 epidemiologic studies investigating the regression of intestinal metaplasia as a primary parameter (a surrogate parameter for cancer prevention) revealed conflicting results. One randomized controlled study indicated that rofecoxib treatment had no significant effect on the regression of intestinal metaplasia,[37] whereas the other nonrandomized study indicated a beneficial effect of celecoxib.[38] The contradictory outcomes of these studies could be partially explained by the differential effects of selective COX-2 inhibitors according to dose, type of drug and duration of exposure,[39] but also, and more importantly, by differences in severity of the target lesion—the extent of coexisting CAG together with intestinal metaplasia—among study patients. The present results indicate that the extent of premalignant lesions as revealed by serum PG levels did not change significantly during the study period despite etodolac treatment. Since the study period was not long, further long-term investigations are warranted to determine the inhibition of progression and/or regression of metaplastic gastritis by COX-2 inhibition. Nonetheless, our results strongly indicate that COX-2 is deeply involved in the growth of initiated cells in the metaplastic stomach and that etodolac treatment leads to a marked delay in cancer development.

We selected early gastric cancer patients with metaplastic gastritis as a target for treatment with a selective COX-2 inhibitor. Several previous studies have demonstrated that the more advanced the stage of *H. pylori*-related CAG, the greater the cancer risk.[31,32,40–44] Subjects with metaplastic gastritis, an end result of long-lasting *H. pylori* infection, are thus considered to be at particularly high risk of gastric cancer. Indeed, our previous longitudinal cohort study found that a group of middle-aged male subjects with metaplastic gastritis based on 2 serum tests—negative results for *H. pylori* antibody and positive results on the PG test—displayed an annual cancer incidence rate of about 0.87%, meaning that 1 cancer developed in 11.5 subjects during every 10-year period.[31,32] Subjects selected for the present study were early cancer patients curatively treated with endoscopic resection and thus appear to constitute a subgroup at even higher risk for gastric cancer among subjects with metaplastic gastritis. A few previous studies have reported an annual incidence of metachronous cancer after endoscopic resection of about 1.3–4.0%,[45,46] while the annual cancer incidence rate in the present study was

>6.0% for the control group. This high incidence rate is probably due to the fact that, in those previous studies, the extent of CAG together with intestinal metaplasia in background stomachs of study patients was not evaluated. Subjects were thus probably heterogeneous in the degree of CAG, which was probably milder as a whole compared to that in subjects in the present study. The observed high incidence rate of metachronous cancer development is evidence for a strategy for cancer prevention in these subjects other than regular and strict follow-up by endoscopy. As described above, subjects with metaplastic gastritis, an *H. pylori*-negative lesion, cannot be treated with *H. pylori* eradication. Furthermore, high expression levels of COX-2 in intestinal metaplasia have been observed even after *H. pylori* eradication.[47] Treatment with a selective COX-2 inhibitor thus appears to represent a reasonable option for subjects with metaplastic gastritis, rather than regular follow-up for cancer.

Although the present study revealed preventive effects of a selective COX-inhibitor, etodolac, on metachronous cancer development in curatively treated gastric cancer patients with metaplastic gastritis, the study shows some limitations. First, the present study was prospectively conducted, but treatment with etodolac was not randomized. However, randomization was not feasible, as most eligible, high-risk cancer subjects were unwilling to remain untreated for long periods, particularly with the knowledge of the results of previous epidemiologic studies that long-term use of NSAIDs, including aspirin, is associated with a reduced risk of gastric cancer.[35,36] In addition, the number of subjects was small because the incidence of serologically diagnosed metaplastic gastritis is quite low, comprising <1% of the middle-aged Japanese population[31,32] and <20% of total gastric cancer cases. Considering the fact that *H. pylori* eradication does not completely eradicate cancer[7–12] and that eradication might be effective in the control of cancer development only among subjects with mild CAG,[7,10] posteradication subjects with extensive CAG and intestinal metaplasia should be considered another possible target for treatment with selective COX-2 inhibitors. However, special attention should be paid to recent evidence that long-term use of COX-2 inhibitors is associated with increased cardiovascular risk, including not only thrombotic events, but also hypertension, congestive heart failure, and arrhythmic events.[48–50] Since the reported cardiovascular toxicity of COX-2 inhibitors is variable among the different drugs and with the dose of each particular drug and, based on past data, etodolac treatment at a dose of 300 mg/day appears to be relatively low in cardiotoxicity,[51,52] the present results warrant a prospective randomized trial. Further careful study using the present dose of etodolac and avoiding inclusion of patients with increased risk of cardiovascular complications would contribute greatly to determining the effectiveness and safety of long-term chemopreventive treatment.

In conclusion, the present results strongly indicate that selective COX-2 inhibitors provide a potent strategy for tertiary cancer prevention in curatively treated gastric cancer patients with metaplastic gastritis, an *H. pylori*-negative premalignant lesion.

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ORIGINAL ARTICLE

Inhibitory effects of Japanese apricot (*Prunus mume* Siebold et Zucc.; *Ume*) on *Helicobacter pylori*-related chronic gastritis

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Objectives: We investigated the correlation between Japanese apricot (JA) intake and *Helicobacter pylori*-related chronic atrophic gastritis (CAG).
Methods: A questionnaire was administered and serum anti-*H. pylori* IgG antibodies measured in 1358 asymptomatic adults. The subjects were divided into high-intake and low-intake groups. Histological and serological evaluation of *H. pylori*-related CAG was performed in 68 non-elderly volunteers.
Results: The *H. pylori*-negative rate did not differ significantly between the high-intake and low-intake groups. Mean antibody titers were lower in the high-intake group, but the difference was not significant. There was no significant difference in the rate of *H. pylori* infection on the basis of JA intake when subjects were stratified by age. Among *H. pylori*-positive non-elderly subjects, antibody titers were significantly lower in the high-intake group ($P=0.041$). Endoscopic tissue biopsy from the 68 volunteers showed less *H. pylori* bacterial load and mononuclear infiltration irrespective of gastric site in the high-intake group. In the high-intake group, antral neutrophil infiltration was significantly less pronounced and corporal atrophy was less extensive. Serological evaluation using serum PG levels also confirmed these histopathological data.
Conclusions: Our findings strongly indicate a preventive effect of JA intake on CAG by inhibiting *H. pylori* infection and reducing active mucosal inflammation.
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Keywords: Japanese apricot; Ume; atrophic gastritis; *H. pylori*; serum pepsinogen

Introduction

It is now widely accepted that progression of chronic atrophic gastritis (CAG), including intestinal metaplasia, resulting from chronic active inflammation of the gastric mucosa by *Helicobacter pylori* infection is a primary mechanism of gastric carcinogenesis in areas of high cancer risk, including Japan (Correa and Houghton, 2007). Although the pathogenic roles of *H. pylori* are not fully elucidated, *H. pylori* eradication leads to histological resolution of CAG, probably prevents progression of CAG and may reduce the incidence of gastric cancer (Kabir, 2009). However, bacterial eradication in all *H. pylori*-infected patients remains difficult because of potential side effects, bacterial resistance to antibiotics and cost. Thus, it is important to find safe and inexpensive agents to control *H. pylori*-related CAG.

Progression of CAG is related to *H. pylori* bacterial factors such as VacA and CagA (Hatakeyama, 2004), host factors like cytokine gene polymorphisms (El-Omar *et al.*, 2000) and environmental factors. Environmental factors, particularly lifestyle and dietary habits, are the most frequent and direct factors to which the gastric mucosa is exposed and have a

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major effect on gastric carcinogenesis. For example, high sodium intake increases gastric mucosal inflammation and the risk of gastric cancer (Nozaki *et al.*, 2002; Shikata *et al.*, 2006), and cigarette smoking is considered to be deeply involved in the transition of CAG to intestinal metaplasia and dysplasia (Kneller *et al.*, 1992; Tredaniel *et al.*, 1997), which are precancerous conditions, in a model of gastric carcinogenesis postulated by Correa (Correa and Houghton, 2007). Conversely, animal experiments and epidemiological studies have shown that vegetables, fruits and green tea lower the risk of gastric cancer (Yu *et al.*, 1995; Kobayashi *et al.*, 2002).

Since ancient times, the Japanese apricot (JA) (*Ume* in Japanese; *Prunus mume* Siebold et Zucc.) has been known to possess various medical benefits. JA processed as an extract, dried or pickled, and made into liquor and soft drinks has been frequently prescribed as a traditional folk remedy for dyspepsia or gastrointestinal infections, including gastroenteritis, the common cold and quick recovery from general fatigue or a stiff neck. Although JA exerts various antibacterial and fungicidal properties, there is very little scientific proof about the effectiveness of JA in the treatment of the above-mentioned disorders. In an *in vitro* study by Fujita *et al.* (2002), JA extracts had a bactericidal effect against *H. pylori*. More recently, Otsuka (2005), in an *in vivo* study using *H. pylori*-infected Mongolian gerbils, reported that JA extracts have anti-*H. pylori* effects and lead to the improvement of *H. pylori*-induced CAG. These findings strongly suggest that JA contains constituents with anti-*H. pylori* effects. In this study, we investigated the effects of JA intake on *H. pylori*-related CAG in residents of Wakayama Prefecture, a well-known JA-growing region in Japan.

Methods

JA intake and H. pylori *infection evaluated by serum antibody level*

This study recruited a total of 1358 adults (mean (s.d.) age: 54.6 (13.2) years; 586 men and 772 women) living in the Wakayama Prefecture, a well-known JA-growing region, who received annual medical check-ups provided by the local community health service. In Japan, these health check-up programs are performed in an effort to detect diseases at an early stage. Therefore, subjects who had specific symptoms requiring medical care were excluded from the program.

Symptom-free subjects underwent the following tests and procedures: physical examination, chest X-ray, electrocardiogram, blood laboratory tests, urinalysis, barium X-ray and fecal occult blood test. They also completed a self-administered questionnaire and an interview to determine general health status. The questionnaire included an assessment of the daily intake of JA, either dried or pickled. The subjects were divided into two groups: high intake ($\geqslant$3 JA daily) and low intake ($<$3 JA daily). This classification is based on the results of a previous study analyzing the effects of a fruit-juice concentrate of JA (CJA) on *H. pylori*-induced gastric lesions in Mongolian gerbils that indicated that daily intake of CJA of around 8.6 mg/day leads to a significant reduction in the number of infected *H. pylori* cells in the gastric epithelium and also an improvement in *H. pylori*-induced active gastritis (Otsuka, 2005). On the basis of the comparison between the body weight of the Mongolian gerbils (50–60 g) and that of the study subjects (50–80 kg), and also on the fact that three JAs are equivalent to around 7.0 g of CJA on the basis of an analysis of citrate content, it is highly probable that daily intake of more than three JAs will exert the same effect in the human stomach as on gerbils.

In all these subjects, *H. pylori* IgG antibody titers were measured by ELISA (MBL Inc., Nagoya, Japan) as described elsewhere (Chen *et al.*, 1997). *H. pylori* antibody titers $\geqslant$30 U/ml were considered positive (+) for *H. pylori* infection and those $<$30 U/ml were considered negative (−).

Correlation between JA intake and gastric mucosal histology

We explained the protocol to 458 *H. pylori*-infected non-elderly subjects and invited them to participate in the study for the evaluation of the effects of JA intake on *H. pylori*-related CAG. Of these potential subjects, 68 non-elderly ($<$65 years) individuals (mean (s.d.) age: 46.7 (8.2) years; 46 men and 22 women) agreed to participate and written informed consent to perform upper gastrointestinal tract endoscopy for histological assessment of CAG was obtained. In brief, endoscopic biopsy of the gastric corpus and antrum was performed (samples were obtained from two sites: one from the lesser curvature of the corpus 4 cm proximal to the gastric angle and the other from the lesser curvature of the antrum). The biopsy samples were embedded in paraffin, cut, stained with hematoxylin and eosin and examined histologically. Histopathological examination on the basis of the Sydney System (Dixon *et al.*, 1996) included *H. pylori* bacterial load, neutrophil infiltration, mononuclear infiltration, atrophy and intestinal metaplasia. In addition, for serological evaluation of the extent of *H. pylori*-related CAG, the serum levels of pepsinogen-I and II (PG-I and PG-II) were measured using a modification (radioimmunoassay beads kit; Dainabott, Tokyo, Japan) of our previously reported radioimmunoassay (Ichinose *et al.*, 1982). Subjects with renal failure, history of gastrectomy or previous *H. pylori* eradication were not included in the analysis. No subject had been prescribed medication that might affect gastrointestinal function, such as proton pump inhibitors or non-steroidal anti-inflammatory drugs, before examination. The ethics committee of Wakayama Medical University approved the study protocols.

Statistical analysis

The data are expressed as mean ± s.d. Data were analyzed using unpaired *t*-test and Fisher's exact text. The level

of statistical significance was $P<0.05$. All analyses were performed using the SPSS 11.0 software package (SPSS Inc., Chicago, IL, USA).

Results

Correlation between JA intake and H. pylori *infection*

In 1358 asymptomatic adults (mean (s.d.) age: 54.6 (13.2) years; 586 men and 772 women), serum anti-*H. pylori* antibody titers were measured for the evaluation of *H. pylori* infection. Table 1 shows the baseline data for subjects in the high- and low-JA-intake groups. There were no significant differences in age, gender or smoking habit between the two groups.

Comparison between the high-intake group (968 subjects) and the low-intake group (390 subjects) showed that the *H. pylori*-negative rate (*H. pylori* antibody titer <30 U/ml) did not differ significantly between the two groups (48.5 versus 48.2%) (Table 1). In the high-intake as compared with the low-intake group, the mean *H. pylori* antibody titer was lower but the difference between groups was not statistically significant (Table 1). We also stratified the subjects by age into non-elderly (30–64 years) and elderly (65–80 years) groups. In both elderly and non-elderly subjects, the *H. pylori*-negative rate and mean antibody titers did not differ significantly between the high-intake and the low-intake group (Table 1).

Then we analyzed data from only the *H. pylori*-positive subjects (*H. pylori* antibody titer ⩾30 U/ml; 701 subjects) (Figure 1). There was no significant difference in the serum *H. pylori* antibody titer level between the non-elderly and the elderly *H. pylori*-infected subjects. Among 243 elderly *H. pylori*-positive subjects, the *H. pylori* antibody titer did not differ significantly on the basis of JA intake (88.5 ± 60.6 U/ml versus 87.9 ± 57.0 U/ml; $P=0.945$). However, in the 458 non-elderly *H. pylori*-positive subjects, the *H. pylori* antibody titers were significantly lower in the high-intake group (83.3 ± 54.8 U/ml versus 95.5 ± 64.6 U/ml; $P=0.041$) (Figure 1). In the subjects in the low- and high-intake groups, there was no significant difference in the antibody titer level between the two age groups.

Effects of JA intake on H. pylori*-related CAG*

Among the 68 non-elderly volunteers who had upper gastrointestinal endoscopy, 31 were in the high-JA-intake group and the remaining 37 were in the low-intake group. There were no significant inter-group differences in age, gender or smoking habit (Table 2). We obtained gastric biopsy samples from the antrum and corpus from these subjects for histological evaluation of the gastric mucosa on the basis of the Sydney System for *H. pylori* bacterial load, neutrophil infiltration, mononuclear infiltration, atrophy and intestinal metaplasia (Table 3). There were no significant inter-group differences in the extent of intestinal metaplasia;

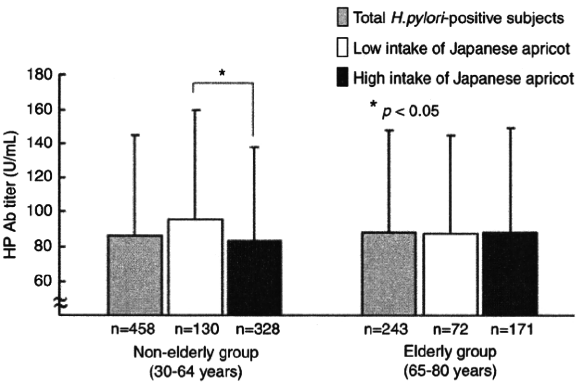

Figure 1 Comparison of *H. pylori* antibody titers on the basis of JA intake, with stratification by age. In *H. pylori*-positive subjects (*H. pylori* antibody titer ⩾30 U/ml), we compared *H. pylori* antibody titers according to JA intake, with stratification for age. In the elderly group (65–80 years), the *H. pylori* antibody titers did not differ significantly on the basis of JA intake (88.5 ± 60.6 U/ml versus 87.9 ± 57.0 U/ml; $P=0.945$). However, in the non-elderly group (30–64 years), the *H. pylori* antibody titers were significantly lower in the high-intake versus the low-intake group (83.3 ± 54.8 U/ml versus 95.5 ± 64.6 U/ml; $P=0.041$). JA, Japanese apricot.

Table 1 Clinico-serological features according to intake of JA ($n=1358$)

	Total subjects	*Low intake of Japanese apricot*			*High intake of Japanese apricot*		
	(n = 1358)	*Total (n = 390)*	*Elderly (n = 114)*	*Non-elderly (n = 276)*	*Total (n = 968)*	*Elderly (n = 275)*	*Non-elderly (n = 693)*
Age (years)	54.6 ± 13.2	53.6 ± 14.0	70.4 ± 3.8	46.6 ± 10.2	55.1 ± 12.8	70.1 ± 3.7	49.1 ± 9.9
Gender (male/female)	586/772	171/219	57/57	114/162	415/553	132/143	283/410
Smoker, *n* (%)	235 (17.3%)	67 (17.2%)	27 (23.7%)	40 (14.5%)	168 (17.4%)	59 (21.5%)	109 (15.7%)
H. pylori Ab titer (U/ml)	47.9 ± 58.8	50.5 ± 62.8	57.1 ± 60.8	47.8 ± 63.5	46.8 ± 57.1	58.2 ± 61.9	42.3 ± 54.5
H. pylori-negative rate	48.4% (657/1358)	48.2% (188/390)	36.8% (42/114)	52.9% (146/276)	48.5% (469/968)	37.8% (104/275)	52.7% (365/693)

Abbreviations: Ab, antibody; JA, Japanese apricot.
Data are expressed as mean ± standard deviation.
There were no significant inter-group differences.
Age groups: Elderly, 65–80 years; non-elderly, 30–64 years.

Table 2 Clinico-serological features according to intake of JA ($n=68$)

	Total subjects ($n=68$)	*Low intake of Japanese apricot* ($n=37$)	*High intake of Japanese apricot* ($n=31$)	P *value*
Age (years)	46.7 ± 8.2	48.2 ± 8.0	44.9 ± 8.3	NS
Gender (male/female)	46/22	24/13	22/9	NS
Smoker, *n* (%)	11 (16.2%)	5 (13.5%)	6 (19.4%)	NS
H. pylori Ab titer (U/ml)	270.6 ± 372.7	365.3 ± 396.7	157.6 ± 311.8	0.021
PG-I (ng/ml)	51.2 ± 21.6	54.4 ± 25.3	47.2 ± 15.5	NS
PG-II (ng/ml)	16.3 ± 9.2	19.8 ± 9.3	12.2 ± 7.2	<0.001
PG-I/II	3.8 ± 1.7	3.0 ± 1.4	4.6 ± 1.6	<0.001

Abbreviations: Ab, antibody; JA, Japanese apricot; NS, not significant; PG, pepsinogen.
Data are expressed as mean ± standard deviation.

Table 3 Histological analysis of gastric mucosa according to intake of JA ($n=68$)

Intake of Japanese apricot	H. pylori	*Neutrophils*	*Mononuclear cells*	*Atrophy*	*Intestinal metaplasia*	*Total*
Antrum						
High intake ($n=31$)	0.5 ± 0.8 **	0.5 ± 1.0 *	1.1 ± 1.1 **	1.5 ± 0.8	0.2 ± 0.6	3.9 ± 3.5 *
Low intake ($n=37$)	1.2 ± 1.0	1.2 ± 1.1	1.9 ± 1.0	1.6 ± 0.7	0.2 ± 0.6	5.8 ± 3.2
Corpus						
High intake ($n=31$)	0.5 ± 0.8 **	0.5 ± 1.0	0.7 ± 1.0 **	0.7 ± 0.7 *	0.0 ± 0.2	2.5 ± 3.3 **
Low intake ($n=37$)	1.4 ± 1.6	1.0 ± 1.1	1.6 ± 0.9	1.1 ± 0.7	0.2 ± 0.5	5.1 ± 3.1

Abbreviation: JA, Japanese apricot.
*$P<0.05$; **$P<0.01$.
Data are expressed as mean ± standard deviation.

however, in the high-intake group, the *H. pylori* bacterial load and mononuclear infiltration scores were significantly lower in both the antrum and corpus as compared with that in the low-intake group. In addition, the scores for neutrophil infiltration in the antrum and atrophy in the corpus were significantly lower. Serum PG was also measured in these volunteers (Table 2). Serum PG-I did not significantly differ between the groups, but PG-II was significantly lower in the high-intake group ($P<0.001$). Hence, the PG-I/II ratio was higher in the high-intake group ($P<0.001$).

Discussion

Our study evaluated the histological and serological effects of JA intake on *H. pylori*-related CAG in residents of Wakayama Prefecture where more than 50% of Japan's JA is grown. The *H. pylori*-negative rate was not increased in the high-intake group. However, the overall, *H. pylori* antibody titers were lower, and in particular, they were significantly lower as compared with that in the low-intake group among the non-elderly (<65 years old) subjects. In general, higher *H. pylori* antibody titers correlate with more active *H. pylori*-related CAG, as shown in clinical and animal studies (Eaton and Krakowka, 1992; Shimizu *et al.*, 1999; Loffeld *et al.*, 2000; Nozaki *et al.*, 2002). In particular, in *H. pylori*-infected Mongolian gerbils, higher antibody titers are associated not only with more active gastritis, but also with a higher incidence of gastric cancer (Shimizu *et al.*, 1999). In light of these previous findings, our results suggest that although JA intake does not eradicate established *H. pylori* infection from the gastric mucosa, it may, by virtue of a direct bactericidal action on *H. pylori* and anti-inflammatory effects, decrease the severity of gastritis.

Histological examination of endoscopic biopsy samples in the non-elderly subjects showed that, compared with the low-intake group, the high-intake group had significantly less *H. pylori* bacterial load, neutrophil infiltration and mononuclear infiltration in the gastric mucosa. As the histological effects of JA intake on *H. pylori*-related CAG were evaluated in a relatively small number of subjects, there is a potential for selection bias. However, the groups of subjects that underwent the analysis do not appear be significantly different from the original cohort, as indicated by the clinical features, except that they included more males. Therefore, these findings are strong histological evidence suggesting that JA intake inhibits *H. pylori* infection and can reduce *H. pylori*-related gastric mucosal inflammation. Furthermore, in the high-intake group, serum PG-II was significantly lower and the PG-I/II ratio was significantly higher as compared with that in the low-intake group. Serum PG-II reflects the activity of *H. pylori*-related gastritis (Plebani *et al.*, 1996; Mardh *et al.*, 2002), and the PG-I/II ratio has been used as a marker for progression of CAG (Samloff *et al.*, 1982;

Miki *et al.*, 1987). This also strongly suggests, from a serological perspective, that JA intake inhibits *H. pylori*-related gastric mucosal inflammation and can effectively prevent the progression of CAG.

In this study, among non-elderly subjects, the high-intake group had significantly lower *H. pylori* antibody titers, but among elderly subjects the *H. pylori* antibody titers did not differ significantly based on JA intake. This probably reflects the natural history of *H. pylori* infection. Compared with elderly subjects with more extensive CAG progression, non-elderly subjects have milder atrophy and higher inflammatory activity, so JA intake probably has a greater inhibitory effect. In other words, the earlier in life that eating JA is adopted, the greater the likelihood of preventing CAG progression and gastric cancer.

The mechanism by which JA inhibits *H. pylori* infection and reduces chronic gastritis activity is presently unknown. However, Miyazawa *et al.* (2006) report that (+)-syringaresinol, a lignan contained in JA extract, inhibits *H. pylori* motility. In addition, Fujita *et al.* (2002) found that JA extract has direct bactericidal activity against *H. pylori*. Thus, JA probably inhibits the progression of *H. pylori*-related CAG by a variety of mechanisms. Correa *et al.* have proposed a multistage model of carcinogenesis due to *H. pylori* infection (Correa and Houghton, 2007) in which CAG is a precancerous condition. According to this model, inhibiting progression to CAG can lead to prevention of gastric cancer, particularly intestinal-type gastric carcinoma. Meanwhile, along with others, we have reported that the incidence of gastric cancer increases gradually with CAG progression (Ohata *et al.*, 2004; Yanaoka *et al.*, 2008a). Therefore, eating JA may be a promising strategy for prevention of gastric cancer.

We have reported that, besides progression of gastric mucosal atrophy, patients with severe gastric mucosal inflammation (high PG-II levels and/or high *H. pylori* antibody titers) are at high risk for gastric cancer, particularly diffuse-type carcinoma (Yanaoka *et al.*, 2008b), and that in these patients *H. pylori* eradication may likely prevent gastric cancer (Yanaoka *et al.*, 2009). In this study, the high-intake group had significantly lower *H. pylori* antibody titers and lower serum PG-II, suggesting that JA intake may be effective in preventing gastric cancer whose mechanism involves *H. pylori*-related active mucosal inflammation. Furthermore, Utsunomiya *et al.* (2005) reported that JA extract is effective in improving insulin resistance. Further studies are needed to confirm whether JA intake inhibits gastric carcinogenesis, but considering that hyperglycemia and insulin resistance are cancer risk factors (Yamagata *et al.*, 2005; Becker *et al.*, 2009), JA intake, in addition to inhibiting CAG progression, may prevent gastric carcinogenesis via this latter route.

Our study has several limitations. Because JA has been considered to be a 'healthy' food with curative or beneficial effects, it is possible that those who have a higher intake of JA may be more likely to have a healthier lifestyle. Although we could not observe significant differences in smoking habits or the *H. pylori*-negative rate between the high- and low-JA intake groups, we cannot exclude the possibility of a secondary association with other unidentified lifestyle factors. Furthermore, the questionnaire in our study did not include an assessment of daily sodium intake for each subject, although high sodium intake enhances gastric mucosal inflammation and is thought to be involved in stomach carcinogenesis as a cancer promoter (Nozaki *et al.*, 2002). However, a simple questionnaire for sodium preference is by no means a reliable indicator of sodium intake in healthy subjects or hypertensive patients (Ohta *et al.*, 2004; Hashimoto *et al.*, 2008). It is well known that JA itself, either dried or pickled, contains a high concentration of sodium (5–20% of net weight); therefore it is highly probable that the high-intake group is a non-sodium-conscious group and JA exerts a potent inhibitory effect on *H. pylori*-related gastritis, even in a group of subjects with high sodium intake.

Our study findings strongly suggest that JA has an inhibitory effect on the *H. pylori*-related active inflammation of the stomach and progression of CAG. This provides a sound basis for further investigation. In Japan, the most important issue in gastric cancer prevention is *H. pylori* infection, which is the major etiological factor in gastric carcinogenesis and which affects up to 60 million people. Promoting dietary habits that protect against gastric cancer, including JA intake, is probably an ideal alternative strategy for gastric cancer prevention. Thus, we are now conducting a follow-up study of the same subjects to analyze the relationship between JA intake and gastric cancer development.

Conflict of interest

The authors declare no conflict of interest.

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REVIEW

CONTROVERSY ON THE MANAGEMENT OF ANTICOAGULANTS AND ANTIPLATELET AGENTS FOR SCHEDULED ENDOSCOPY

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Guidelines on the management of anticoagulants and antiplatelet agents for endoscopy were established by Japan Gastroenterological Endoscopy Society (JGES) in 2005. However, the permeation of the JGES guideline is reported to possibly be low. One of the important causes of this problem is the confusing situation of gaps between the guidelines of various societies. Additionally, our ongoing investigation has revealed another important cause, which is the current daily clinical practice that cessation periods before endoscopy were determined by non-gastroenterological specialists who might be unfamiliar with the JGES guidelines. Considering the low permeation of the guidelines for non-gastroenterological specialists prescribing these agents, we propose that close coordination between various specialists is mandatory to fill the gap between endoscopists and non-gastroenterological specialists.

Key words: anticoagulant, antiplatelet agent, cessation, endoscopy, complication.

INTRODUCTION

Many patients receive anticoagulants and antiplatelet agents to prevent cardiovascular, cerebrovascular or venous thrombolic events.[1–4] In our previous study, approximately 15% of patients who underwent endoscopy were receiving anticoagulants or antiplatelet agents.[5] Additionally, the rapid spread of drug-eluting stents in coronary arteries, requiring dual antiplatelet therapy, raise the proportion of patients receiving combined antithrombotic therapy. These prophylactic agents evidently reduce the risks of thromboembolic events. At the same time, these agents induce the risk of gastrointestinal bleeding, especially for patients receiving combined antithrombotic therapy.[6–10] This dilemma between the risks of thromboembolic events and gastrointestinal bleedings has been a major concern for endoscopists. Also, many endoscopists have faced this dilemma than ever before.

PRESENT SITUATION SURROUNDING GUIDELINES IN JAPAN

Until recently, cessation periods for anticoagulants and antiplatelet agents were determined based on the lifetime of platelets. Consequently, without solid evidence, cessation periods for an irreversible inhibitor agent (e.g. aspirin, ticlopidine) were recommended to be 7–10 days for invasive procedures. In 2005, guidelines on the management of anticoagulants and antiplatelet agents for endoscopic procedures were established by the Japan Gastroenterological Endoscopy Society (JGES),[11] referring to the American Society for Gastrointestinal Endoscopy (ASGE) guidelines. These guidelines recommended shorter cessation periods for aspirin, ticlopidine and dual antiplatelet therapy (3, 5 and 7 days, respectively) than ever before, based upon a study of the time-course of primary hemostasis after the cessation.[12] However, these guidelines show a practical cessation period for these two agents only. Additionally, as our previous study revealed, the permeation of these guidelines to prescribing doctors with various specialties can be low.[5]

The possible cause of the low permeation is the confusing situation of gaps between the guidelines of various societies (Table 1). Both the JGES guidelines and the ASGE guidelines classify procedural risks and thromboembolic risks into low-risk and high-risk procedures. They also give us recommendations of cessation periods of anticoagulants and antiplatelet agents for each category based on a combination of procedural risks and thromboembolic risks. However, the details of these guidelines are somewhat different. Cessation before endoscopic biopsy is not recommended in the ASGE guidelines. In contrast, cessation before endoscopic biopsy is principally recommended in the Japanese guidelines, including the JGES guideline. Moreover, even between Japanese guidelines, there are gaps concerning cessation period. By November in 2009, the JGES guidelines and the previous version of Japanese Circulation Society (JCS) guidelines recommended 3 days and 7 days cessation for aspirin; and 5 days and 10–14 days cessation for ticlopidine, respectively.[13] Although the JCS guidelines were revised in 2009 based upon the same evidence referred to in the JGES guideline,[14] they recommend the same cessation periods for these two agents for low-risk procedures only. Moreover, they recommend longer cessation periods for high-risk

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Table 1. Differences between the guidelines of various societies concerning cessation before endoscopy

	Low-risk procedures	High-risk procedures
JGES	Discontinue aspirin 3 days, ticlopidine 5 days and combination of both 7 days, respectively. Discontinue warfarin 3–4 days. Check INR < 1.5 before high-risk procedures.	
JCS (version issued in 2004)	Discontinue aspirin 7 days, ticlopidine 10–14 days and cilostazol 3 days, respectively.	
JCS (version revised in 2009)	Discontinue aspirin 3 days, ticlopidine 5 days and combination of both 7 days, respectively.	Discontinue aspirin 7 days, ticlopidine 10–14 days and cilostazol 3 days, respectively.
	Discontinue or warfarin. Check INR < 1.5 before procedures.	
ASGE	Continue.	Continue aspirin or NSAIDs. Discontinue clopidogrel 7–10 days. Discontinue warfarin 3–5 days.

ASGE, American Society for Gastrointestinal Endoscopy; INR, international normalized ratio; JCS, Japanese Circulation Society; JGES, Japan Gastroenterological Endoscopy Society; NSAIDs, non-steroidal anti-inflammatory drugs.

procedures. Altogether, there is no solid evidence to establish unified guidelines.

FACT-FINDING STUDY

To find out other possible causes of the low permeation, we conducted a more detailed fact-finding study about current daily clinical practice regarding the management of these agents for scheduled endoscopy. This fact-finding study was conducted at the Department of Endoscopy and Endoscopic Surgery of the University of Tokyo on two days of the week (Wednesday and Thursday) from June 2009 to November 2009 before the revised JCS guidelines were issued. Subjects were limited to outpatients. Among 1878 patients who underwent scheduled endoscopy, 253 patients (13.5%) receiving anticoagulants or antiplatelet agents were enrolled into this study using a questionnaire that was handed out before endoscopy. The patients sent back the questionnaires approximately 14 days after endoscopy. The following questions were included in the questionnaire.

- What anticoagulants or antiplatelet agents are you prescribed?
- For what comorbidity are you prescribed each agent?
- What specialty does your doctor prescribing each agent have?
- How long are you ordered to stop each agent before and after endoscopy?
- What specialty does the doctor who determined your cessation period have?
- Are you prescribed any anti-ulcer agents or other agents affecting digestive organs?
- Have you experienced any additional symptoms before and during 2 weeks after endoscopy?

Among 253 patients who were receiving anticoagulants and antiplatelet agents, 208 patients (82.2%, 71.5 ± 9.3 years, range 42–94 years, male/female 131/77) sent back valid responses to the questionnaires.

We defined the following as antiplatelet agents: cyclooxygenase inhibitors (e.g. aspirin), phosphodiesterase inhibitors (e.g. cilostazol), purinergic receptor antagonists (e.g. ticlopidine), serotonin receptor antagonists (e.g. sarpogrelate), eicosapentaenoic acid preparations (e.g. icosapentate), and prostaglandin preparations. Endoscopic procedures we investigated were 144 esophagogastroduodenoscopies (EGD) and 64 colonoscopies with and without invasive procedures. Invasive procedures we investigated were defined as those with biopsy or therapeutic endoscopies, including polypectomy and endoscopic mucosal resection (EMR). We principally do not carry out therapeutic endoscopies on EGD for outpatients, so in this study, all therapeutic procedures were carried out on colonoscopies. To investigate complications including gastrointestinal bleeding, we checked medical records in addition to subjective symptoms that were obtained from questionnaires.

The endoscopies were ordered by various doctors with various specialties, including gastroenterology, cardiology and neurology. All patients received explanations of the risks and benefits of the endoscopies and were provided with written informed consent forms by the doctors in charge. Written informed consent forms for this study were sent back with the questionnaires. This study was approved by the ethics committee of our institution.

Proportion of prescribed agents

Among 208 patients, 148 patients (71.2%) were receiving a single agent and 60 patients (28.8%) more than two agents. Forty patients (19.2%) were receiving warfarin as anticoagulant. The most common antiplatelet agent was aspirin in 136 patients (65.4%), followed by prostaglandin preparations, eicosapentaenoic acid preparations, ticlopidine and clopidogrel. Ninety-eight patients (47.1%) were receiving antiulcer agents. Among them, 71 patients (72.4%) were receiving proton pump inhibitors (48.9%) or H2 receptor antagonists (23.5%).

Proportion of pre-existing comorbidities of patients

The most common comorbidity requiring anticoagulants or antiplatelet agents was ischemic heart disease in 65 patients (31.2%), followed by arrhythmia and cerebrovascular disturbance in 37 (17.8%) and 34 patients (16.3%), respectively (Fig. 1). Among 65 patients who had ischemic heart disease, 41 patients (63.1%) had a mechanical stent in the coronary artery. The prevalence rate of ischemic heart disease was 55% in patients receiving combined antithrombotic therapy.