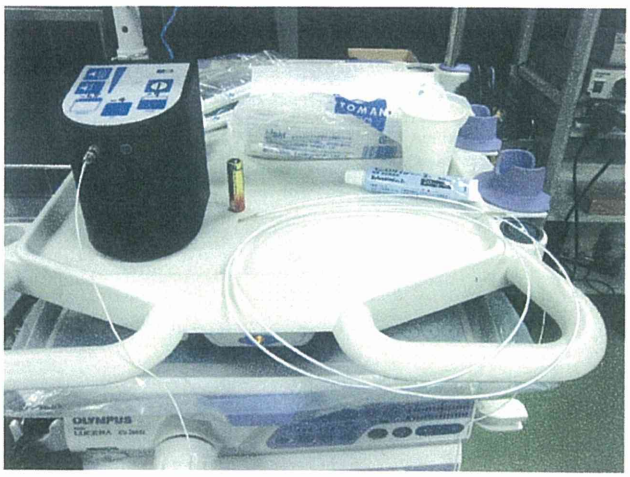

Figure 1. The DOP-US system consisted of a battery-powered portable transceiver and a disposable probe. It converted blood flow signals at the tip of the probe into sound output from a speaker.

Rabeprazole 20 mg (Eisai Co Ltd, Tokyo, Japan) was administered to all patients for 8 weeks.[3] Second-look endoscopy was not performed in any of the patients.

Outcome measures

The detectability of DOP-US signals in post-ESD ulcers was evaluated as the primary endpoint. Incidence of delayed bleeding, procedure time, and adverse events were assessed as secondary endpoints. Delayed bleeding was defined as hematemesis or melena that required endoscopic hemostasis and decreased hemoglobin count by more than 2 g/dL from day 2 until day 30. The procedure time was measured from the start of the Doppler examination until the end of the procedure and it included the time required to perform coagulation for areas with a DOP-US+ or active bleeding. Adverse events were evaluated according to the National Cancer Institute Common Terminology Criteria for Adverse Events version 3.0.

RESULTS

Demographics of the patients with the lesions, blood vessel appearance, Doppler findings, and procedure data are provided in Table 1. A total of 13 oozing bleeding sites were observed in 10 patients and 1 had a DOP-US+. Conspicuous bleeding had already stopped during the ESD procedure; therefore, oozing bleeding just after ESD was minor. All sites were coagulated, bleeding was stopped, and the DOP-US+ became silent. Of 136 NBVVs, 24 (18%) were DOP-US+ and were coagulated. After coagulation (once at 19 sites, twice at 3 sites, and 3 times at 2 sites), all signals became silent. The remainder of the 112 NBVVs (82%) and 8 adherent clots were DOP-US− (Fig. 2); thus, they were left untreated. A DOP-US+ was heard at 7 areas that had no bleeding stigmata, and the areas were coagulated until the signals became silent (Fig. 3, Video 1). No delayed bleeding occurred in the 10 patients within 30 days. The median time required for performing the Doppler procedure was 34 minutes, but it improved with experience so that only 18 and 19 minutes were required in patients 9 and 10, respectively. There were no procedure-related adverse events that were more severe than grade 3 in National Cancer Institute Common Terminology Criteria for Adverse Events version 3.0.

DISCUSSION

In this study, the use of DOP-US changed endoscopic management of post-ESD ulcers in 10 patients. A total of 32 DOP-US+ areas in the 10 patients with post-ESD ulcers were treated under DOP-US guidance, and none of the 112 DOP-US negative (DOP-US−) NBVVs rebled without endoscopic treatment.

Among the 32 areas with DOP-US+, 7 had no endoscopic sign of bleeding stigmata. Such areas were located mostly at the periphery of the ulcers. In 2 of 7 areas that had a DOP-US+ but no endoscopic finding of bleeding stigmata, vessels were identified in the submucosa after washing out and watching the region carefully. In post-ESD ulcers, the submucosa in the central area was almost removed by ESD, whereas the submucosa in the peripheral area remained partially intact. We speculate that the vessels in the central area were already cut and coagulated during ESD, but the vessels in the peripheral area remained viable beneath the submucosa and were missed by conventional observation. Such nonvisible vessels could become a potential cause of delayed bleeding.

We found a DOP-US+ in 24 of 139 NBVVs (17%), but more than 80% of the NBVVs were DOP-US−, and there was no delayed bleeding, although they were left untreated. Post-ESD ulcers were fresh and were not coated with fibrinopurulent exudates, as are peptic ulcers; therefore, even small NBVVs could be observed on the ulcer base. However, routine coagulation of all NBVVs is currently recommended at the end of ESD, even if there is no evidence of bleeding. Taking all of this into consideration, we might be overtreating many NBVVs in post-ESD ulcers that have no risk of delayed bleeding. In patient 10, an 8-cm specimen was removed for a 6-cm tumor. Although none of the 23 NBVVs in large ulcers was treated because they did not have a DOP-US+, no delayed bleeding occurred. Wong[9] suggested that if active blood flow in bleeding lesions has ceased, for example, as a result of spontaneous intravascular thrombosis, the risk of recurrent bleeding is reduced. We observed a few NBVVs that appeared as thick pulsating vessel stumps, which were not treated because there was no Doppler signal; however, delayed bleeding did not occur from these vessels. We suspect that such vessels would be spontaneously organized and disappear after a while. Repeated electrical coagulation was required for persistent signals in 3 DOP-US+ NBVVs. Confirmation of the disappearance of the

TABLE 1. Summary of results in this study

	Tumor				Vessel appearance and Doppler signal						
Patient	Location	Type	UI	Size (mm)	Oozing bleeding	Nonbleeding visible vessel	Adherent clot	Area without stigmata of recent bleeding	Endoscope	Transparent hood	Procedure time (min)
1	M, LC	IIa	–	25	○	●○○○○○○		●	GIF-Q260J	+	37
2	L, PW	IIc	+	10		●○○○○○○	○○○○		GIF-Q260J	+	33
3	M, LC	IIc	–	35	○○○○	●●●●○○○○○○ ○○○○	○○		GIF-2TQ260M	+	31
4	L, LC	IIc	–	20		●●●○○○○○○○ ○○○	○		GIF-H260Z	+	42
5	M, LC	IIc	+	25	●○○○	○○○○○○○○○○ ○○○○	○	●	GIF-Q260J	+	49
6	M, LC	IIa	–	40	○	●●●●●●●●○ ○○○○○○○○○ ○○○			GIF-2TQ260M	+	39
7	L, GC	IIa	–	40	○○	●○○○○○○○○ ○○○○○○○○		●	GIF-2TQ260M	+	26
8	L, PW	IIc	–	22	○	●●●●●○○○○		●●	GIF-2TQ260M	+	34
9	U, PW	IIa	–	35		●○○○○○○○○ ○○○○○		●●	GIF-2TQ260M	+	18
10	M, LC	IIa	–	57		○○○○○○○○○ ○○○○○○○○○ ○○○○○			GIF-2TQ260M	+	19

AW, Anterior wall; *GC*, greater curvature; *L*, lower third; *LC*, lesser curvature; *M*, middle third; *PW*, posterior wall; *U*, upper third; *UI*, ulcer scar; ●, positive Doppler US signal; ○, negative Doppler US signal.

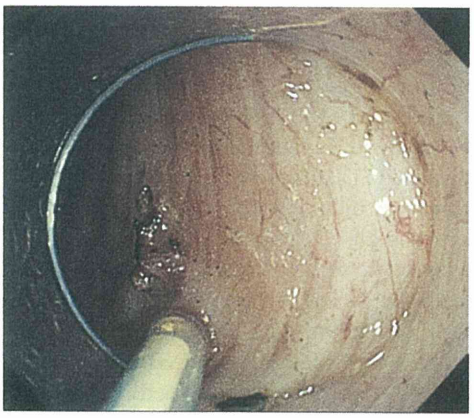

Figure 2. The NBVV was observed in the center of the post-ESD ulcer, and because there was no Doppler signal, the vessel was not treated.

blood flow signal by electrical coagulation with DOP-US might also contribute to risk reduction for delayed bleeding. We also expect that the use of DOP-US at the time of the first ESD procedure significantly reduces the incidence of delayed bleeding; therefore, routine second-look endoscopy might not be necessary as standard practice.

Post-ESD ulcers were larger than ordinary peptic ulcers (in particular, we included lesions >2 cm); therefore, it required more than 30 minutes for Doppler examination at the beginning of the study. The transparent hood that is used commonly in ESD was a good accessory for the Doppler procedure because it could be fixed to the proximal mucosa to ensure that the probe was in stable contact with the ulcer base. The multibending, double-channel videoendoscope (EVIS-2TQ260M; Olympus Medical Systems) was also beneficial for the procedure. We searched for DOP-US signals with a probe that was inserted through 1 channel of the videoendoscope, and we coagulated the DOP-US+ area by a hemostatic forceps through another channel. When using an ordinary single-channel videoendoscope, we sometimes missed the DOP-US+ area during replacement of the Doppler probe with the hemostatic forceps. Moreover, the videoendoscope was equipped with a dedicated water-jet channel that was connected to an electrical pump, so that the blood or mucous clots on the ulcer could be flushed out without withdrawing the devices that occupied the working channels. Although the Doppler examination can be performed with a conventional videoendoscope, the multibending function of the videoscope facilitated approaching the ulcer that was located in an area that was difficult to reach by conventional endoscopy.[10]

In conclusion, we detected Doppler US signals efficiently in post-ESD ulcers. Our instrumental setting and maneuver warrant further investigation to clarify whether DOP-US can reduce delayed bleeding and avoid unnec-

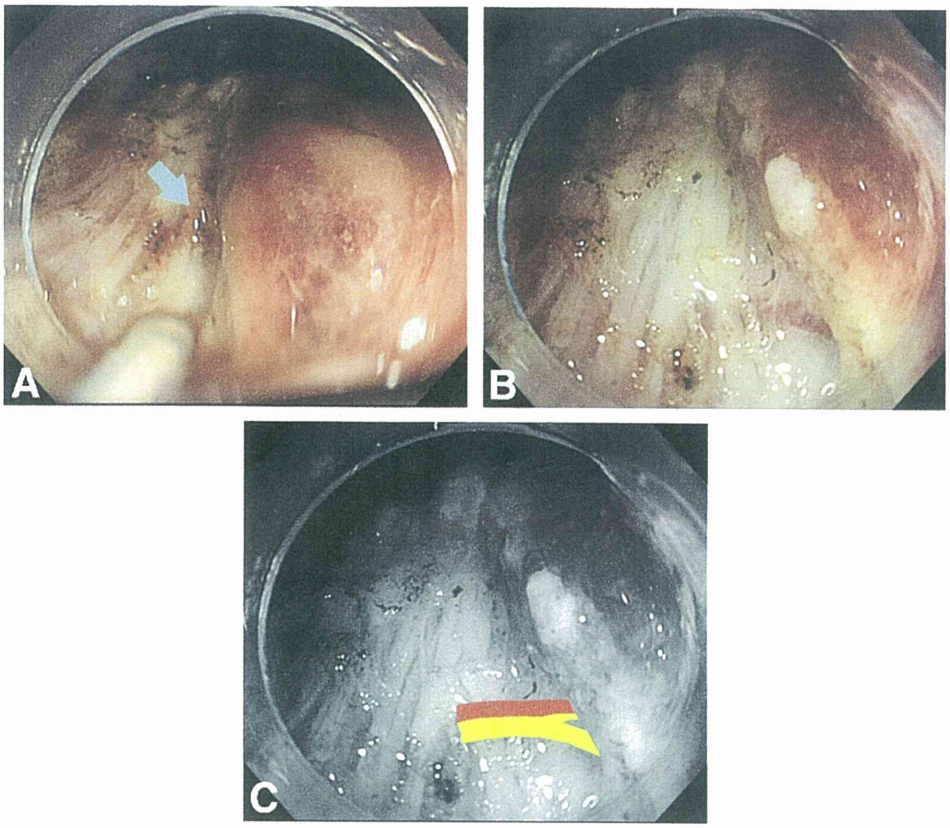

Figure 3. A, A DOP-US+ was detected at the periphery of the post-ESD ulcer, although the area had no obvious endoscopic appearance of bleeding stigmata (*arrow*). **B**, After washing the area with a water jet, the vessel beneath the submucosa became visible. **C**, The reddish vessel was more perceptible, but there was a strong pulsatile signal in an area adjacent to the reddish vein (yellow), which indicated the presence of an accompanying artery (red).

essary coagulation of NBVVs in post-ESD ulcers in patients treated for EGC.

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Helicobacter pylori Eradication Prevents Extension of Intestinalization Even in the High-Risk Group for Gastric Cancer

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Key Words

Helicobacter pylori eradication · Gastric cancer · Atrophic gastritis · CDX2

Abstract

Background/Aims: CDX2 is associated with the intestinal phenotype in the gastrointestinal tracts and is expressed in the intestinal type of gastric cancer. *Helicobacter pylori*-associated atrophic gastritis is characterized by aberrant expression of CDX2. The aim was to investigate the effects of eradication to the expression of genes related to the gastric and intestinal phenotype including *CDX2*. We compared the effect of eradication between the patients at high risk for gastric cancer and controls. ***Methods:*** 20 patients with endoscopic resection for early gastric cancer and 12 sex- and age-matched controls were studied. *CDX2* and mucin mRNA expressions were examined using whole biopsy specimens and microdissected gastric glands taken from corpus lesser and greater curves before and 1 year after eradication. ***Results:*** *CDX2* and *MUC2* expressions in the cancer group were significantly higher than in the controls and were significantly decreased after eradication. *MUC5AC* ($p = 0.01$) and *MUC6* ($p = 0.02$) expression significantly increased in the control group; the difference between the two groups became significant after eradication. *CDX2* expression in the glands without goblet cells was detectable and disappeared after eradication. ***Conclusion:*** *H. pylori* eradication can reverse gastric phenotype and diminish aberrant *CDX2* expression in the early stage of intestinalization.

Introduction

The extent and severity of *Helicobacter pylori*-induced atrophic gastritis, particularly corpus atrophy, is the most important risk factor for the development of gastric cancer [1–3]. Although recent papers have reported a prophylactic effect of *H. pylori* eradication on the development of gastric cancer, the molecular pathogenesis of prevention remains unclear.

Mucins are heavily glycosylated proteins. Twelve core proteins for human mucins (MUC1, 2, 3, 4, 5AC, 5B, 6, 7, 8, 9, 11, 12) have been identified [4–7]. The normal gastric mucosa shows cell type-specific expression of MUC5AC

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and MUC6 and does not express MUC2. Both cancer and intestinal metaplasia (IM) are associated with alterations in the pattern of mucin expression; loss of expression of MUC5AC and increased mucin heterogeneity have been reported in gastric cancer and underexpression of MUC5AC and MUC6 along with de novo expression of MUC2 have been described in IM.

CDX proteins function as intestine-specific transcription factors and act as master regulators of intestinal development and differentiation [8–10]. In Cdx2-transgenic mice, aberrant Cdx2 expression induces IM and plays a significant role in the genesis and progression of gastric carcinoma [11]. In particular, loss of CDX2 expression leads to focal gastric differentiation in the colon [12], while aberrant expression of CDX2 in the upper gastrointestinal tract is a key event in the pathogenesis of Barrett's mucosa in the esophagus and of IM in the stomach [13, 14]. CDX2 is expressed in adenocarcinomas from various organs such as the stomach, colon and pancreas, and may be clinically useful in predicting the outcome of patients with advanced cancer [15–19]. Here we investigated *CDX2* mRNA expression in metaplastic and non-metaplastic gastric glands among patients with early intestinal-type gastric cancer compared with control subjects using fixed-point biopsy samples. We also assessed the effects of *H. pylori* eradication on gastric and intestinal phenotypes and *CDX2* expression.

Methods

This was a case-control study of patients before and after endoscopic submucosal dissection (ESD) for early gastric cancer and non-cancer controls. Patients were enrolled for the study between October 2006 and April 2008.

Patients

Patients with ESD for early stage, non-cardia intestinal-type gastric cancer without lymph node metastasis were enrolled as the high-risk group for gastric cancer. The control group consisted of subjects who had been previously diagnosed as *H. pylori*-positive gastric ulcer or atrophic gastritis. Exclusion criteria included prior *H. pylori* eradication, use of anti-secretory or non-steroidal anti-inflammatory drugs (NSAIDs), hemorrhagic diseases, insulin-dependent diabetes mellitus, cirrhosis, or renal failure. Demographic data collected at study entry included age, gender, smoking habit, alcohol consumption and drug treatments. Drinking and smoking were defined as 'regular' when consumption was >35 g for ethanol or 5 cigarettes per day, respectively. The study was approved by the Osaka Medical Center for Cancer and Cardiovascular Diseases Ethical Committee and Kawasaki Medical School Ethical Committee, and written informed consent was obtained from each patient.

Biopsy Samples

Endoscopies were performed by experienced endoscopists after patients had fasted for 12 h. Two specimens from each sample site, the greater and lesser curves of the corpus, were taken using the endoscopic forceps (FB231K(A); Olympus, Tokyo, Japan) under the endoscopic examinations before and 1 year after eradication; one was used as a whole sample and the other for laser-captured microdissection. The biopsy samples were immediately frozen with liquid nitrogen and stored at −80°C until use.

Laser-Captured Microdissection

The frozen samples obtained at endoscopy were embedded in optimal cutting temperature compound (Sakura Finetek USA, Inc., Torrance, Calif., USA) and cut into serial 8-μm sections. Before microdissection, up to 8 sections from each block were mounted on slides and one slide was stained with Alcian blue and the others were stained using HistGene LCM Frozen Section Staining Kit (Arcturus Bioscience, Mountain View, Calif., USA). The cryostat sections were laser-microdissected with a PixCell II laser-microdissection system (Arcturus Engineering, Mountain View, Calif., USA). Both glands with goblets cells (metaplastic glands) and without (non-metaplastic glands) were isolated. Goblet cells were identified by their characteristic shape with an expanded apical portion and a nucleus at the base of it referring to the slide stained with Alcian blue.

RNA Extraction and Quantitative Polymerase Chain Reaction

Total RNA was extracted from whole biopsy samples using RNeasy Mini kit (Qiagen, Hilden, Germany) and microdissected tissue using a Pico Pure RNA Isolation kit (Arcturus Bioscience). The cDNA syntheses were performed using SuperScriptIII First Strand Synthesis System (Invitrogen, Carlsbad, Calif., USA). Quantitative reverse transcription-polymerase chain reaction (RT-PCR) analysis of *CDX2, MUC5AC, MUC6, MUC2* and *β-actin* mRNA expression was performed using the TaqMan inventoried primers of each gene (Applied Biosystems, Foster City, Calif., USA) and 7500 real-time PCR System (Applied Biosystems) employing TaqMan gene expression assay according to the manufacturer's instruction (Applied Biosystems). Real-time PCR was performed with cDNA for both target genes and the endogenous control using TaqMan Universal PCR Master Mix (Applied Biosystems). Each amplification reaction was performed in triplicate and the average of the threshold cycles was used. The amount of target was obtained by normalization to an endogenous reference (β-actin) and relative to a calibrator. If the microdissection materials contained too small targets for non-metaplastic or metaplastic glands or the average of the threshold cycles for β-actin was >33, the samples were excluded.

Immunohistochemistry

Immunohistochemical staining for CDX2 and mucins was performed using the Vectastain ABC-AP kit (Vector Laboratories, Burlingame, Calif., USA) using previously described methods [20–22]. Four adjacent sections 6-μm thick were cut onto polylysine-coated glass slides. Sections were incubated with mouse monoclonal Cdx2 antibody (1:500, BioGenex, San Ramon, Calif., USA) overnight at 4°C and with diluted primary mouse monoclonal antibodies against MUC2, MUC5AC, and MUC6 (Novocastra Laboratories, Newcastle, UK) at a dilution of 1:200, 1:500, and 1:500, respectively at room temperature for 1 h.

Diagnosis of H. pylori

Venous blood samples were analyzed for specific IgG *H. pylori* antibodies with an enzyme-linked immunosorbent assay (ELISA) kit using the E plate test (Eiken Kagaku, Inc., Tokyo, Japan). Patients were considered to be infected with *H. pylori* if the serum test was positive combined with evidence of chronic gastritis or atrophy with *H. pylori* on histopathological examination. Eradication was confirmed by negative histological examination of gastric biopsies, together with a negative ^{13}C-urea breath test (^{13}C-UBT) following the completion of eradication therapy for 6–8 weeks.

Eradication of H. pylori

Patients were treated with a 7-day regimen consisting of amoxicillin (500 mg tid), clarithromycin (200 mg tid) and a proton pump inhibitor twice daily (total dose = omeprazole 40 mg, lansoprazole 60 mg, or rabeprazole 20 mg), which was the standard approved first-line regimen in Japan. The patients with unsuccessful eradication were retreated with the regimen of changing clarithromycin to metronidazole (250 mg tid).

Statistical Analyses

Values are expressed as the mean ± SD or the median with a 25–75% range, whichever was appropriate depending on whether the data were normally distributed. Mantel-Haenszel χ^2 analysis and the unpaired t test were performed to measure differences in demographic and clinical characteristics. Statistical analyses for significant differences of parameters were performed using the non-parametric Mann-Whitney U test between the two groups and the Wilcoxon signed rank test for paired data. A two-sided p value <0.05 was considered statistically significant. All statistical computations were performed using SPSS (SPSS, Inc., Chicago, Ill., USA).

Results

Twenty-five *H. pylori*-positive patients with ESD for early gastric cancer (cancer group) and 25 controls who were *H. pylori*-positive and matched to the cancer group by gender and age were enrolled in the study. Three patients in the cancer group and 8 patients in the control group were lost to follow-up. Eradication failure occurred in 6 patients in the cancer group and 7 in the control group; 4 cancer patients and 3 controls of these were successfully retreated with the second regimen. One control patient was excluded to match to the cancer group by gender and age. The final study groups consisted of 20 *H. pylori*-positive patients with ESD for early gastric cancer (cancer group) and 12 *H. pylori*-positive controls matched to the cancer group by gender and age. Demographic and clinical characteristics of the study groups are shown in table 1. The locations of the early gastric cancers were: 7 in the lower stomach, 7 in the middle stomach, and 4 in the upper stomach. The remaining 2 patients had malignant lesions located in two different locations.

Table 1. Demographic and clinical characteristics of the patients undergoing eradication

	Controls (n = 12)	Cancer (n = 20)	p values
Age, mean (SD)	65.2 (6.4)	65.7 (9.1)	0.85[a]
Male/female	4/8	6/14	1.0[b]
Current smokers	0 (0%)	4 (20%)	0.27[b]
Regular alcohol intake	5 (41.7%)	10 (50%)	0.73[b]

p values calculated using [a] unpaired t test, [b] Mantel-Haenszel χ^2 analysis.

Whole Biopsy Samples

Using the entire biopsy samples, the *CDX2* mRNA levels positively correlated with *MUC2* mRNA levels ($r = 0.86$, $p < 0.001$). *CDX2* and *MUC2* expression in the cancer group were significantly higher than those in the controls (median *CDX2* 0.75×10^{-2} vs. 0.1×10^{-2}; *MUC2* 0.32×10^{-1} vs. 0.02×10^{-1}, $p = 0.01$). Eradication was associated with a significant decrease in expression of *CDX2* ($p = 0.009$) and *MUC2* ($p = 0.04$) in the cancer group thus reducing the difference between the two groups (fig. 1). *MUC5AC* ($p = 0.01$) and *MUC6* ($p = 0.02$) expressions were significantly increased only in the control group (median *MUC5AC* 4.1–10; *MUC6* 0.57–0.9) and were significantly higher in the controls than those in the cancer group after eradication (fig. 2).

Microdissected Gastric Glands

Using the microdissected gastric glands, expressions of *CDX2* and *MUC2* were significantly higher and *MUC5AC* and *MUC6* expressions were lower in the metaplastic glands than in the non-metaplastic glands both before and after eradication in the total subjects (table 2).

MUC2 expression in the metaplastic glands was significantly lower ($p = 0.03$) in the cancer group than in the controls. However, there was no significant difference in the other genes between the two groups before and after eradication (table 3). *CDX2* expression in the non-metaplastic glands was detectable and disappeared after eradication in each group (table 3; fig. 3).The other genes in the non-metaplastic or metaplastic glands and *CDX2* in the metaplastic glands expressed without significantly change after eradication.

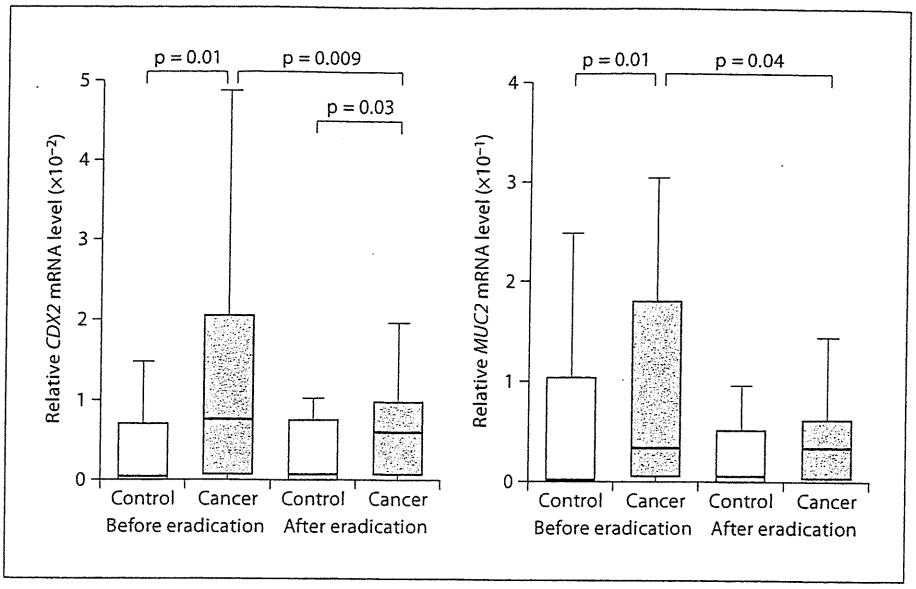


Fig. 1. Comparisons of *CDX2* and *MUC2* expressions between the control group and the cancer group before and after eradication. Horizontal bar = median; box = 25th–75th interquartile range; vertical lines = range of values. mRNA levels are expressed relative to the control gene β-actin. p values were calculated using the non-parametric Mann-Whitney U test between the two groups and the Wilcoxon signed rank test for paired data.

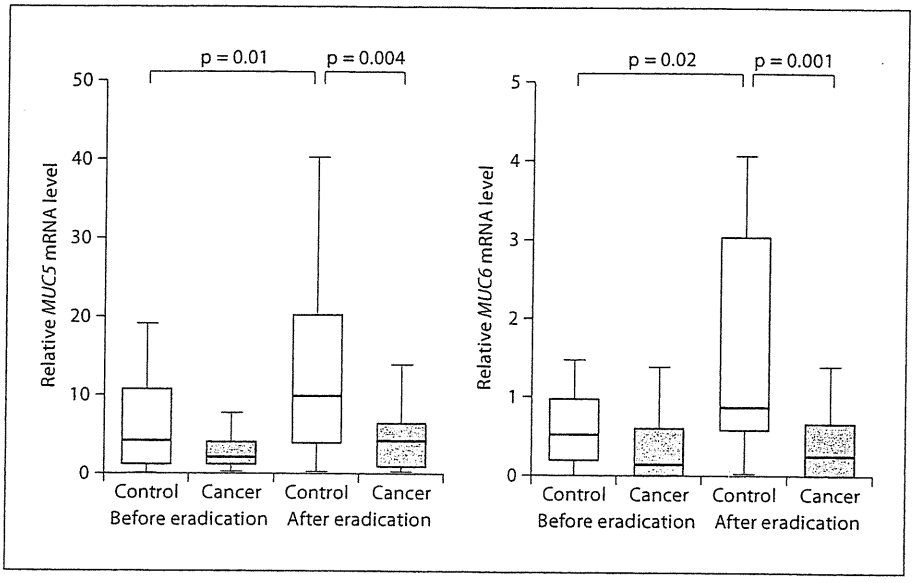


Fig. 2. Comparisons of *MUC5AC* and *MUC6* expressions between the control group and the cancer group before and after eradication. Horizontal bar = median; box = 25th–75th interquartile range; vertical lines = range of values. mRNA levels are expressed relative to the control gene β-actin. p values were calculated using the non-parametric Mann-Whitney U test between the two groups and the Wilcoxon signed rank test for paired data.

Immunohistochemical Staining

In the non-metaplastic glands, MUC5AC expression was detected in foveolar epithelium and mucous neck cells and MUC6 expression was detected in the mucous cells of the neck zone. MUC2 expression displayed a vacuolar staining in the goblet cells. CDX2 as well as MUC2 was detected in the metaplastic glands and was at the adjacent small area in some cancer patients. CDX2 protein expression as well as mucins were associated with mRNA levels, and *H. pylori* eradication decreased CDX2 and MUC2 expression and increased MUC5AC and MUC 6 expressions like as mRNA levels (fig. 4).

Discussion

MUC2 and CDX2 expressions were significantly higher in the fundic regions of gastric mucosa of the cancer patients compared to the non-cancer controls. This difference primarily reflected the higher proportion of glands with IM in the cancer patients compared to the *H. pylori*-infected controls. Interestingly, eradication increased *MUC5AC* and *MUC6* mRNA levels only in the controls. In addition, the difference between those gastric phenotypic genes expression in the two groups also became significant, and the results of immunohistochemi-

Table 2. Comparisons of mRNA levels in the non-metaplastic and metaplastic glands before and after eradication

	Non-metaplastic glands	Metaplastic glands	p values
Before			
CDX2	0 (0–18)	50 (30–120)	<0.00001
MUC2	0 (0–25)	650 (420–970)	<0.00001
MUC5AC	11,080 (4,360–19,595)	840 (50–4,270)	<0.00001
MUC6	70 (10–210)	0 (0–50)	0.01
After			
CDX2	0	20 (10–120)	<0.00001
MUC2	0	335 (130–1,440)	<0.00001
MUC5AC	12,160 (6,490–33,728)	445 (220–1,755)	<0.00001
MUC6	90 (45–365)	0 (0–45)	<0.00001

Values are expressed relative to the control gene β-actin as the median with a 25–75% range. p values were calculated using the non-parametric Mann-Whitney U test.

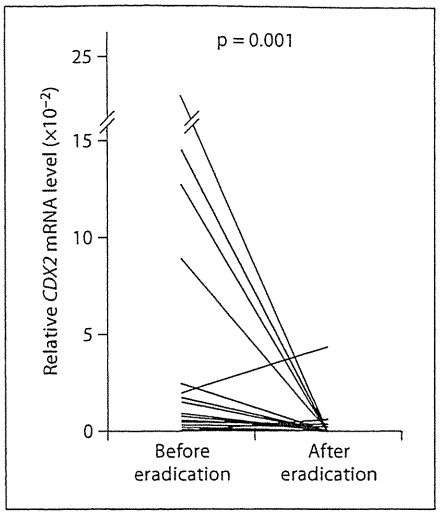


Fig. 3. *CDX2* expression in the non-metaplastic glands of the total subjects before and after eradication. mRNA levels are expressed relative to the control gene β-actin. p values were calculated using the Wilcoxon signed rank test for paired data.

cal stainings were associated with mRNA levels. These results confirm and extend our previous results using immunostainings [20–22]. In our previous study, residual corpus gastritis after eradication was more frequently detected in the cancer group than in the controls and in the mucosa with incomplete IM than in those without incomplete IM. Ravizza [23] reported that complete IM in the antrum regressed 2 or 3 years after eradication, however, incomplete IM remained unchanged. These results suggest that eradication therapy prior to development of incomplete IM is the best strategy to heal the gastritis and to regain the gastric phenotype which should also minimize gastric cancer risk.

Here we show that *CDX2* expression in the non-metaplastic glands was detectable and disappeared after eradication. We isolated the glands with goblets cells (metaplastic glands) and without (non-metaplastic glands) identified by Alcian blue staining. It is possible that adjacent glands without goblet cells are part of metaplastic glands which are possibly incomplete metaplasia glands. Gastric glands without goblet cells expressing CDX2 possibly represent the extension of intestinalization of gastric glands and is consistent with prior clinical studies using immunostaining [24–26]. CDX2 expression was detectable in gastric mucosa infected with *H. pylori* and without obvious metaplastic glands [24, 25], and a small amount of CDX2 expression has also been described in isolated gastric-type glands [24, 25]. These results are consistent with the notion that aberrant *CDX2* expression may be a key event in the initiation of intestinalization of gastric glands. It remains to be determined whether non-metaplastic glands expressing CDX2 are involved in carcinogenesis or are simply markers for an increased risk of developing gastric cancer.

Wong et al. [27] reported a beneficial effect from eradication only in the subgroup of patients without atrophy, IM or dysplasia, suggesting that the benefit of *H. pylori* eradication diminishes once atrophy or IM are present. In contrast, a Japanese study group recently reported a marked decrease in cancer risk following eradication. Their trial included 544 patients with endoscopic treatment for early gastric cancer, and the hazard ratio for metachronous gastric cancer was 0.339 (95% CI 0.157–0.729; p = 0.003) at 3-year follow-up [28]. We selected the patients with recent history of ESD for early gastric cancer as the high-risk group for gastric cancer. We showed that *CDX2* aberrant expression at the metaplastic glands without goblet cells disappeared after eradication even in the cancer group. We speculated that eradication may prevent extension of intestinalization even in the high-risk group for gastric cancer. It is reported that CDX2 expression and intestinal trans-differentiation in the gastric mucosa can be suppressed by an increase in acid secretion that often follows *H. pylori* eradication presumably due to removal of the postulated acid inhibitory factors [29–32]. Further studies are required to elucidate the

Table 3. Comparisons of mRNA levels in the non-metaplastic and metaplastic glands between the control group and the cancer group before and after eradication

		Control	Cancer	p values*
Non-metaplastic glands				
Number of samples[1]	before	10	24	
	after	13	31	
CDX2	before	0 (0–10)	10 (0–20)	0.32
	after	0	0	0.54
	p values	0.02	0.01	
MUC2	before	0 (0–35)	0 (0–38)	
	after	0	0	0.96
	p values	0.40	0.11	0.67
MUC5AC	before	6,390 (3,545–27,780)	15,530 (9,415–28,650)	0.51
	after	16,260 (6,440–42,490)	11,745 (6,490–34,570)	
	p values	0.40	0.43	0.48
MUC6	before	150 (40–550)	60 (3–208)	0.22
	after	110 (40–600)	90 (40–320)	0.95
	p values	0.40	0.19	
Metaplastic glands				
Number of samples[1]	before	5	19	
	after	5	15	
CDX2	before	110 (60–415)	40 (20–123)	0.23
	after	20 (20–140)	20 (10–120)	0.56
	p values	0.07	0.28	
MUC2	before	1,150 (750–5,190)	510 (180–855)	0.03
	after	390 (140–1,620)	280 (150–1,425)	0.59
	p values	0.14	0.20	
MUC5AC	before	1,170 (0–5,510)	840 (95–3,898)	0.66
	after	200 (100–670)	470 (330–8,890)	0.94
	p values	0.27	0.35	
MUC6	before	0 (0–105)	10 (0–105)	0.23
	after	20 (0–160)	0 (0–25)	0.11
	p values	0.47	0.13	

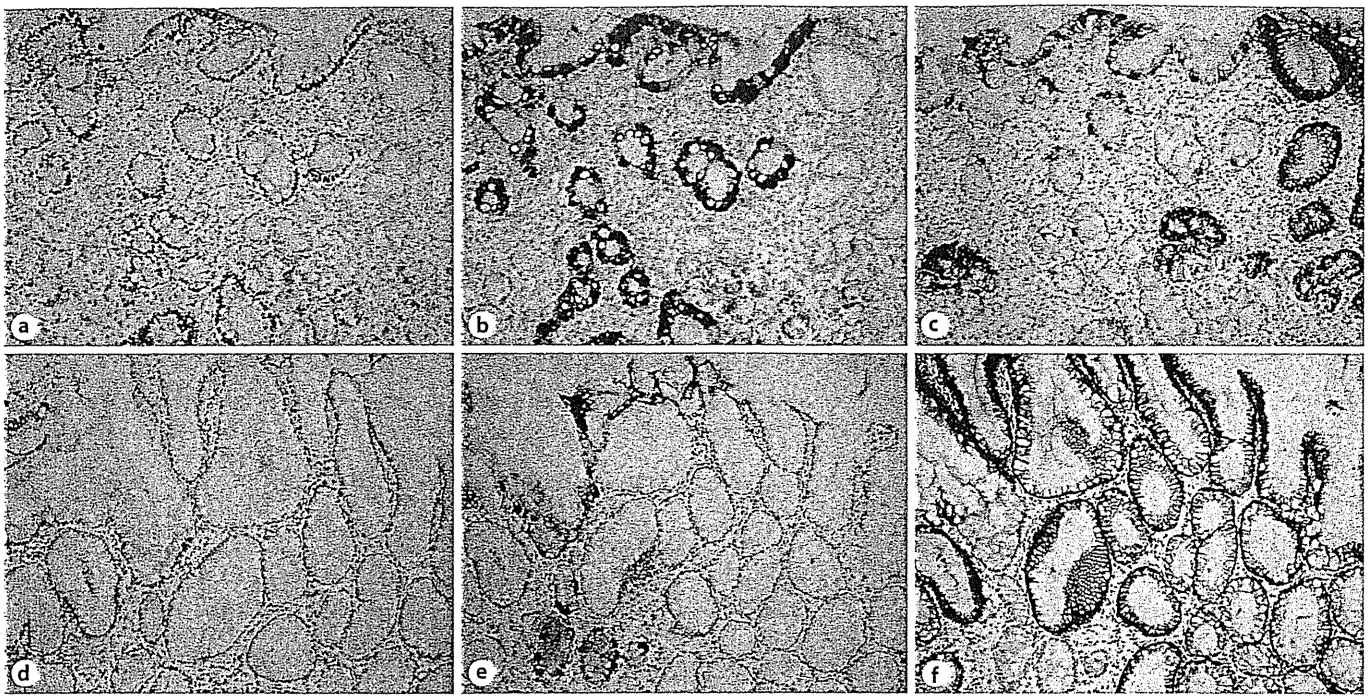
Values are expressed relative ($\times 10^{-3}$) to the control gene β-actin as the median with a 25–75% range.

* p values for the comparisons between the cancer group and the controls were calculated using the non-parametric Mann-Whitney U test. p values for the comparisons between before and after eradication were calculated using the Wilcoxon signed rank test.

[1] Number of samples corrected by laser capture containing enough non-metaplastic or metaplastic glands in the cancer group and the controls. The samples contained too small targets for non-metaplastic or metaplastic glands were excluded.

molecular mechanisms how eradication suppress aberrant *CDX2* expression.

MUC2 and CDX2 were repressed in the metaplastic glands isolated from patients who had undergone mucosal resection of early gastric cancer compared to the controls. Tsukamoto et al. [25] analyzed *MUC2* and *CDX2* mRNA levels in isolated gastric glands from surgically resected antral mucosa and demonstrated that *MUC2* and *CDX2* expressions were progressively upregulated with intestinalization from the gastric type to the gastric/intestinal-mixed type to the intestinal type. In our previous immunohistochemical studies, there is a significant association between types of IM and atrophic scores or serum pepsinogen levels [33]. The most incomplete IM (types II and III) preserving gastric mucin was the gastric and intestinal mixed (GI) type, whereas the complete type expressing MUC2 and CD10 was the intestinal (I) type. Incomplete or gastric/intestinal-mixed type IM was detectable in the mucosa of gastric cancer patients significantly more frequently (58 vs. 38%, $p < 0.001$) than in the controls [33].



Color version available online

Fig. 4. CDX2 (**a**, **d**), MUC2 (**b**, **e**) and MUC5AC (**c**, **f**) immunohistochemical staining of serial sections of the patient with gastric cancer in the corpus greater curve before (**a–c**) and after eradication (**d–f**). Orig. magnif. ×40.

Moreover, CDX2 expression increased in patients in the ascending order of those without IM, those with complete IM and those with incomplete IM ($p < 0.001$) [34]. Although both *MUC5AC* and *MUC6* gene expressions were not significantly different between the two groups, the lower levels of *MUC2* and *CDX2* mRNA in the cancer group of the present study may reflect a higher proportion of those with gastric/intestinal-mixed type IM.

In summary, we first indicated that *CDX2* aberrant expression was detected at the gastric glands without goblet cells in the corpus and disappeared after *H. pylori* eradication. *H. pylori* eradication reversed the gastric phenotype only in the control group. We propose that *CDX2* expression is a feature denoting the early phases of intestinalization of gastric glands and eradication may prevent extension of intestinalization even in the high-risk group for gastric cancer. Depending on the extent and severity of corpus gastritis/atrophy, *H. pylori* eradication can reverse corpus atrophy and have the greatest benefit in reducing gastric cancer risk.

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SURGERY TODAY

Original Article

Survival of Patients Treated by an Autonomic Nerve-Preserving Gastrectomy for Early Gastric Cancer

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Abstract

Purpose. Autonomic nerve preservation in a gastrectomy for gastric cancer improves the postoperative quality of life. We retrospectively examined the survival of patients treated by an autonomic nerve-preserving gastrectomy in comparison to the survival of the patients treated by a conventional gastrectomy.

Methods. The survival of 385 patients treated by an autonomic nerve-preserving gastrectomy for clinical early gastric cancer (the ANP group) was compared with that of 285 patients treated by a conventional gastrectomy (non-ANP group).

Results. Among the ANP group, the numbers of patients with tumor invasion to the mucosa, submucosa, and muscularis propria were 210, 166, and 9, respectively, whereas the numbers of patients with lymph node metastasis grades of N0, N1, and N2 were 360, 21, and 4, respectively. The overall 5-year survival rate of the ANP group was 94.7%, which was superior to that of the non-ANP group (90.4%; $P = 0.003$). The 5-year survival rates of patients with lymph node metastasis were 94.9% and 91.8% in the ANP and non-ANP groups, respectively ($P = 0.733$). Only 3 patients in the ANP group died from gastric cancer.

Conclusions. The survival of patients treated by an autonomic nerve-preserving gastrectomy was equivalent to that of patients treated by a conventional gastrectomy, thus suggesting that an autonomic nerve-preserving gastrectomy could be a useful procedure for the treatment of early gastric cancer.

Key words Gastric cancer · Gastrectomy · Autonomic nerve preservation · Survival

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Introduction

Function-preserving surgery for early gastric cancer is now widely performed in Japan.[1] There are various types of function-preserving operations, including those involving a reduced extent of gastrectomy, autonomic nerve preservation, sphincter preservation, and formation of a neostomach.[2] Because the preservation of the vagus nerve has been demonstrated to improve the postoperative quality of life in patients who undergo either a vagotomy and/or gastrectomy,[3–14] we have performed an autonomic nerve-preserving gastrectomy for early gastric cancer since December 1994. Although autonomic nerve preservation has been considered to maintain the curability of patients,[15,16] the long-term survival rate after an autonomic nerve-preserving gastrectomy has not been fully assessed to date. We retrospectively examined the survival of patients after an autonomic nerve-preserving gastrectomy for early gastric cancer.

Patients and Methods

Between December 1994 and July 2003, 385 patients were treated by an autonomic nerve-preserving gastrectomy for clinical early gastric cancer at our institute (ANP group). The indications for this operation included tumor invasion into the mucosal or submucosal layer (T1), and the absence of lymph node involvement and distant metastases according to clinical and surgical findings (N0/M0). All patients underwent gastrointestinal fiberscopy, a gastrointestinal series, and computed tomography for the preoperative evaluation. If regional lymph node metastasis was suspected by the intraoperative findings, a frozen-section analysis of the lymph node was performed and the patients with positive nodes were excluded from the study. With regard to the specific procedures, a distal gastrectomy was

performed in 210 cases, a proximal gastrectomy was performed in 31 cases, a total gastrectomy was performed in 17 cases, and a pylorus-preserving gastrectomy was performed in 127 cases. Ten patients were treated by laparoscopy-assisted surgery. Lymph node dissection was performed for D1+α, D1+β or D2, where α refers to lymph nodes 7 and 8a in cases of lower third cancer, and β refers to lymph nodes 7, 8a, and 9. Cases with local and segmental resection and lymph node dissection less than D1 were excluded. Staging and classification were determined according to the general rules for surgical and pathological gastric cancer studies in Japan.[17] The control group was 285 patients treated by a conventional gastrectomy for early gastric cancer based on their clinical stages between 1991 and 1998 (non-ANP group), because we began performing an autonomic nerve-preserving gastrectomy in 1994 and have performed this procedure for almost all patients with a clinical T1 N0 M0 stage after 1999.

Operative Procedure

The preserved autonomic nerves included the hepatic branch originating from the anterior trunk of the vagus nerve and the celiac branch, the plexus surrounding the common hepatic artery and the splenic artery, and the pancreatic branch and the hepatic branch originating from the posterior trunk of the vagus nerve. The celiac branch was followed upward from the root of the left gastric artery or downward from the posterior trunk of the vagus nerve, taped, and preserved. The left gastric artery was divided at the peripheral side of the confluence of the celiac branch (Fig. 1).

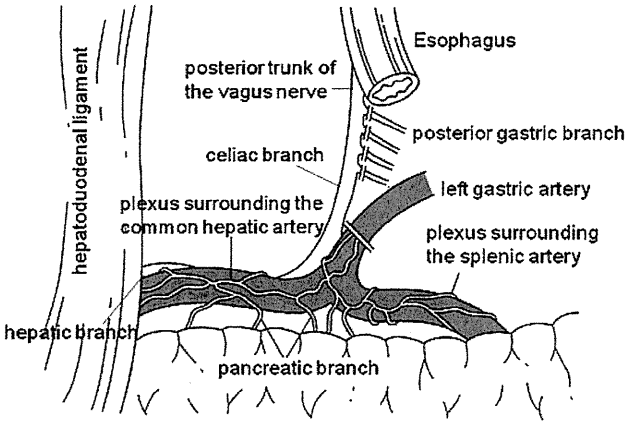

Fig. 1. Preserved autonomic nerves and cutting lines (*double lines*) for the left gastric artery and the posterior gastric branches

Statistical Analysis

The clinicopathological features and survival rates of the ANP group were compared with those of the non-ANP group. The median follow-up time was 5.7 years. Statistical analyses were conducted using the Statcel version 2.0 software program (OMS, Tokyo, Japan). Statistically significant differences were determined using the χ^2 test or Student's *t*-test. The survival rates were calculated using the Kaplan–Meier method and the log-rank test. The level of significance was set at $P < 0.05$.

Results

Clinicopathological Features

Table 1 shows the clinicopathological data for both groups. Fewer patients in the ANP group were treated by a total gastrectomy in comparison to the non-ANP group. In the histological analyses, the numbers of patients with tumor invasion to the mucosa, submucosa, muscularis propria, and subserosa were 210, 166, 9, and 0 in the ANP group and 166, 98, 15, and 6 in the non-ANP group, respectively. Although the non-ANP group contained significantly more patients with tumors exhibiting deeper invasion than the ANP group, the extent of lymph node metastasis and tumor staging were similar in the two groups. The number of dissected lymph nodes was greater in the ANP group than in the non-ANP group. In the ANP group, the number of patients with lymph node metastasis was 25 (6.5%) and the number of patients over stage T1 N0 M0 was 32 (8.3%).

Survival

The overall 5-year survival rates were 94.7% in the ANP group and 90.4% in the non-ANP group ($P = 0.003$) (Fig. 2). The 5-year survival rates of patients at stages IA, IB, and II were 94.8%, 96.2%, and 83.3% in the ANP group, and 89.5%, 96.2%, and 83.3% in the non-ANP group, respectively (Fig. 3). The survival rate in the ANP group was superior to that in the non-ANP group for stage IA ($P = 0.003$). Because there was no significant difference in the disease-specific survival rates for stage IA (99.7% vs 98.5%, $P = 0.571$, Fig. 4), the difference in the survival rates of the stage IA patients between the ANP and non-ANP groups may have been caused by death from other diseases. In contrast, the 5-year survival rates of the patients in the ANP group were 94.9% in those without lymph node metastasis and 91.8% in those with metastasis, respectively, with no significant difference (Fig. 5).

Table 1. Clinicopathological findings of patients who underwent a gastrectomy with or without the preservation of the autonomic nerves

	ANP (n = 385)	Non-ANP (n = 285)	P value
Age, years (range)	62.6 (25–88)	62.0 (28–85)	0.451
Sex			0.436
Male	251	194	
Female	134	91	
Tumor location			0.053
U	44	31	
M	244	158	
L	97	96	
Tumor size (mm)	27.5	28.3	0.579
Lymph node dissection			0.391
D1	85	71	
D2	300	214	
Operation method			<0.001
DG	210	222	
PG	31	7	
TG	17	32	
PPG	127	24	
Depth of tumor invasion			0.001
pM	210	166	
pSM	166	98	
pMP	9	15	
pSS	0	6	
Lymph node metastasis			0.894
pN0	360	268	
pN1	21	15	
pN2	4	2	
fStage			0.297
IA	353	251	
IB	26	28	
II	6	6	
Histologic type			0.124
Differentiated	246	202	
Undifferentiated	138	83	
Unknown	1	0	
Number of dissected lymph nodes (range)			
All	37.8 (5–110)	32.4 (2–127)	<0.001
Group2[a]	13.9 (0–52)	10.9 (0–72)	<0.001

U, upper third; M, middle third; L, lower third; DG, distal gastrectomy; PG, proximal gastrectomy; TG, total gastrectomy; PPG, pylorus-preserving gastrectomy; M, mucosa and/or muscularis mucosa; SM, submucosa; MP, muscularis propria; SS, subserosa

[a] Group 2 lymph nodes refer to Nos. 7, 8a, 9, and 11

Although 26 patients died in the ANP group and 50 patients died in the non-ANP group, only 3 and 5 deaths, respectively, were disease-specific. Among these patients, only one in each group had lymph node metastasis (Table 2). The mortality was only one patient in each group.

Discussion

In gastric cancer patients, there are two types of postgastrectomy syndrome that are classified based on their etiology, namely, postgastrectomy syndrome from resection of the stomach and injury to the vagus nerve from lymph node dissection.[18] Many limited gastrectomy techniques have been developed to reduce the incidence of postgastrectomy syndrome, including pylorus-preserving gastrectomy, proximal gastrectomy, segmental gastrectomy, and local resection. The hepatic and celiac branches of the vagus nerve innervate the region from the pylorus to the large intestine as far as the distal portion of the transverse colon, the biliary tract, and the other upper abdominal organs. Preservation of the vagus nerve minimizes the loss of digestive and absorptive functions, thereby improving recovery of postoperative bodyweight and reducing diarrhea.[5,10,13–15] Furthermore, vagus nerve preservation decreases the incidence of cholelithiasis[3,13,14] and pre-

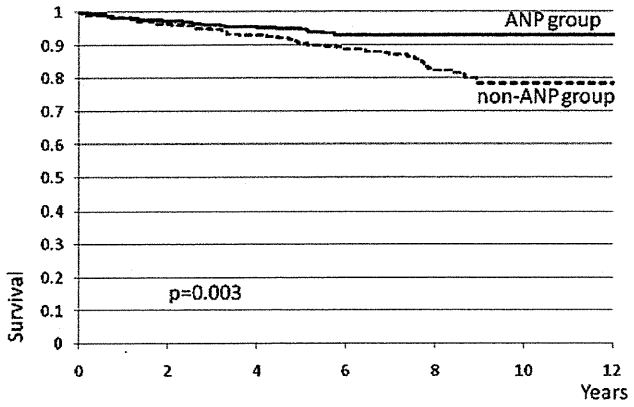

Fig. 2. Survival curves of the patients who underwent a gastrectomy with (*ANP*) and without (*non-ANP*) the preservation of the autonomic nerves. The overall 5-year survival rates are 94.7% in the ANP group and 90.4% in the non-ANP group ($P = 0.003$, log-rank test)

serves pancreatic insulin release.[9,11] We previously reported the superiority of this procedure.[10,13]

We designated our nerve-preserving procedure an "autonomic nerve-preserving gastrectomy," although it has also been referred to as a vagus nerve-preserving gastrectomy in previous reports. The reason for this designation is that the procedure preserves the plexus surrounding the common hepatic artery and the splenic artery, the pancreatic branch, and the hepatic branch originating from the posterior trunk of the vagus nerve as well as the sympathetic nervous system from the celiac ganglia.[19] It seems reasonable that the preservation of both the sympathetic and parasympathetic nervous systems is important for maintaining the function of the upper gut after a gastrectomy.[20,21]

A gastrectomy with extensive nodal dissection appears to prevent recurrence and improve cancer-specific survival in early gastric cancer patients with

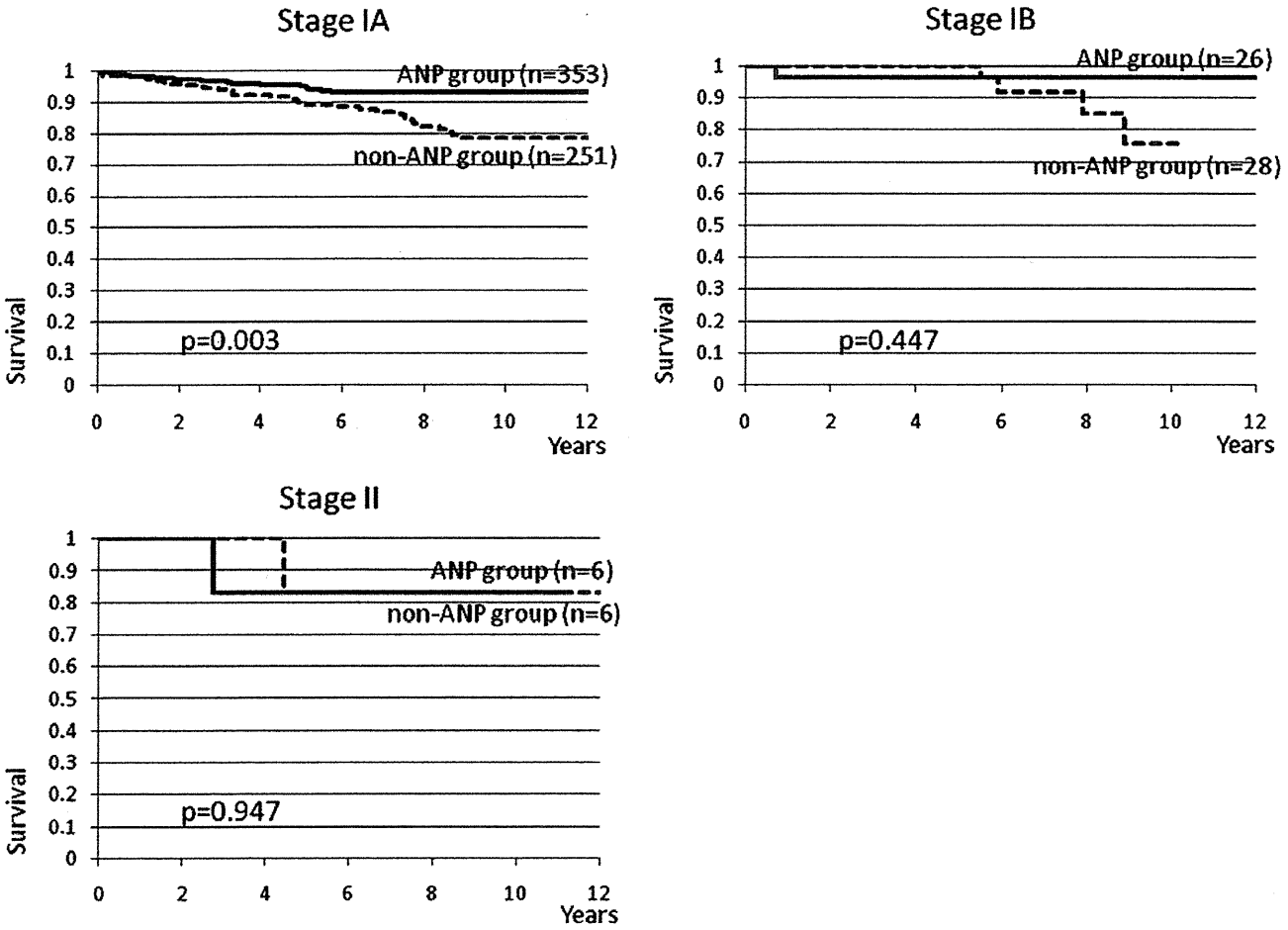

Fig. 3. Survival curves for stage IA, IB, and II patients who underwent a gastrectomy with (*ANP*) and without (*non-ANP*) the preservation of the autonomic nerves. The survival rate in the ANP group is superior to that in the non-ANP group for stage IA ($P = 0.003$, log-rank test). The survival rates between the ANP and non-ANP groups do not differ significantly for stages IB ($P = 0.433$, log-rank test) and II ($P = 0.947$, log-rank test)

Table 2. Patient mortality due to gastric cancer

pT	pN	fStage	Lesion of nodal metastasis	No. of nodal metastases	Organ of recurrence
ANP group					
M	N0	IA	—	0	Remnant stomach, liver, LN
SM	N0	IA	—	0	Liver
SM	N2	II	Nos. 1, 3, 4d, 6, 7, 8a, 9, 11	25	Peritoneum, LN
Non-ANP group					
M	N0	IA	—	0	Brain
M	N0	IA	—	0	Mediastinum, LN, bone
M	N0	IA	—	0	Unknown
M	N0	IA	—	0	Unknown
M	N1	IB	No.4d	1	Peritoneum

M, mucosa and/or muscularis mucosa; SM, submucosa; LN, lymph node

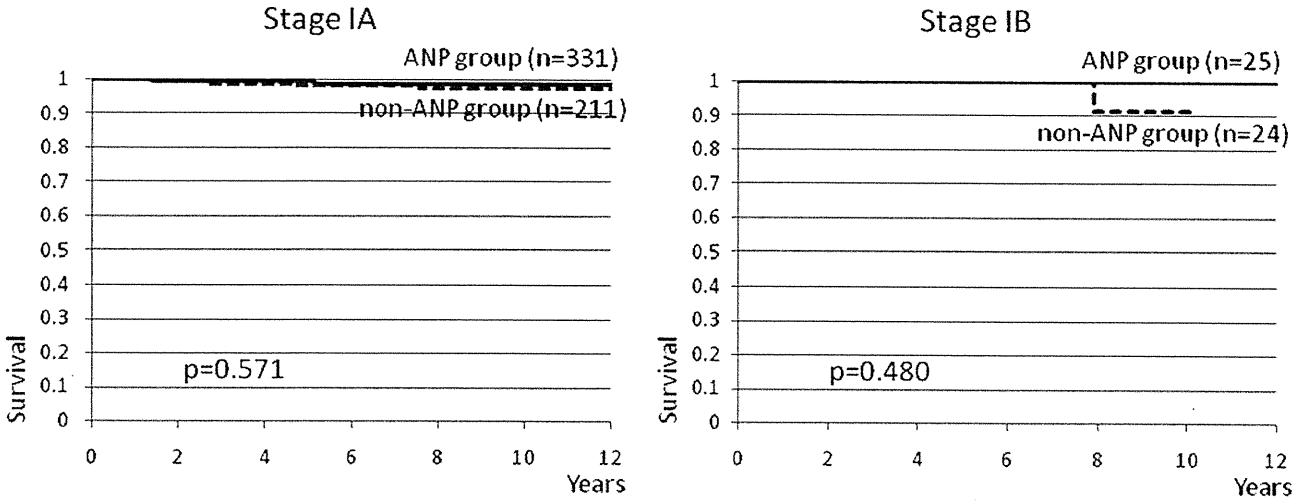

Fig. 4. Disease-specific survival curves for stage IA and IB patients who underwent a gastrectomy with (*ANP*) and without (*non-ANP*) the preservation of the autonomic nerves. The survival rates between the ANP and non-ANP groups do not differ significantly for stages IA ($P = 0.571$, log-rank test) and IB ($P = 0.480$, log-rank test)

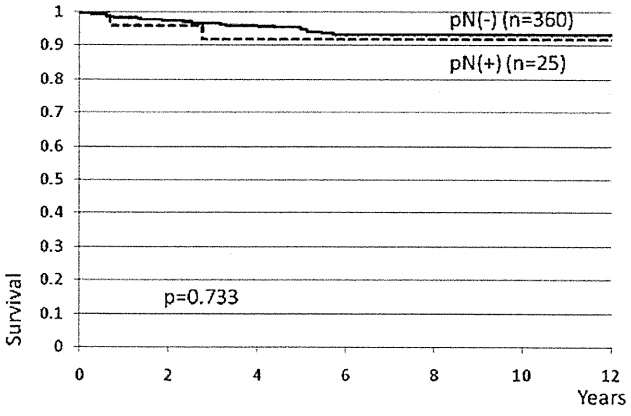

Fig. 5. Five-year survival rates of the patients in the ANP group are 94.9% in those without lymph node metastasis and 91.8% in those with metastasis ($P = 0.733$, log-rank test)

nodal metastasis in comparison to a gastrectomy with a limited lymph node dissection.[22] However, a recent study reported no significant difference in the survival rates between the standardized D2 lymphadenectomy and the D2 plus para-aortic lymphadenectomy in gastric cancer surgery.[23,24] The purpose of a nerve-preserving gastrectomy is to maintain both the postoperative quality of life and the curability of the patient. The left gastric artery and the common hepatic artery are enveloped in connective tissue, and the lymphatics along the arteries encircle this connective tissue. The nerves are dispersed within the connective tissue surrounding the arteries.[15,16] Therefore, an autonomic nerve-preserving gastrectomy may provide a curative operation even for patients with intracapsular microscopic metastases.[15,16] The number of dissected lymph nodes was actually greater in the ANP group than in the non-ANP group

although the dissection levels of both groups were equal. We achieved technical improvement of the dissection by confirming the location of the autonomic nerves. Although macroscopic diagnosis of lymph node metastases is possible, a previous study found that 15 of 158 gastric cancer patients with macroscopically negative nodes had lymph node metastases, and the false-negative rate was 3.8%.[25] In the present study the false-negative rate was 6.5%, and the survival of such patients was same in both groups. In addition, only one patient experienced recurrence in comparison to the four patients who had metastasis to lymph nodes near the celiac branch, such as No. 1 or No. 7. Therefore, our retrospective study indicates that an autonomic nerve-preserving gastrectomy did not reduce the patient survival rate, even in patients with microscopic lymph node metastases, thus suggesting that this procedure can eliminate lymphatic invasion (including microscopic metastases) as effectively as a conventional gastrectomy.

This study was a retrospective analysis and therefore had some degree of bias. Given that significantly more patients in the non-ANP group had tumors with histologically greater invasion, the surgeons may have selected a conventional gastrectomy when advanced cases were suspected based on intraoperative findings. In the present study, although the most important factor was lymph node metastasis, no significant differences with regard to the extent of the lymph node metastasis and staging were observed between the two groups. A prospective randomized trial is therefore necessary in the future to solve and elucidate the problems identified in our study.

In conclusion, an autonomic nerve-preserving gastrectomy did not reduce the survival of patients with early gastric cancer as compared with a conventional gastrectomy. Therefore, an autonomic nerve-preserving gastrectomy appears to be a useful function-preserving procedure for the treatment of clinical early gastric cancer. In addition, we recently started to perform a laparoscopy-assisted autonomic nerve-preserving gastrectomy,[26] and this method is expected to be both a function-preserving and minimally invasive treatment modality.

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Safety of carbon dioxide insufflation for upper gastrointestinal tract endoscopic treatment of patients under deep sedation

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Abstract
Background It is well known that carbon dioxide (CO_2) is absorbed faster in the body than air and also that it is rapidly excreted through respiration. This study aimed to investigate the safety of CO_2 insufflation used for esophageal and gastric endoscopic submucosal dissection (ESD) in patients under deep sedation.
Methods Patients with either early gastric or esophageal cancers that could be resected by ESD were enrolled in this study from March 2007 to July 2008 and randomly assigned to undergo ESD procedures with CO_2 insufflation (CO_2 group) or air insufflation (air group). A TOSCA measurement system and TOSCA 500 monitor were used to measure and monitor both transcutaneous partial pressure of CO_2 ($PtcCO_2$) and oxygen saturation (SpO_2).
Results The study enrolled 89 patients and randomly assigned them to a CO_2 group (45 patients) or an air group (44 patients). The mean CO_2 group versus air group measurements were as follows: $PtcCO_2$ (49.1 ± 5.0 vs. 50.1 ± 5.3 mmHg; nonsignificant difference [NS]), maximum $PtcCO_2$ (55.1 ± 6.5 vs. 56.8 ± 7.0 mmHg; NS), $PtcCO_2$ elevation (9.1 ± 5.4 vs. 11.4 ± 5.6 mmHg; $p = 0.054$), SpO_2 (99.0 ± 0.7% vs. 99.0 ± 1.0%; NS), minimum SpO_2 (96.5 ± 2.4% vs. 95.4 ± 3.3%; $p = 0.085$), and SpO_2 depression (2.4 ± 2.3% vs. 3.3 ± 2.9%; NS). The $PtcCO_2$ and SpO_2 measurements were similar in the two groups, but the CO_2 group was better than the air group in $PtcCO_2$ elevation and minimum SpO_2.
Conclusions The findings demonstrated CO_2 insufflation to be as safe as air insufflation for upper gastrointestinal tract ESDs performed for patients under deep sedation without evidencing any adverse effects.

Keywords Carbon dioxide insufflation · Deep sedation · Endoscopic submucosal dissection · Transcutaneous partial pressure of carbon dioxide · Upper gastrointestinal tract

Several recent studies investigating colonoscopy and endoscopic retrograde cholangiopancreatography (ERCP) have reported that carbon dioxide (CO_2) insufflation reduces abdominal pain and discomfort caused by bowel hyperextension and can be used as safely as air insufflation [1–6]. It is well known that CO_2 is absorbed faster in the body than air and that it also is rapidly excreted through respiration unless some type of pulmonary dysfunction exists [1, 2]. To date, almost all endoscopic procedures have been performed using air insufflation, although it has led to some problems of abdominal pain and discomfort in routine examinations and perforation-related subcutaneous or mediastinal emphysema and pneumoperitoneum in endoscopic treatments [7, 8].

With the relatively recent development and increasingly widespread use of endoscopic submucosal dissection (ESD) as a minimally invasive treatment, performance of ESD for early gastrointestinal (GI) neoplasm in the esophagus, stomach, and colorectum has increased dramatically [9–16]. Quite naturally, the number of complications also has increased as a direct result, including perforations that occur during the technically difficult ESD procedure itself and the delayed bleeding experienced afterward [7, 8, 14, 17, 18]. In fact, the reported ESD perforation rate is 7% for cases involving the esophagus,

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4% for cases involving the stomach, and 5% for cases involving the colorectum [10, 14, 15]. Perforation can cause peritonitis and mediastinitis, and possibly also thromboembolism due to blood flow congestion (compartment syndrome) when significant pneumatic leakage results in excess internal pressure [19–24]. It is anticipated that such associated problems will be minimized by further use of CO_2 insufflation.

Colonoscopy with conscious sedation and the use of CO_2 insufflation has become more generally accepted since the demonstration of the safety and effectiveness of CO_2 insufflation in a previously published reported [5]. We previously conducted a case–control study that showed CO_2 insufflation to be both safe and effective for colorectal ESD with conscious sedation [25]. However, the safety of CO_2 insufflation has not been established for upper GI tract endoscopic treatment such as ESD with deep sedation in which CO_2 retention and decreased oxygenation are more important factors than in colonoscopy performed with conscious sedation.

This study aimed to investigate the safety of CO_2 insufflation for esophageal and gastric ESDs with deep sedation. Both operations are lengthy procedures.

Materials and methods

Patients

We prospectively assessed the safety of CO_2 insufflation for upper GI tract ESDs performed with the patient under deep sedation compared with air insufflation from March 2007 to July 2008 at the National Cancer Center Hospital (NCCH) in Tokyo, Japan. The study enrolled 89 patients with either early gastric or esophageal cancer that could be resected by ESD and randomly assigned them to undergo ESD procedures with CO_2 insufflation (CO_2 group) or air insufflation (air group).

The study excluded patients with severe pulmonary disease including either chronic obstructive pulmonary disease (COPD) or disease resulting in less than 80% of vital capacity (%VC) or less than 70% of the forced expiratory volume in 1 s as a percentage of the forced vital capacity (FEV1%), patients with severe cardiovascular disease including NYHA III or IV heart failure or arrhythmia with any treatment history, patients with hepatic or renal dysfunction, and patients with a change in insufflation methods from CO_2 to air or from air to CO_2 for any reason during their ESDs.

Endoscopic procedures

All ESD procedures were performed with Olympus video endoscopes and a standard videoendoscope system (EVIS LUCERA; Olympus Optical Co., Ltd., Tokyo, Japan). For ESD procedures, an insulation-tipped diathermic knife (IT-knife; Olympus) was used from March to October 2007 and an improved IT-knife (IT-knife 2; Olympus) from November 2007 to July 2008 [11, 26, 27].

First, marking dots were made around the lesion using a needleknife (Olympus). This was followed by injection of diluted epinephrine with normal saline (1:200,000) to lift the submucosal layer and allow the tip of the IT-knife or IT-knife 2 to be inserted into the submucosal layer. A small initial incision then was made by a needleknife, and a complete circumferential mucosal incision around the periphery of the marking dots was performed with the IT-knife or IT-knife 2. After an additional submucosal injection, the submucosal layer beneath the lesion was directly dissected using the same IT-knife or IT-knife 2.

Although all ESDs were generally performed in this manner, we sometimes used not only other devices such as an argon plasma coagulation probe for the marking dots and a bipolar needleknife (B-knife; XEMEX Co., Tokyo, Japan) for the initial incision and submucosal dissection [15, 28], but also another injection solution, sodium hyaluronate (MucoUp; Johnson & Johnson Co., Ltd., Tokyo, Japan) diluted with normal saline (1:1), especially for esophageal ESDs [12, 29–31]. The final objective was to achieve successful en bloc resections for precise pathologic evaluations.

Patients received midazolam, propofol, or both for deep sedation, and oxygen (O_2) was administered nasally (2 l/min) during ESD. Initially, 3–5 mg of midazolam was used for induction of venous anesthesia, with an additional 1–3 mg given repeatedly as necessary based on the judgment of the individual endoscopist. Propofol was administered initially at a dosage of 20 mg for induction, with another 0.1–0.5 mg/kg/h given continuously for maintenance depending on the condition of the patient.

CO_2 insufflation and transcutaneous measurements

A CO_2 regulator prototype (Olympus) connected to a CO_2 bottle was used for CO_2 insufflation until the Olympus UCR (Fig. 1) became commercially available in Japan in May 2008 [25]. During the procedure, CO_2 insufflation was set at a constant rate of 1.2 l/min, which is a moderate level. In upper GI endoscopy, the UCR has three insufflation levels, which can be controlled by the use of three types of connecting tubes. These insufflation amounts are almost equivalent to the original three regulation levels of the EVIS LUCERA (Olympus).

Measurement of the arterial partial pressure of CO_2 (partial pressure of carbon dioxide [PCO_2] and arterial partial pressure of carbon dioxide [$PaCO_2$]) is an invasive, intermittent, and unpleasant process widely used for