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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

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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

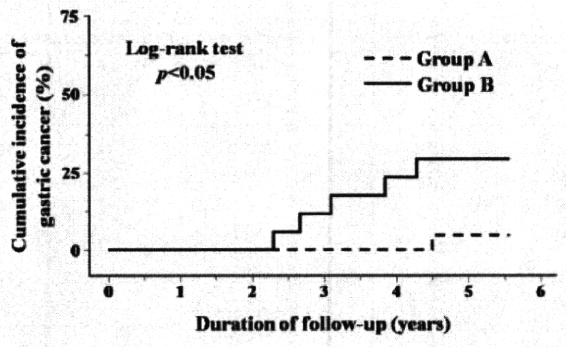

Figure 1. Kaplan–Meier analysis of metachronous cancer development in patients with early gastric cancer resected endoscopically. Group A received etodolac treatment (300 mg/day), while Group B did not receive any treatment. Both groups were followed for up to 5 years. Cancer incidence rates were 898/100,000 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

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>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;

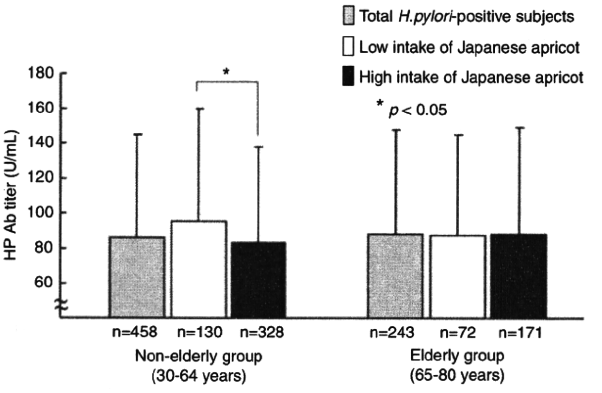

Figure 1 Comparison of *H. pylori* antibody titers on the basis of JA intake, with stratification by age. In *H. pylori*-positive subjects (*H. pylori* antibody titer ⩾30 U/ml), we compared *H. pylori* antibody titers according to JA intake, with stratification for age. In the elderly group (65–80 years), 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 anti-ulcer 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.

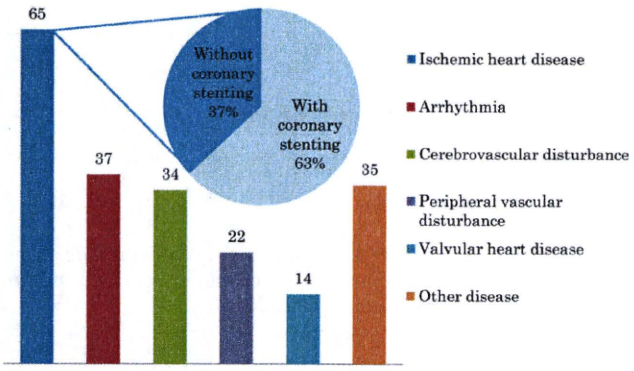

Fig. 1. Comorbidities of 208 patients taking anticoagulants or antiplatelet agents. Among 65 patients with ischemic heart disease, 41 patients (63%) had a mechanical stent in the coronary artery.

Cessation period

Histograms of cessation periods before and after endoscopy are shown in Figures 2 and 3, respectively. Most patients underwent endoscopy without cessation or after a cessation period of 6–7 days. For details, patients receiving single antithrombotic therapy tended to undergo endoscopy after a cessation period of 6–7 days principally, and patients receiving combined antithrombotic therapy tended to undergo endoscopy without cessation. However, patients who underwent endoscopy with invasive procedures after the cessation period tended to restart antithrombotic therapy the next day after endoscopy.

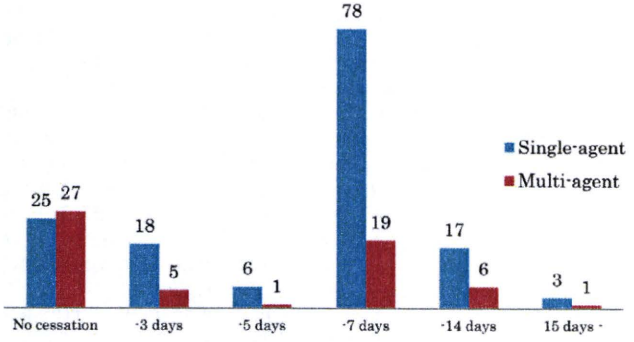

Fig. 2. Preoperative cessation period of patients receiving single-agent or multi-agent antithrombotic therapy. Among 208 patients, 206 patients sent back valid responses to our questionnaire on preoperative cessation periods.

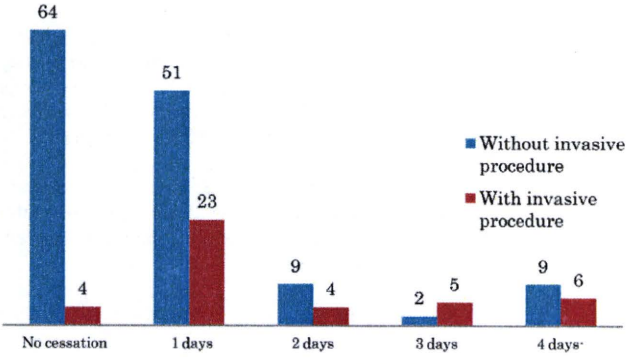

Fig. 3. Postoperative cessation period of patients who underwent invasive or non-invasive procedures. Among 208 patients, 177 patients sent back valid responses to our questionnaire on postoperative cessation periods.

Specialty of doctors who determined cessation periods

Cessation periods before endoscopy were determined by cardiology specialists for 57.7% of the patients. For only 4.8% of the patients, cessation periods before endoscopy were determined by gastroenterological specialists (Fig. 4). By contrast, for 36.5% of the patients, cessation periods after endoscopy were determined by gastroenterological specialists, including operators of endoscopes.

Complications

In this study, no symptom related to thromboembolic events and gastrointestinal bleeding was observed. No patients underwent invasive procedures without cessation.

DISCUSSION

In our previous study, we revealed the low permeation of the JGES guidelines in the cessation period of aspirin alone, ticlopidine alone and the combination of both. Most patients receiving aspirin alone or ticlopidine alone underwent endoscopy after a cessation period of 6–7 days. Among patients receiving the combination of aspirin and ticlopidine, most patients underwent endoscopy without cessation. These results indicated a large discrepancy between the clinical daily practice and the recommendations of the JGES guidelines. The present study also revealed this discrepancy.

Considering the results of the present study, the low permeation of the guidelines is caused by the daily clinical practice that cessation periods are mainly determined by prescribing doctors with non-gastroenterological specialties. Furthermore, this means that risk estimation of comorbidities for cessation is principally left to prescribing doctors. The present study revealed the limitations of the guidelines for gastroenterology specialists themselves to decide cessation periods considering comorbidities. We propose that enhancement of coordination between various specialists is mandatory to manage anticoagulants and antiplatelet agents appropriately.

The present study also revealed the current daily practice of the cessation period after endoscopy. In the JGES guidelines, restarting antithrombotic therapy is recommended shortly after endoscopy as far as low risks of bleeding are confirmed after endoscopy. However, there are no solid criteria to judge whether or not risks of bleeding are low. In the present study, 74 patients, including 23 patients who underwent invasive procedures, restarted antithrombotic therapy the next day after endoscopy. Considering that they all did not experience gastrointestinal bleeding after endoscopy, restarting the next day after endoscopy may be reasonable.

Still, we have another problem, which is the necessity for cessation before minimally invasive biopsy. Although

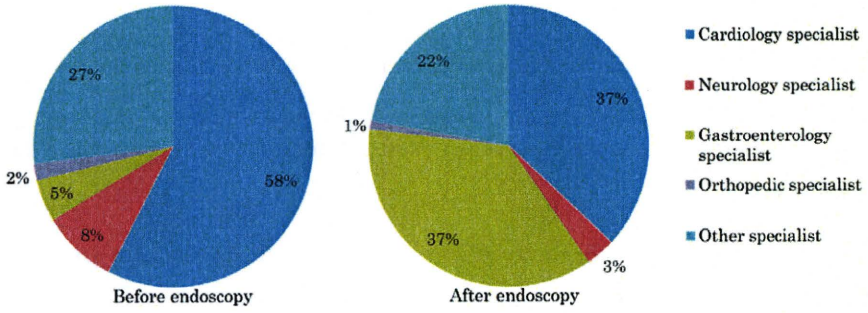

Fig. 4. Specialties of doctors who determined cessation periods before and after endoscopy.

Western guidelines recommend biopsy without cessation, Japanese physicians principally secure cessation before biopsy and the JGES guidelines also recommend this. We speculate that the most important reason is racial differences, as mentioned in the JGES guidelines.[11] However, after invasive procedures, thromboembolic events during cessation are reported to result in lethal outcomes rather than bleeding events.[15] Additionally, there are not enough data on racial differences to conclude that bleeding events are more life-threatening than thromboembolic events for Japanese compared to Western people. Although we need further accumulation of data concerning this problem, minimally invasive biopsy without cessation might be acceptable for Japanese.

Taken as a whole, the present study revealed further details of clinical daily practice concerning the management of anticoagulants and antiplatelet agents for scheduled endoscopy. However, it has some limitations. To exclude selection bias in a single-center design and insufficient number of patients, we need further investigation in a multi-center design. We do believe that in a few years it will be possible to correct the current gap between endoscopists and non-gastroenterological specialists in the management of anticoagulants and antiplatelet agents for scheduled endoscopy.

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A second-look endoscopy after endoscopic submucosal dissection for gastric epithelial neoplasm may be unnecessary: a retrospective analysis of postendoscopic submucosal dissection bleeding

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Background: Endoscopic submucosal dissection (ESD) is one of the curative endoluminal surgical procedures for gastric epithelial neoplasms. There has been little research on bleeding after gastric ESD.

Objective: To investigate cases of post-ESD bleeding and to verify whether a second-look endoscopy after ESD is effective in the prevention of delayed bleeding.

Design: A retrospective study with consecutive data.

Setting: A single tertiary referral center.

Subjects: A total of 454 gastric epithelial neoplasms (386 early gastric cancers and 68 gastric adenomas).

Interventions: ESD and second-look endoscopy.

Main Outcome Measurements: Predictors on post-ESD bleeding by univariate analysis, incidence of post–ESD bleedings, and the timing of those before and after second-look endoscopy.

Results: Post–ESD bleeding occurred in 26 (5.7%) lesions. Gross type (flat or depressed type) was the only factor influencing post-ESD bleeding. All cases of post-ESD bleeding occurred within 14 days after ESD (median 2; range 0-14), and bleeding tended to occur from the lower and upper stomach earlier and later, respectively. In 19 lesions with delayed bleeding more than 24 hours after ESD, the maximum delayed bleeding rates before and after the second-look endoscopy were 2.8% and 2.5%, respectively.

Limitations: A retrospective, single-center analysis.

Conclusions: A second-look endoscopy after gastric ESD may contribute little to the prevention of delayed bleeding. (Gastrointest Endosc 2010;71:241-8.)

Endoscopic submucosal dissection (ESD) has been increasingly established as a promising endoluminal technique for GI epithelial neoplasms. Unlike conventional techniques such as EMR, ESD consists of circumferential mucosal incision and submucosal dissection, which have made it possible, even in a large tumors or tumors with ulcerative findings, to resect in en bloc fashion with tumor-free margins.[1-5] By using ESD, we have performed curative resection for node-negative early gastric cancer (EGC)[6] instead of open surgery.

One of the major concerns about gastric ESD is postoperative bleeding. It occurs in approximately 5% of the patients who undergo gastric ESD.[7-10] In almost all cases, endoscopic hemostasis is effective in stopping bleeding if an emergency endoscopy is properly performed. Therefore, it is necessary to determine the nature of the post-ESD bleeding and the appropriate management. In practice, a second-look endoscopy is routinely performed the next day or later after ESD in most hospitals in Japan to check for the possibility of postoperative bleeding. However, performing a second-look endoscopy without any signs of bleeding has not yet been validated, and, to the contrary, bleeding even after the confirmation of hemostasis sometimes occurs. Because there have been few reports concerning post-ESD bleeding or its

Abbreviations: EGC, early gastric cancer; ESD, endoscopic submucosal dissection; GA, gastric adenoma; PPI, proton pump inhibitor.

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prevention,[10] we retrospectively reviewed our gastric ESD cases to verify the clinicopathological features of post-ESD bleeding and to confirm whether a second-look endoscopy was necessary for preventing delayed bleeding.

PATIENTS AND METHODS

A total of 476 lesions with a histologic diagnosis of gastric epithelial neoplasms (406 EGCs and 70 gastric adenomas [GAs]) were consecutively treated with ESD in our hospital from December 2003 to November 2008. Among them, 454 lesions (386 EGCs and 68 GAs) were included after exclusion of 14 lesions in a remnant stomach after gastrectomy or in a gastric tube after esophagectomy and 8 lesions in which perforation occurred during ESD caused by their specific physiological conditions.

ESD was principally indicated for possible node-negative EGCs according to the criteria of Gotoda et al[6] based on endoscopic findings including chromoendoscopy with biopsy. In cases of possible node-positive EGCs, ESD was only indicated when the lesions were considered to be technically removable from the gastric wall and patients desired to undergo ESD after sufficient information was provided by both endoscopists and surgeons. In cases of GAs, ESD was only indicated when it was possible that the lesion contained foci of cancer or the patients strongly desired the lesion to be resected. All patients provided written informed consent before undergoing treatment.

ESD procedures

The ESD technique was described elsewhere.[1-5] In brief, after circumferential marking, submucosal injection was performed below the tumor to create a submucosal fluid cushion. Next, circumferential cutting of the mucosal layer with muscularis mucosa approximately 5 mm outside the marking was performed. Subsequently, the submucosal layer beneath the tumor was dissected to detach it from the gastric wall. A mixture of 10% glycerin, 5% fructose, and 0.9% saline solution (Glyceol; Chugai Pharmaceutical Co, Tokyo, Japan) containing 0.005% indigo carmine and 0.0005% epinephrine was used as submucosal fluid.[11] Hyaluronic acid was additionally used when the lesion could not rise enough by using Glyceol alone.[12,13] The Flexknife (KD-630L; Olympus, Tokyo, Japan) was selected as the main electrosurgical knife.[3,14] Other knives, such as the IT knife (KD-610L; Olympus),[1,2] Hookknife (KD-620LR; Olympus),[4] Splash-needle (DN-2618A; Pentax, Tokyo, Japan),[5] and a needle-knife, were used in some cases instead of the Flexknife, according to the tumor characteristics and/or operator preference. Hemostatic forceps (HDB2422 W; Pentax) were used for bleeding during ESD or for visible vessels on the mucosal defect after removal.[15]

Capsule Summary

What is already known on this topic

- Endoscopic submucosal dissection (ESD) facilitates en bloc resection with tumor-free margins in gastric cancers but is associated with a postoperative bleeding rate of 5%.

What this study adds to our knowledge

- In a retrospective study of 454 cases of gastric ESD, postoperative bleeding occurred in 26, all within 14 days.
- Delayed bleeding rates before and after the second-look endoscopy were 2.8% and 2.5%, respectively.

The patient was usually allowed to eat a light meal the day after ESD. In principle, a second-look endoscopy was performed once within 1 week to check whether there was a recent hemorrhage or a possible bleeding spot (a nonbleeding visible vessel) that should be treated on the mucosal defect. The day of the second-look endoscopy was decided by the operator according to the patient's condition or the operation day once during the hospitalization. If the hospitalization was uneventful, patients were discharged within 1 week after ESD. Principally, the patients receiving anticoagulants and/or antiplatelet drugs were instructed to stop taking them for 1 week before and 1 week after ESD. When bleeding or nonbleeding visible vessels were seen (sometimes in removing adherent clots by forceps or water jet) on the second-look endoscopy, prophylactic hemostasis was performed. Clipping with hemostatic clips (HX-610-135 or HX-610-090L; Olympus) was performed for large nonbleeding vessels, and thermocoagulation with hemostatic forceps was performed for bleeding vessels or small nonbleeding vessels or in locations where it was difficult to place a clip because of consolidation of the ulcer bed. Between the day of ESD and the first day of feeding (2 days in most cases), 20 mg omeprazole twice daily was administered intravenously. Thereafter, from the day of feeding to at least 2 weeks after ESD, according to the decision of physicians in charge, one of the following proton pump inhibitors (PPIs) was administered orally: 10 mg rabeprazole, 20 mg omeprazole, or 30 mg lansoprazole once daily (these were thought to be equally effective for acid suppression).[16] A follow-up endoscopy was performed 2 months after ESD. Patients were also asked to contact the physicians in case of hematemesis or melena, even after discharge. When perforation or post-ESD bleeding occurred, the schedules for discharge including food intake were changed according to the patient's condition. In cases of possible post-ESD bleeding, the patient underwent an emergency endoscopy, and endoscopic hemostasis was performed on bleeding spots or nonbleeding visible vessels, mainly by clipping or thermocoagulation.

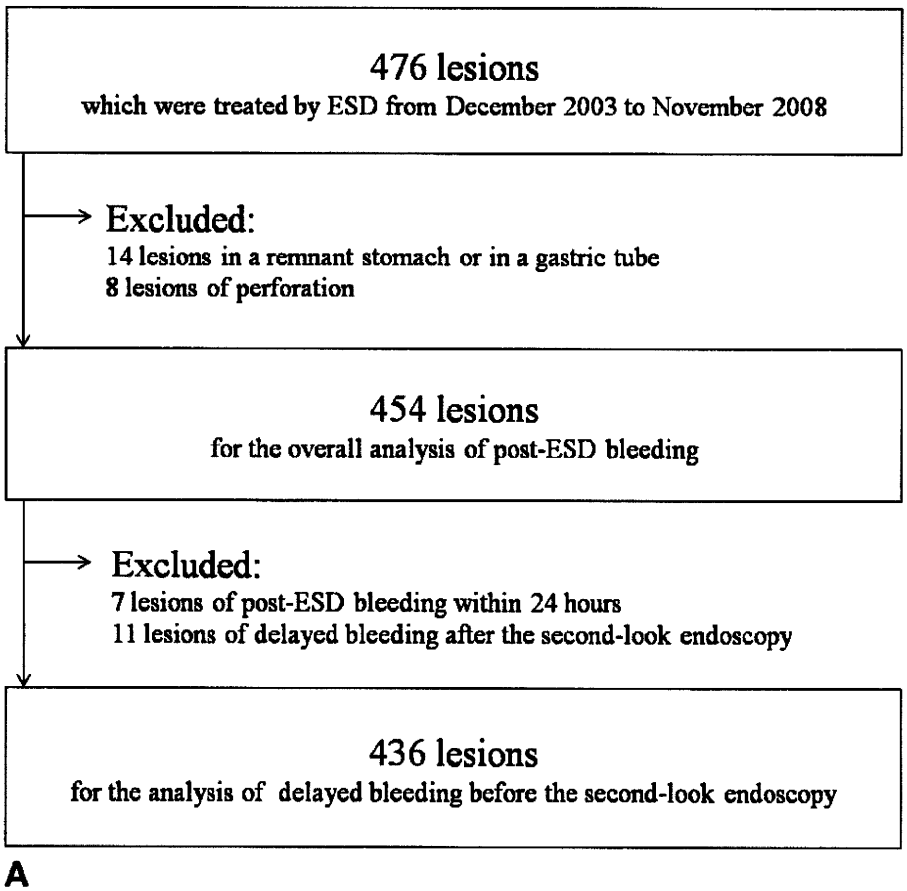

Figure 1. A, Flowchart showing the inclusion in the analysis of delayed bleeding before the second-look endoscopy. **B,** Flowchart showing the inclusion in the analysis of delayed bleeding after the second-look endoscopy. (Continued on next page)

Data analysis

Post-ESD bleeding was defined as massive bleeding from the mucosal defect after ESD, as diagnosed by the emergency endoscopy, which was performed because of hematemesis or melena. To investigate factors influencing post-ESD bleeding, the following variables were analyzed: age, sex, comorbidities (hypertension, diabetes mellitus, heart disease, chronic renal failure, and liver cirrhosis), and the use of anticoagulants and/or antiplatelet drugs (patient-related factors); the location (upper third, middle third, or lower third), circumference (anterior wall, posterior wall, lesser curve, or greater curve), gross type (0-I/IIa, 0-IIb/IIc, or combined type), ulcerative findings in the submucosal layer (endoscopically present or absent), the resection style (en bloc or piecemeal), tumor size (maximum diameter of the resected tumor actually measured), tumor depth (mucosal tumor or submucosal invasive tumor), and histologic type (intestinal type, diffuse type, or adenoma) (lesion-related factors); the period of ESD (early [2003-2005] and late [2006-2008]); and the operator's experience with gastric ESD ($\leq$30 cases, 31-100 cases, and $>$100 cases) (operator-related factors).

Delayed bleeding was defined as post-ESD bleeding diagnosed more than 24 hours after ESD. From the consecutive cases, rates of overall post-ESD bleeding and delayed bleeding before and after the second-look endoscopy were investigated to determine the usefulness of the second-look endoscopy. The maximum follow-up duration was 56 days when almost all the artificial ulcers were considered to be cured.[17] Flow charts for inclusion in each analysis are shown in Figure 1.

Statistical analysis

Univariate analysis was performed by using the Student *t* test for age and tumor size; the Fisher exact test for probability for sex, comorbidities, the use of anticoagulants and/or antiplatelet drugs, ulcerative findings, resection style, tumor depth, and the period of ESD; and the χ^2 test for location, circumference, gross type, histologic type, and the operator's experience. Statistical significance was set at a P value of $<.05$, and if there were more than 1 predictor with a significant difference by univariate analysis, multivariate analysis by using a logistic regression