Category: Case report

Short title (running head): Retrievable stent graft for evaluation of spinal cord ischemia for patch aneurysm

Word counts

Abstract: 94 words

Text body: 1384 words

Abstract (94/100 words)

Patch aneurysms after thoracoabdominal aortic aneurysm repair are a serious late complication. We treated a patient with patch aneurysm (origin at the artery of Adamkiewicz) involving a portion of an implanted graft from a previous operation. First, thoracic endovascular aneurysm repair was planned. A retrievable stent graft was inserted and motor evoked potentials (MEPs) were monitored in order to evaluate spinal cord ischemia. Significant changes in the MEPs were observed and permanent stent graft placement was abandoned. Later, open surgery was performed. The patient showed no postoperative paraplegia and was discharged in good condition.

Patch aneurysms after thoracoabdominal aortic repair are an alarming late complication. The incidence has been reported as 7.1%.[1] Thoracic endovascular aneurysm repair (TEVAR) creates new options for patients having a very high risk for morbidity and mortality, such as patch aneurysm patients during conventional operations. Although TEVAR is a less invasive procedure to treat patients with patch aneurysm, spinal cord ischemia is a major concern when the stent graft covers segmental arteries, particularly the artery of Adamkiewicz.

We report our experience with a patient with a planned TEVAR of a patch graft aneurysm. Before the permanent placement of a stent graft at the portion of the patch aneurysm at the origin of the artery of Adamkiewicz (which supplies the spinal cord), a retrievable stent graft was inserted and MEPs were monitored in order to evaluate spinal cord ischemia.

CASE REPORT

A 60-year-old man with a history of chronic aortic dissection presented with dilatation of the thoracoabdominal aorta. Previous surgery included extent II thoracoabdominal aortic aneurysm (TAAA) repair and reconstruction of arteries T9 through T11, which were reattached by the patch technique to a prosthetic graft 15 years

ago. Computed tomography (CT) examination showed an aneurysmal formation of the intercostal patch anastomosis. In addition, CT examination showed the artery of Adamkiewicz originating from the T10 intercostal artery (Fig. 1). Although open surgical repair is the traditional treatment for these lesions, such repairs carry substantial mortality and morbidity risks.[2-4] We considered TEVAR to be a less invasive and safer approach for the patient. However, since all three intercostal arteries were patent and the artery of Adamkiewicz originated from these arteries, the risk of paraplegia was our major concern. Before deploying the stent graft device for permanent implantation for the patch aneurysm, we decided to place a retrievable stent graft and record the amplitude of MEPs before and during the retrievable stent graft placement. The retrievable stent graft was developed by a modification of the self-expanding Matsui-Kitamura (MK) stent (Kitamura Inc., Kanazawa, Japan). All wires were 0.4 mm Nitinol wire (Memoalloy, Tokin Inc., Tokyo, Japan). The basic framework of the sea anchor-shaped stent was collected in a bundle and connected to the pushing rod. The stent fabric, an expanded PTFE sheet 0.1 mm in thickness, was attached by 5-0 polypropylene sutures. The length of the retrievable stent graft was 150 mm and the expanded diameter was 30 mm in this case. The retrievable stent graft was inserted into a 20 Fr Cook KTI sheath (Cook Medical, Bloomington, IN, USA) to deliver it to the

descending aorta.

A cerebrospinal fluid (CSF) drainage catheter was introduced into the intrathecal space on the day before the surgery. During the operation, CSF pressure was routinely monitored and was allowed to drain spontaneously if the CSF pressure increased above 10 mmHg.

The induction of anesthesia was achieved with intravenous doses of propofol administered using the target-controlled infusion method,[5] remifentanil (0.25 μg/kg/min), and dexmedetomidine hydrochloride (2 μg/kg/hr). Anesthesia was maintained with propofol administered using the target-controlled infusion method, remifentanil (0.25 μg/kg/min) and dexmedetomidine hydrochloride (0.2 μg/kg/hr). Muscle relaxation was induced with 25 mg of rocuronium bromide once at the time of anesthesia induction. The retrievable stent graft was placed. The graft was 150 mm long and the patch aneurysm was 70 mm long; therefore, the graft could sufficiently cover both the proximal and distal landing zones. We used low-dose epinephrine (0.01-0.03 μg/kg/min) to induce hypertension (over 120 mmHg of systolic pressure) during the examination of MEPs. The amplitude of MEPs decreased significantly during the retriever placement, suggesting critical spinal cord ischemia (Fig. 2). The retrievable stent graft was removed and permanent implantation of the stent graft was abandoned.

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The amplitude of MEPs returned soon after having collected the retrievable stent graft. We removed the CSF drainage catheter on the day after the operation after having confirmed that there was no paraplegia. The patient complained of weakness in the lower extremities after waking up from anesthesia, and needed one week to recover from his lower extremity symptoms. Two months after this operation, open surgery was performed, including reconstruction of all three intercostal arteries. The patient showed no postoperative paraplegia.

DISCUSSION

TEVAR is preferred for high risk candidates, such as patch aneurysm, since open repair is associated with considerable morbidity and mortality.[2-4] Although TEVAR is less invasive for these high-risk candidates, stent graft deployment could induce interruption of the blood flow in critical intercostal arteries originating from the patch aneurysm and may cause spinal cord ischemia. In our case, preoperative CT examination showed that the artery of Adamkiewicz originated from an intercostal branch which included reconstructed intercostal arteries. Ishimaru et al reported that in 16 patients with aneurysms located in the middle and distal segment of the descending aorta, retrievable stent grafts were placed temporarily before stent graft deployment.

They recorded the amplitude or latency of evoked spinal cord potentials (ESCPs) and concluded that a retrievable stent graft was useful as a predictor of spinal cord ischemia in candidates for stent graft repair of thoracic aortic aneurysms.[6] After this thesis, we reported that the patient underwent TEVAR for ulcer like projection (ULP) of chronic aortic dissection. A CT image showed that the artery of Adamkiewicz originated from the Th 9 level which was located close to the ULP. A retrievable stent graft was used to predict the possibility of spinal cord ischemia. In this study, we recorded MEPs instead of ESCPs because MEPs are easier to monitor than ESCPs.[7] Moreover, monitoring MEPs is a highly reliable technique to assess spinal cord ischemia during TAAA repair.[8] We attempted to evaluate spinal cord ischemia using a retrievable stent graft before permanent placement TEVAR in this case. MEP amplitudes were significantly decreased. We decided to abandon the permanent placement of a stent graft and switched to open surgery. Paraplegia was preventable by this procedure in the present case.

Although TEVAR is considered suitable for the treatment of patch aneurysms because of its low invasiveness, in some cases there is an increased risk of paraplegia. In this case in particular, the artery of Adamkiewicz originated from the patch aneurysm, which was clearly detected by preoperative CT examination, and open surgery was

selected. We conclude that the use of a retrievable stent graft is a promising technique to determine the suitability of TEVAR versus open surgery for intercostal patch aneurysm.

Conflicts of interest: None declared.

Funding sources: None declared.

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Figure legends

Fig. 1

Three-dimensional CT images of the patch aneurysm located at the previous site of reattachment of the intercostal arteries. Arteries T9 through T11 were all patent. The red arrow shows that the artery of Adamkiewicz originated from the T10 intercostal artery.

Fig. 2

A1, Digital subtraction angiography of patch aneurysm located at the previous site of reattachment of the intercostal arteries.

A2, A retrievable stent graft was placed to completely cover the patch aneurysm.

B, Significant changes in amplitude and latency of MEP recordings were observed before and after retrievable stent graft placement. *BP,* systemic arterial pressure in millimeters of mercury.

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Fig. 1

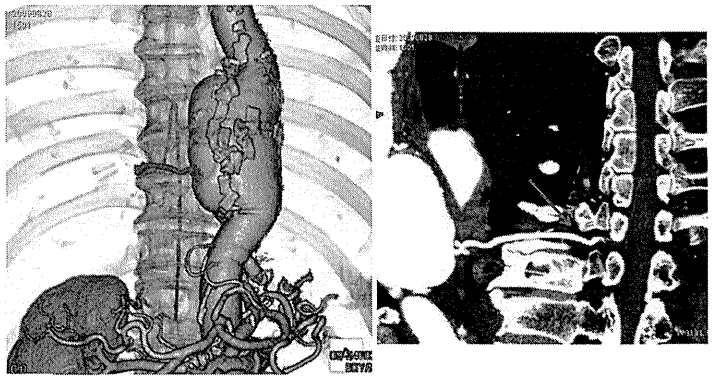
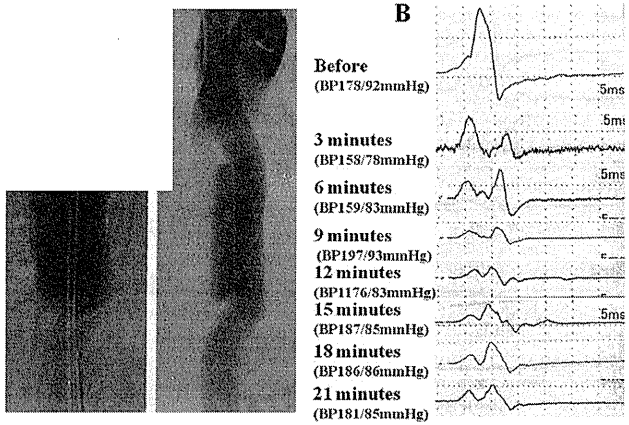


Fig. 2



Murine Double Minute 2 and Its Association with Chemoradioresistance of Esophageal Squamous Cell Carcinoma

HIROSHI OKAMOTO[1,3], FUMIYOSHI FUJISHIMA[3], YASUHIRO NAKAMURA[2], MASASHI ZUGUCHI[1], GO MIYATA[1], TAKASHI KAMEI[1], TORU NAKANO[1], KAZUNORI KATSURA[1], SHIGEO ABE[1], YUSUKE TANIYAMA[1], JIN TESHIMA[1], MIKA WATANABE[3], AKIRA SATO[1], NORIAKI OHUCHI[1] and HIRONOBU SASANO[2,3]

[1]*Division of Advanced Surgical Science and Technology and* [2]*Department of Pathology, Graduate School of Medicine, Tohoku University, Aoba-ku, Sendai, Miyagi, Japan;* [3]*Department of Pathology, Tohoku University Hospital, Aoba-ku, Sendai, Miyagi, Japan*

Abstract. *Background: Definitive chemoradiotherapy (dCRT) has been established as the standard treatment for esophageal squamous cell carcinoma (ESCC). However, many patients develop persistent or recurrent disease following dCRT. We investigated factors related to chemoradioresistance and treatment outcomes in patients with ESCC who underwent salvage esophagectomy after dCRT. Patients and Methods: We selected 38 patients with persistent disease and 24 with recurrent disease who underwent salvage esophagectomy after dCRT, immunolocalized p53, p16, p27, murine double minute 2 (MDM2), cyclin D1, Ki-67, and epidermal growth factor receptor, and correlated the findings with clinicopathological features. Results: MDM2 positivity was significantly higher among patients with persistent disease than among those with recurrent disease ($p<0.0001$). In addition, negative p16 expression was a predictor of poor prognosis among patients with persistent disease. Conclusion: MDM2 overexpression plays an important role in chemoradioresistance of ESCC; furthermore, negative p16 expression can predict poor prognosis of patients with persistent disease.*

Definitive chemoradiotherapy (dCRT) has become the standard treatment for esophageal carcinoma (1-3), and results of some previous studies, including our own, have shown comparable clinical outcomes between patients undergoing dCRT and those undergoing surgery alone (4, 5). However, it is also true that 34.2%-56.0% of patients who undergo dCRT experience persistent or recurrent disease (2, 4-6), which generally results in adverse clinical outcomes. We have aggressively performed salvage esophagectomy after dCRT since October 2001 to improve the chances of survival among these patients (6-9). However, patients who undergo salvage esophagectomy after dCRT still exhibit a high rate of morbidity and mortality (6, 7, 9). If we could determine the chemoradiosensitivity of disease in such patients at the time of diagnosis, we could dramatically improve treatment strategies and clinical outcomes. In addition, if cases with a poor prognosis after salvage esophagectomy could be identified at an earlier clinical stage, much more stringent follow-up and administration of more aggressive adjuvant therapy could also confer clinical benefits to these patients.

Salvage esophagectomy is a rather restricted procedure because of its high-risk nature. To the best of our knowledge, a detailed evaluation of surgical pathology specimens obtained during salvage esophagectomy, following dCRT, has not been previously reported. Therefore, in this study, we retrospectively evaluated the clinicopathological and immunohistochemical features of esophageal squamous cell carcinoma (ESCC) specimens obtained from patients who underwent salvage esophagectomy after dCRT in order to explore the factors related to chemoradioresistance among patients with ESCC. We immunolocalized p53, p16, p27, murine double minute 2 (MDM2), cyclin D1, Ki-67, and epidermal growth factor receptor (EGFR) because all of these are known prognostic factors for ESCC and/or are reportedly related to chemoradioresistance (10-16).

Correspondence to: Hiroshi Okamoto, MD, Division of Advanced Surgical Science and Technology, Graduate School of Medicine, Tohoku University, 1-1 Seiryo-machi, Aoba-ku, Sendai, Miyagi 980-8574, Japan. Tel: +81 227177214, Fax: +81 227177217, e-mail: hi-ok-0531@med.tohoku.ac.jp

Key Words: Esophagus, squamous cell carcinoma, definitive chemoradiotherapy, salvage esophagectomy, murine double minute 2, p16, Ki-67.

Patients and Methods

Patients. We performed 68 salvage esophagectomies following dCRT at the Tohoku University Hospital (Sendai, Japan) between September 2001 and November 2008. Two cases of distant metastasis, three cases of initial endoscopic treatment, and one case of previous CRT for head and neck cancer were excluded. Therefore, 62 patients with ESCC were included in the study, out of whom 38 had persistent disease and 24 had recurrent disease. The definitions of persistent and recurrent disease used in this study are described in the next section.

dCRT and salvage esophagectomy. The dCRT protocol in our study basically followed that of the Japan Clinical Oncology Group (JCOG) trial 9906 (2). In brief, the protocol comprised of two cycles of intravenous cisplatin (40 mg/m^2) infusion on days 1 and 8 and continuous 5-fluorouracil (400 mg/m^2) infusion over 24 h on days 1-5 and 8-12 every five weeks with concurrent radiotherapy (60 Gy administered in 30 fractions over a period of eight weeks, including a 2-week rest period after the administration of 30 Gy). The radiotherapy administered is three-dimensional. Gross tumour volume (GTV) included the primary tumour and metastatic lymph nodes evaluated by endoscopy, computed tomography (CT), and 2-[^{18}F]fluoro-2-deoxy-D-glucose positron-emission tomography (FDG-PET), if necessary. The clinical target volume included the GTV and the supraclavicular, mediastinal, and celiac axis lymph node regions. When the tumours were located in the upper third of the esophagus, the celiac axis lymph node region was excluded. After 40 Gy, an extra boost of radiation was administered to GTV *via* an oblique approach (20 Gy administered in 10 fractions). Additional chemotherapy was administered until May 2004. This comprised of two cycles of 80 mg/m^3 of cisplatin on day 1 and a continuous infusion of 800 mg/m^2 of 5-fluorouracil on days 1-5, every four weeks. Clinical evaluation by endoscopy with biopsy, CT, and FDG-PET (if necessary) were performed one month after treatment completion. Patients who were evaluated as having an incomplete response at this time and who subsequently underwent salvage esophagectomy were defined as persistent cases. Patients who were evaluated as having a complete response (CR) at this time, but whose tumours subsequently recurred in the same location and who underwent salvage esophagectomy later were defined as recurrent cases. Recurrence was confirmed by biopsy. A total of 24 out of the 38 patients with persistent disease and 15 out of the 24 patients with recurrent disease underwent the JCOG 9906 protocol. The other patients underwent treatment according to different protocols followed by other hospitals or other Departments (Radiation Oncology, Clinical Oncology, or Otolaryngology) of the Tohoku University Hospital. The treatments that the patients received are summarized in Table 1. Some patients required a dose decrease or a delay in chemotherapy because of adverse side-effects. All patients received the complete scheduled radiation dose.

Salvage esophagectomy was usually performed under thoracoscopic guidance. One- or two-field lymph node dissection was performed for tumours of the cervical esophagus or the middle or lower thirds of the esophagus, and 3-field lymph node dissection was performed for tumours of the upper third of the esophagus.

Immunohistochemical staining and pathological evaluation. Surgical specimens were fixed in 10% formalin and representative sections were embedded in paraffin wax. Excluding specimens in which no residual tumour cells were observed microscopically, the specimens of 33 patients with persistent disease and 22 patients with recurrent disease were evaluated immunohistochemically. Immunohistochemical staining was performed using the streptavidin–biotin complex method, as follows. Serial 4-μm-thick sections from the most representative area of each specimen were de-paraffinized in xylene, rehydrated in a graded ethanol series, and then immersed in 3.0% hydrogen peroxide in methanol for 10 min at room temperature (RT) to block endogenous peroxidase activity. For antigen retrieval, the slides for p53 were heated in a microwave at 95°C for 15 min in 0.01 M citrate buffer (pH 6.0). The slides for p16, p27, MDM2, cyclin D1, and Ki-67 were heated for 5 min in 0.01 M citrate buffer (pH 6.0) using an autoclave at 121°C. The slides for EGFR were incubated in 0.05% protease in Tris-HCL buffer (pH 7.6) at 37°C for 10 min. The slides were incubated in 1% normal rabbit (for mouse monoclonal antibody) or goat (for rabbit monoclonal antibody) serum for 30 min at RT to reduce non-specific antibody binding. Subsequently, the slides were incubated at 4°C overnight with mouse monoclonal antibody against p53 (DO-7, diluted 1/100; Nichirei Biosciences Inc., Tokyo, Japan), p16 (G175-1239, diluted 1/100; BD Biosciences, Franklin Lakes, NJ, USA), p27 (SX53G8, diluted 1/800; Dako, Glostrup, Denmark), MDM2 (SMP14 diluted 1/1000; Santa Cruz Biotechnology Inc., CA, USA), Ki-67 (MIB-1, diluted 1/300; Dako), EGFR (31G7, used as delivered, product code 413701; Nichirei Biosciences Inc.), and rabbit monoclonal antibody against cyclin D1 (SP4, used as delivered, product code 413521; Nichirei Biosciences Inc.). The next day, the sections were incubated with biotinylated anti-mouse or anti-rabbit immunoglobulin (Nichirei Biosciences Inc.) as secondary antibodies and incubated with peroxidase-labeled streptavidin (Nichirei Biosciences Inc.) for 30 min at RT. The antigen–antibody complexes were visualized with 3,3'-diaminobenzidine, and the slides were counterstained with Mayer's haematoxylin, dehydrated in a graded ethanol series, and cleared in xylene.

The staining and pathological findings were evaluated independently by two of the authors (HO and FF) who were blinded to the patients' clinical data. The histopathological findings were classified according to the seventh edition of the Union for International Cancer Control system (17). The percentage of p53-, p27-, MDM2-, Ki-67-, and cyclinD1-positive nuclei was determined for more than three regions of the deepest area of the tumour and 1000 viable tumour cells were evaluated at a magnification of ×400 by microscopy. For p16, the percentage of cells with positive nuclei and positive cytoplasm was determined, and for EGFR, the percentage of cells with positive membranes was determined. The cutoff values for abnormal expression were as follows: p53, ≥10% (13); p16, ≤5% (16); p27, ≥10% (15); MDM2, ≥20% (18); cyclin D1, ≥10% (14); Ki-67, ≥39% (12). Scoring for EGFR was performed using the immunoreactive score (IRS) obtained by multiplying the intensity score (0=no staining, 1=faint staining, 2=moderate staining, 3=strong staining) by the extent score (0=none, 1=<10%, 2=10%-50%, 3=>50%-80%, 4=>80%), and ranged from 1 to 12. It was decided that an IRS ≥6 was indicative of abnormal expression (10). Histopathological tumour regression was classified into five categories according to the Japanese Classification of Esophageal Cancer, tenth edition (19) as follows: grade 3, markedly effective (no viable residual tumour cells); grade 2, moderately effective (less than one-third residual tumour cells); grade 1, slightly effective (1b, one-third to two-thirds residual tumour cells; 1a, more than two-thirds residual tumour cells); grade 0, ineffective (no therapeutic effect observed).

Table I. *Summary of treatments.*

Patients with persistent disease	n=38
JCOG9906 protocol	24 (63.2%)
Cisplatin/5-FU/50 Gy	1 (2.6%)
Cisplatin/5-FU/60 Gy	1 (2.6%)
Cisplatin/5-FU/64 Gy	1 (2.6%)
Nedaplatin/5-FU/60 Gy	2 (5.3%)
Nedaplatin/5-FU/64 Gy	2 (5.3%)
Nedaplatin/5-FU/70 Gy	6 (15.8%)
Cisplatin/5-FU/DOC/70 Gy	1 (2.6%)
Patients with recurrent disease	**n=24**
JCOG9906 protocol	15 (62.5%)
Cisplatin/5-FU/60 Gy	2 (8.3%)
Cisplatin/5-FU/64 Gy	1 (4.2%)
Cisplatin/5-FU/70 Gy	1 (4.2%)
Nedaplatin/5-FU/60 Gy	2 (8.3%)
Nedaplatin/5-FU/69.6 Gy	1 (4.2%)
Nedaplatin/5-FU/70 Gy	1 (4.2%)
Nedaplatin/DOC/68.4 Gy	1 (4.2%)

JCOG, Japan Clinical Oncology Group; 5-FU, 5-fluorouracil; DOC, docetaxel.

Statistical analysis. Continuous data were analysed using the Student's *t*-test or the Mann–Whitney *U*-test. Categorical data were evaluated using Pearson's chi-square test, Fisher's exact test, or the Mann–Whitney *U*-test as appropriate. Normality was assessed using the Shapiro–Wilk test. Equality of variances was evaluated using the F test. Overall curves were determined by the Kaplan–Meier method, and a log-rank test was used to compare the survival curves. The patient survival time was determined from the date of salvage surgery until death or the last follow-up examination. All statistical analyses were performed using JMP Pro Version 9.0.2 (SAS Institute Inc., Cary, NC, USA). Two-tailed *p*-values <0.05 were considered statistically significant.

This study was approved by the Ethical Committee of Tohoku University Hospital (accession number 2011-596).

Results

Comparison of clinicopathological features and survival outcomes between patients with persistent and recurrent disease. The median follow-up time for patients with persistent and recurrent disease was 12.5 months (range=0-102 months) and 34.5 months (range=4-102 months), respectively. Twelve out of 38 patients with persistent disease and three out of 24 patients with recurrent disease underwent non-curative resection (R1/R2). The clinicopathological features of the patients with persistent and recurrent disease are shown in Table II. Pathological tumour depth, lymph node status, and tumour stage were significantly more advanced among patients with persistent disease than among those with recurrent disease. In terms of tumour differentiation, poorly-differentiated tumours were more frequently observed in patients with recurrent disease than in those with persistent disease. The 3- and 5-year overall survival (OS) rates for all 62 patients were 35.1% and 28.0%, respectively. The survival outcomes of patients with persistent and recurrent disease are compared in Figure 1. The 3- and 5-year OS rates were 28.2% and 20.6%, respectively, for patients with persistent disease and 45.8% and 41.3%, respectively, for patients with recurrent disease. The OS rate of patients with persistent disease was significantly worse than that of patients with recurrent disease (p=0.044).

Table II. *Clinicopathological features of patients with persistent and recurrent disease.*

Variable	Persistent disease (n=33) (%)	Recurrent disease (n=22) (%)	*p*-Value
Mean age±SD	63.9±7.8	65.6±9.1	0.28
(Range), years	(51-80)	(44-82)	
Gender			
Male	28 (84.8)	20 (90.9)	0.69
Female	5 (15.2)	2 (9.1)	
Location			
Cervix	0 (0.0)	3 (13.6)	0.14
Upper	4 (12.1)	1 (4.5)	
Middle	19 (57.6)	10 (45.5)	
Lower	10 (30.3)	8 (36.4)	
Radiation			
60 Gy	23 (69.7)	16 (72.7)	0.81
>60 Gy	10 (30.3)	6 (27.3)	
Additional chemotherapy			
Not performed	28 (84.8)	15 (68.2)	0.19
Performed	5 (15.2)	7 (31.8)	
Histological type			
Well differentiated	3 (9.1)	1 (4.5)	0.06
Moderately differentiated	27 (81.8)	13 (59.1)	
Poorly differentiated	3 (9.1)	8 (36.4)	
pT			
1	1 (3.0)	8 (36.4)	0.0005
2	6 (18.2)	4 (18.2)	
3	23 (69.7)	5 (22.7)	
4	3 (9.1)	5 (22.7)	
pN			
0	13 (39.4)	18 (81.8)	0.0019
1-3	20 (60.6)	4 (18.2)	
pStage			
I	4 (12.1)	11 (50.0)	0.015
II	10 (30.3)	5 (22.7)	
III	17 (51.5)	6 (27.3)	
VI	2 (6.1)	0 (0.0)	
Lymphatic invasion			
Negative	17 (51.5)	12 (54.5)	0.83
Positive	16 (48.5)	10 (45.5)	
Venous invasion			
Negative	7 (21.2)	8 (36.4)	0.22
Positive	26 (78.8)	14 (63.6)	
Residual tumour			
R0	21 (63.6)	19 (86.4)	0.064
R1/R2	12 (36.4)	3 (13.6)	
Tumour regression grade			
0	1 (3.0)		
Ia	20 (60.6)		
Ib	9 (27.3)		
2	3 (9.1)		

SD, Standard deviation.

Comparison of marker expression between patients with persistent and recurrent disease. Marker expression among the patients with persistent and recurrent disease is summarized in Figure 2. The MDM2 positivity rate (p<0.0001) and the IRS for EGFR (p=0.030) were significantly higher among patients with persistent disease than among those with recurrent disease. On the other hand, the Ki-67 positivity rate tended to be higher among patients with recurrent disease than among those with persistent disease (p=0.062). None of the other markers exhibited any significant correlations with persistent or recurrent disease. Tumour cells positive for MDM2, p16, Ki-67, and EGFR expression are illustrated in Figure 3.

Correlations between marker expression and clinicopathological features. Among the patients with persistent disease, EGFR expression was correlated with advanced pathological stage (p=0.036, data not shown) and lymphatic invasion (p=0.024, data not shown). No other significant correlations were observed.

Survival analysis of clinicopathological findings and marker expression among patients with persistent and recurrent disease. Among patients with persistent disease, survival analysis showed that pathological tumour depth, pathological stage, lymphatic invasion, residual tumour, and p16 status were significant prognostic factors for OS (Table III and Figure 4). Among patients with recurrent disease, pathological tumour depth, pathological stage, lymphatic invasion, and residual tumour were significant prognostic factors for OS (Table III).

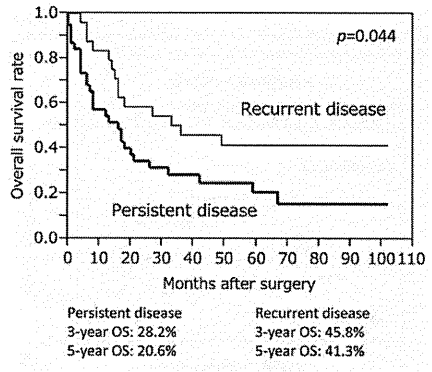


Figure 1. *Comparison of survival outcomes between patients with persistent and recurrent disease. Overall survival (OS) was significantly worse among patients with persistent disease than among those with recurrent disease (p=0.044).*

Discussion

The oncoprotein MDM2 inhibits p53 by directly blocking its transcriptional activity or ubiquitinating p53 to promote p53 resolution in cytoplasmic proteasomes (20, 21). MDM2 overexpression induced by ionizing radiation inhibits mediation of cell-cycle arrest in the G_1 phase and apoptosis by p53 (22, 23), which may explain why some tumours resist radiotherapy or CRT. On the other hand, Ki-67 is a widely known marker of cell proliferation. In this study, tumour MDM2 positivity was significantly higher among patients with persistent disease than among those with recurrent disease. In addition, Ki-67 positivity tended to be higher among those with recurrent disease. Therefore, the biological behaviour of persistent disease appears to differ from that of recurrent disease with regard to resistance to chemoradiation. Persistent disease is usually characterized by marked chemoradioresistance, whereas recurrent disease occurs in patients who have once been evaluated as having a complete clinical response. Considering that tumours with increased resistant to CRT are more frequently identified as persistent rather than recurrent, high levels of MDM2 seem to play a critical role in chemoradioresistance of ESCC cells. Ikeguchi *et al.* (11) reported that the correlation between MDM2 expression in ESCC and shorter survival was more marked for patients who underwent postoperative adjuvant CRT than for those who did not. These results together with those in our present study clearly indicate that MDM2 expression in ESCCs that display chemoradioresistance is already high before treatment and remains stable or increases after CRT. If this observation is valid, we may be able to determine chemoradiosensitivity in patients with ESCC by examining MDM2 expression in biopsy specimens obtained before treatment, or by examining the increase in MDM2 positivity in biopsy specimens obtained after induction CRT; however, this awaits further investigations for clarification. Recently, the effects of the MDM2 inhibitor Nutlin-3 (24) were clinically evaluated, especially with regard to the treatment of leukemia (25, 26). Nutlin-3 inhibits MDM2 and causes cell-cycle arrest, apoptosis, and senescence through the

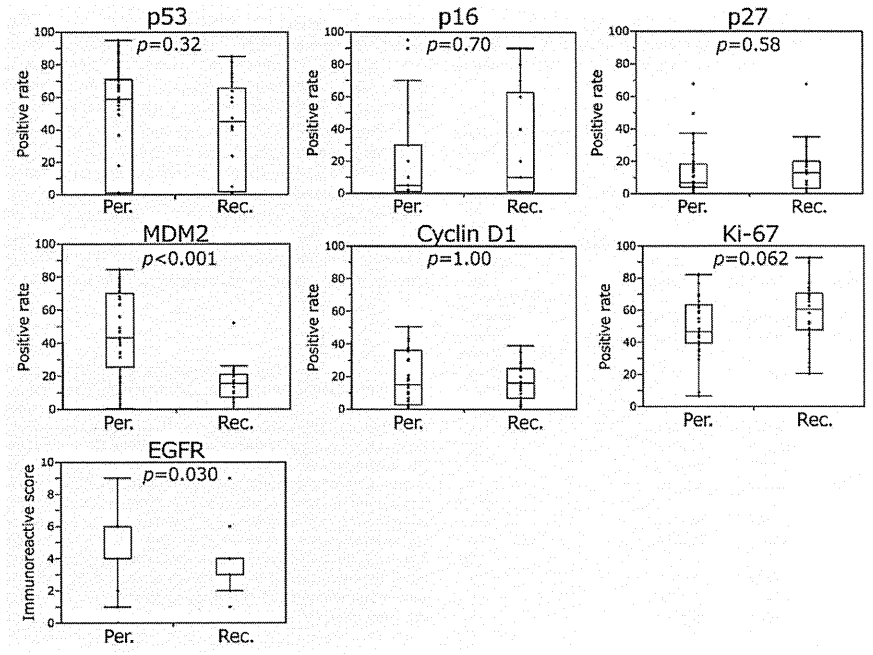


Figure 2. *Comparison of marker expression between patients with persistent and recurrent disease. Murine double minute 2 (MDM2) positivity rate (p<0.0001) and the immunoreactive score of epidermal growth factor receptor (EGFR) (p=0.030) were significantly higher among patients with persistent disease than among those with recurrent disease. On the other hand, the Ki-67 positivity rate tended to be higher among patients with recurrent disease than among those with persistent disease (p=0.062). Per., Persistent disease; Rec., recurrent disease.*

accumulation and activation of p53. Arya *et al*. (27) reported that Nutlin-3 improved the radiosensitivity of laryngeal squamous carcinoma cells. Therefore, CRT in conjunction with Nutlin-3 may contribute to an improvement in response rate among patients with ESCC.

It appears that recurrent tumour cells have good chemoradiosensitivity but remain in the esophageal wall and regrow in the same location of the esophagus as that of the primary tumour. Considering that the tumours of patients with recurrent disease tend to exhibit high Ki-67 expression, these tumours seem to have a high proliferative capacity. Patients evaluated as having a clinical complete response included those in whom carcinoma cells actually remained in the esophageal wall. Therefore, early detection of recurrence may lead to an improved prognosis. When early-stage recurrence is found, a good prognosis can be expected after salvage esophagectomy, as shown in this study. Moreover, endoscopic treatment can be considered to preserve the esophagus.

Regarding EGFR, IRS was significantly higher in patients with persistent disease than in patients with recurrent disease. This may be because the pathological stage of tumours was significantly more advanced in patients with persistent disease than in those with recurrent disease and because EGFR expression was correlated with advanced pathological stage among patients with persistent disease.

p16 is a cyclin-dependent kinase inhibitor (28). Inactivation of p16 has been observed in several human malignancies, including ESCC (29, 30). In this study, p16 expression was significantly correlated with the survival of patients with persistent disease. Although R0 resection contributes to survival after salvage esophagectomy (7, 9), this finding suggests that patients who undergo salvage

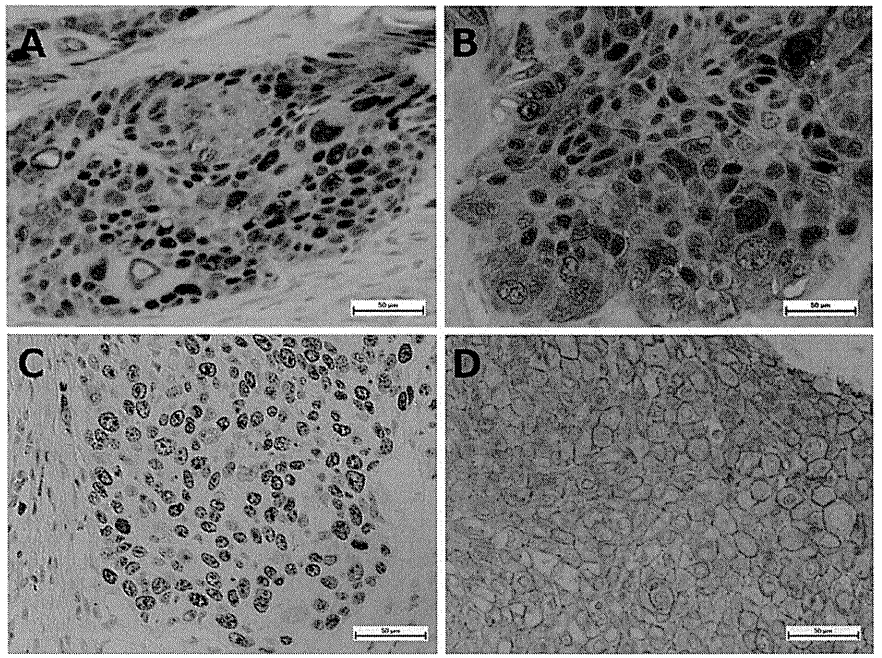


Figure 3. *Immunohistochemical staining of esophageal squamous cell carcinoma Tumour cells positive for murine double minute 2 (A), p16 (B), Ki-67 (C), and epidermal growth factor receptor (D) expression (×400 magnification).*

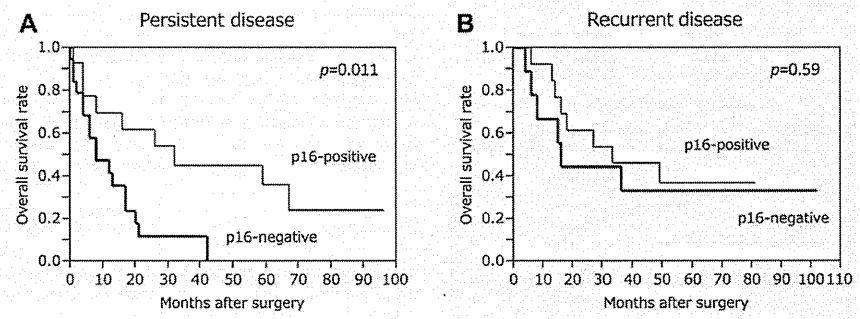


Figure 4. *Kaplan–Meier curves of patients prepared on the basis of p16 expression Among patients with persistent disease, overall survival was significantly shorter in those with negative p16 expression than in those with positive p16 expression (A); however, no statistically significant differences were observed in survival according to p16 status among patients with recurrent disease (B).*

Table III. *Survival analysis of clinicopathological findings and marker expression among patients with persistent and recurrent disease.*

Variable	Persistent disease				Recurrent disease			
	No.	3-Year OS (%)	5-Year OS (%)	*p*-Value	No.	3-Year OS (%)	5-Year OS (%)	*p*-Value
Age (years)								
<60	11	38.4	25.6	0.20	4	25.0	25.0	0.65
≥60	22	19.3	12.9		18	44.4	38.1	
Gender								
Male	28	22.4	18.0	0.39	20	40.0	35.0	0.92
Female	5	50.0	0.0		2	50.0	50.0	
Location								
Cervix/Upper	4	25.0	25.0	0.65	4	25.0	25.0	0.66
Middle/Lower	29	26.3	15.8		18	44.4	38.1	
Radiation								
60 Gy	23	29.4	19.6	0.23	16	37.5	31.3	0.66
>60 Gy	10	30.0	15.0		6	50.0	50.0	
Additional chemotherapy								
Not performed	28	23.6	17.7	0.25	15	40.0	40.0	0.93
Performed	5	40.0	20.0		7	42.9	28.6	
Histological type								
Well/Moderately-differentiated	30	25.4	20.3	0.94	14	42.9	42.9	0.83
Poorly-differentiated	3	33.3	0.0		8	37.5	25.0	
pT								
1/2	7	83.3	55.6	0.0062	12	58.3	48.6	0.046
3/4	26	12.1	8.1		10	20.0	20.0	
pN								
0	13	42.3	31.7	0.096	18	44.4	38.1	0.50
1-3	20	14.8	7.4		4	25.0	25.0	
pStage								
I/II	14	46.8	35.1	0.034	16	56.3	49.2	0.0005
III/VI	19	11.8	5.9		6	0.0	0.0	
Lymphatic invasion								
Negative	17	43.2	34.6	0.019	12	66.7	58.3	0.029
Positive	16	7.3	0.0		10	10.0	10.0	
Venous invasion								
Negative	7	42.9	21.4	0.49	8	50.0	50.0	0.65
Positive	26	21.9	16.4		14	35.7	26.8	
Residual tumour								
R0	21	41.8	27.9	<0.0001	19	47.4	41.5	0.006
R1/2	12	0.0	0.0		3	0.0	0.0	
TRG								
0/1a	21	23.8	14.3	0.31				
1b/2	12	28.5	28.5					
p53								
Negative	9	13.9	13.9	0.50	7	42.9	42.9	0.49
Positive	24	30.0	20.0		15	40.0	32.0	
p16								
Negative	19	11.8	0.0	0.011	9	33.3	33.3	0.59
Positive	14	45.1	36.1		13	46.2	36.9	
p27								
Negative	18	34.2	34.2	0.47	10	30.0	30.0	0.41
Positive	15	20.0	6.7		12	50.0	40.0	
MDM2								
Negative	7	14.3	14.3	0.43	15	33.3	25.0	0.18
Positive	26	30.5	20.3		7	57.1	57.1	
Cyclin D1								
Negative	14	44.5	14.8	0.33	7	28.6	28.6	0.45
Positive	19	15.8	15.8		15	46.7	38.9	
Ki-67								
<39	8	42.9	0.0	0.53	4	25.0	25.0	0.60
≥39	25	21.7	21.7		18	44.4	38.1	
EGFR								
Negative	19	21.2	21.2	0.95	18	38.9	32.4	0.64
Positive	14	33.3	11.1		4	50.0	50.0	

OS, Overall survival; TRG, tumour regression grade; MDM2, murine double minute 2; EGFR, epidermal growth factor receptor.

esophagectomy for tumours with low p16 expression may clinically benefit from stricter perioperative management, aggressive adjuvant therapy, and careful follow-up. In the present study, p16 expression was evaluated only after dCRT. Therefore, the expression of this marker in pretreatment biopsy specimens remains to be evaluated.

A positive correlation between tumour regression grading (TRG) and ESCC prognosis has been reported (13), but no correlation between TRG (0/1a *vs.* 1b/2) and patient survival was detected in this study. The period from dCRT to salvage esophagectomy varied among patients, and only three specimens from patients with persistent disease were TRG2. Moreover, TRG3 specimens were excluded. We believe this to be the reason why TRG was not necessarily correlated with survival in this study. Further investigation of ESCC specimens obtained after neoadjuvant CRT in patients with a fixed interval between chemoradiotherapy and surgery is required.

In conclusion, to the best of our knowledge, this is the first study to undertake a detailed evaluation of surgical pathology specimens obtained during salvage esophagectomy following dCRT. Our findings indicate that overexpression of MDM2 plays an important role in the chemoradioresistance of ESCC cells and that low or lack of p16 expression has the potential to predict poor prognosis among patients with persistent disease after dCRT. We believe that these results and those of further investigations will contribute to the development of a new treatment strategy for ESCC.

Conflicts of Interests

The Authors declare that they have no conflicts of interest.

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ORIGINAL ARTICLE

Reactive Increase in Gastric Mucus Secretion Is an Adaptive Defense Mechanism Against Low-Dose Aspirin-Induced Gastropathy

K. Iijima · T. Iwabuchi · N. Ara · T. Koike · H. Shinkai · Y. Kamata · T. Ichikawa · K. Ishihara · T. Shimosegawa

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Abstract
Background Gastric mucus is considered to play an essential role in gastric mucosal defense mechanisms, especially when irritants are present in the stomach.
Aim To investigate the relationship between low-dose aspirin-induced gastropathy and gastric secretory function, especially gastric mucus secretion, in healthy volunteers.
Methods Thirty male, asymptomatic, *Helicobacter pylori pylori*-negative healthy volunteers were asked to take 100 mg of enteric-coated aspirin (Bayaspirin) once a day for 10 days. Endoscopic examination was performed before and 3 and 10 days after drug administration. The extent of endoscopically assessed gastric mucosal injury was semi-quantitatively evaluated according to the modified Lanza score. The pentagastrin-stimulated gastric juice was collected for 10 min during the endoscopic examination and subjected to analysis for gastric acid (mEq/10 min) or mucus (mg hexose/10 min) output.
Results Overall, the 10-day aspirin treatment significantly increased gastric mucus secretion from 0.8 (interquartile range 1.7) to 1.6 (1.6) mg hexose/10 min ($P < 0.05$), with a concomitant and significant decrease in the gastric acid/mucus ratio from 4.3 (5.2) to 2.9 (4.7) ($P < 0.01$). Subsequent analysis of two subgroups of volunteers categorized according to their endoscopic status ("severe gastropathy" vs. "modest gastropathy") revealed that changes in gastric secretory parameters occurred exclusively in those subjects without severe gastric injury; there was no alteration in these parameters in subjects with severe gastric injury.
Conclusions The results of this study suggest that the reactive increase in gastric mucus secretion is an adaptive defense mechanism against low-dose aspirin-induced gastropathy. In some individuals, such a response may be insufficient to prevent the development of severe mucosal injury and even ulcers and their complications.

Keywords Low-dose aspirin · Gastropathy · Gastric adaptation · Gastric mucus secretion

Abbreviations

COX	Cyclooxygenase
EGT	Endoscopic gastrin test
GSRS	Gastro-intestinal symptom rating scale
LDA	Low-dose aspirin
MLS	Modified Lanza score
NSAID	Non-steroidal anti-inflammatory drug

Introduction

Aspirin has long been used as an effective antipyretic analgesic in the treatment of a wide spectrum of conditions and diseases. It is also now widely administered at relatively low doses (100 mg/day in most Japanese patients) as an antithrombotic drug for the prevention of cerebrovascular and cardiovascular disease. Despite the definite benefits of these antithrombotic effects, aspirin, even at a low dose, is known to cause upper gastro-intestinal (GI) complications, such as hemorrhagic gastritis and gastroduodenal ulcers [1, 2]. Our current understanding of the gastric mucosa is that its integrity is dependent on an equilibrium between aggressive factors and protective mechanisms [3–5]. Consequently, a good understanding of how these factors contribute to aspirin-induced epithelial disruption is important for establishing a preventive strategy for upper GI complications.

The gastric mucus serves as the first line of mucosal defense against noxious luminal acid by creating an unstirred layer on the mucosal surface that both supports the maintenance of a near-neutral pH at the surface and acts as a physical barrier [3–5]. The turnover of surface mucus biosynthesis can ultimately be reflected by an increase or decrease in the amount of soluble mucus secreted in the gastric lumen [6, 7]. In clinical study of patients taking long-term low-dose aspirin (LDA) for cardiovascular and/or cerebrovascular disease, we recently found that the level of gastric mucus secretion was significantly higher in chronic LDA-takers than in non-aspirin controls [8]. In addition, the higher level of mucus secretion was more prominent in LDA-takers without severe gastric injury than in those with severe gastric injury [8]. Based on the results of this observational study, we speculated that the higher level of mucus secretion in LDA-takers might be a reactive response to repetitive oral administration of LDA and that the reactive mucus secretion might play a pivotal role in gastric mucosal protection against luminal irritants. However, to prove that gastric mucus secretion is actually changed by the administration of lDA, we needed to conduct a subsequent study to measure gastric mucus secretion both prior to and after the administration of LDA in an appropriate subject group, such as healthy volunteers.

It is well-known that the gastric mucosa can become more tolerant or adaptive in response to the prolonged administration of noxious agents, such as aspirin [9]. In a number of studies in humans, a high dose of aspirin (1–2.5 g per day) was shown to consistently induce maximal gastric injury within 3 days of treatment initiation, but thereafter the lesions tended to resolve despite continued administration of the drug [9–11]. However, the precise mechanism of this adaptation to aspirin under depleted prostaglandin biosynthesis due to the inhibition of the cyclo-oxgenase (COX) [12, 13] enzyme remains to be clarified. In addition, although the aspirin-induced gastric mucosal injury and subsequent adaptive response showed a dose–response relationship [14], few time-course studies on LDA-induced gastric mucosal injury have been reported [15, 16].

In this study involving healthy volunteers, we performed a time-course study to monitor gastric mucosal injury and gastric secretory parameters during a 10-day administration of 100 mg aspirin. Our aim was to determine the relationship between the gastric mucosal adaptive response and gastric secretory parameters.

Methods

Thirty male asymptomatic and healthy volunteers (mean age 26.7 years, range 19–42 years), all of whom were non-smokers were enrolled in this study. All subjects had *Helicobacter pylori pylori*-negative status determined by the ^{13}C-urea breath test. None of the subjects had a history of peptic ulcer or *H. pylori* eradication, and none was taking any drugs before entry into the study or during the study period. All subjects were asked to take 100 mg of enteric-coated aspirin (Bayaspirin, Bayer, Tokyo) once in the morning each day for 10 days. Each participant underwent three endoscopic examinations: one before (day 0) and two (on day 3 and day 10) after the initiation of drug administration. Each endoscopic examination was performed after an overnight fast, and the drug was administered 2 h before the examination. In each endoscopic examination, gastric secretory function was evaluated with the endoscopic gastrin test (EGT) technique described below, and the extent of endoscopically assessed gastric mucosal injury was semi-quantitatively evaluated. The study was approved by the Tohoku University School of Medicine Ethics Committee (2009-356), and each subject gave written informed consent.

Gastric Aspiration with the EGT Technique

The details of EGT have been reported previously [17]. Briefly, the subjects were injected intramuscularly with pentagastrin at a dose of 6 μg/kg (pentagastrin; Sigma, St. Louis, MO) about 15 min before the endoscopic examination. Following insertion of the endoscope into the stomach, the gastric fluid which had pooled in the stomach was aspirated and discarded. The gastric juice newly secreted between 20 and 30 min after the pentagastrin injection and which pooled in the upper part of the participant's stomach, with the individual lying in a left lateral decubitus position, was aspirated and collected under direct visualization during the routine endoscopic examination. During the examination, the subjects were asked not to swallow their saliva. After the collection of gastric juice, the endoscope was removed. The volume of gastric juice collected in the 10-min period was recorded, and the collected gastric juice was then divided into two aliquots, with one aliquot subjected to analysis for gastric acid secretion and the other to analysis for gastric mucus secretion. The laboratory investigators were blinded to the subjects' medical information.

K. Iijima (✉) · T. Iwabuchi · N. Ara · T. Koike · H. Shinkai · T. Shimosegawa
Division of Gastroenterology, Tohoku University Graduate School of Medicine, 1-1 Seiryo-machi, Aobaku, Sendai, Miyagi 980-8574, Japan
e-mail: kiijima@med.tohoku.ac.jp

Y. Kamata · T. Ichikawa
Department of Pathological Biochemistry, Kitasato University School of Allied Health Sciences, Sagamihara, Kanagawa, Japan

K. Ishihara
Kitasato Junior College of Health and Hygienic Sciences, Niigata, Japan

Assessment of Gastric Acid Secretion

The H^+ concentration of the gastric juice was determined by titration. Acid output in the 10-min period was calculated by multiplying the volume by the H^+ concentration, and the EGT value was expressed as milliequivalents per 10 min. We have previously shown that the EGT values correlate very well with the peak acid output determined by conventional methods (correlation efficient 0.92) with high reproducibility (coefficient of variation 5.6 %) [17].

Extraction and Isolation of Mucin in Gastric Juice

The collected gastric juice was centrifuged at 1,500 *g* for 30 min at room temperature to remove contaminating debris. The mucin, a major component of gastric mucus, in the gastric juice samples was extracted and isolated using a previously described method which enables mucin to be successfully isolated and condensed from the gastric juice without contamination by non-mucin glycoproteins, such as serum-type glycoproteins. Absolute ethanol (6 mL) was added to 2 mL of the supernatant obtained from the gastric juice to a final concentration of 75 % ethanol (v/v). The resultant suspension was maintained at 4° overnight to complete the precipitation, after which the precipitate was collected by centrifugation (8,000 *g* for 30 min at 4 °C). The pellet was dissolved in distilled water (2 mL), and its hexose content was measured by the phenol–sulfuric acid method. The mucin content of the gastric juice was expressed as the amount of hexose in the solution obtained by the precipitation method (μg/mL). The total mucus output was determined by multiplying the mucin concentration by the volume of gastric juice collected in the 10-min period [18].

Endoscopic Evaluation of Gastric Mucosal Injury

The grade of gastric mucosal injury was assessed according to the modified Lanza score (MLS) [19, 20]. In our analysis, the scoring system was based on the number of erosions and/or ulcers despite the presence or absence of hemorrhaging because petechial hemorrhages are generally of little clinical significance [21]. Gastric mucosal injury was graded into six groups, ranging from 0 to 5, with grade 0 = no erosion, grade 1 = one to two lesions of erosion localized in one area of the stomach, grade 2 = three to five lesions of erosion localized in one area of the stomach, grade 3 = six to nine lesions of erosion localized in one area of the stomach or no more than ten lesions in two areas of the stomach, grade 4 = erosions in three areas of the stomach or no fewer than ten lesions in the whole stomach, and grade 5 = a gastric ulcer defined as a mucosal defect >5 mm in diameter.

During endoscopy, more than 40 endoscopic pictures covering the whole area of the stomach were saved in the database. These were subsequently used by two endoscopists (KI, TI), who were blinded to other information on the subjects, to independently grade the MLS. In cases of disagreement, a consensus was reached through joint review of the endoscopic pictures.

Evaluation of Gastrointestinal Symptoms

Gastrointestinal symptoms were assessed using the GI symptom rating scale (GSRS) [22]. The GSRS contains 15 items rated on the 7-point Likert scale, where score 1 represents "no discomfort" and score 7 represents "very severe discomfort." The items have been divided into five major GI symptoms: abdominal pain, reflux, indigestion, diarrhea, and constipation. The GSRS was used before each endoscopic examination and the scores were averaged for each major GI symptom.

Statistics

Based on our recent report [8], the number of subjects required to detect an absolute difference in gastric mucus secretion at a two-sided alpha level of 0.05 with 80 % power was calculated to be $n = 26$. In addition to various gastric secretory parameters derived directly from the determination of the gastric aspirates, we calculated the ratio of total acid output relative to total mucus output (acid/mucus ratio), which reflects the importance of the equilibrium between aggressive and protective factors in the pathogenesis of gastric mucosal injury [8]. Because the distribution of some raw data was considerably skewed, continuous data as well as non-parametric data were expressed as the median and inter-quantile range (IQR), and the statistical significance of differences was determined by the Wilcoxon rank sum test. A P value of <0.05 was considered to be statistically significant.

Results

Of the 30 subjects who were initially enrolled in this study, one individual was subsequently excluded from the analysis due to a moderate level of endoscopically assessed gastric mucosal injury at entry. The remaining 29 subjects completed the study protocol without problematic side effects. In all gastric aspirations with the EGT technique, a sufficient amount of gastric fluid was collected for the analysis of both gastric acid and mucus secretion.

Endoscopic Gastric Injury

The administration of 100 mg aspirin to healthy volunteers induced a variety of endoscopic gastric mucosal reactions. While the aspirin did not cause any discernible mucosal damage in some of the volunteers, it did cause severe mucosal damage, even gastric ulceration, in others during the experimental period. Overall, the median MLS showed a modest but significant increase by 3 days after first administration of the drug, from 0.0 (IQR 1.0) at day 0 to 1.0 (IQR 1.0) at day 3 ($P < 0.01$); thereafter, there was no further increase in the MLS between days 3 and 10 [1.0 (IQR 1.0) vs. 1.0 (IQR 1.3); not significant) (Fig. 1a; Table 1).

Since a wide range of gastric mucosal injuries were observed following the administration of LDA, all subjects were subsequently divided into one of two subgroups according to their endoscopic status on day 10—i.e., one group comprising subjects (23/29, 79 %) showing only modest endoscopic evidence of mucosal damage (MLS $\leq$2 on day 10) and the other group comprising subjects (6) with severe gastric mucosal injury (MLS $\geq$4 at day 10) (there was no subject presenting a MLS of 3 on day 10). Further analysis revealed that there were substantial differences in the time-course of LDA-induced gastropathy between the two subgroups. Subjects with modest gastric injury showed an initial significant increase in MLS from 0.0 (IQR 1.0) on day 0 to 1.0 (IQR 1.0) on day 3; thereafter, however, the score tended to decrease, reaching 1.0 (IQR 1.5) on day 10 ($P = 0.11$); this MLS on day 10 was no longer significantly different from that on day 0 (Fig. 1b; Table 2). Subjects with severe gastric injury also showed an initial, modest increase in MLS from 0.0 (IQR 1.0) on day 0 to 1.5 (IQR 2.0) on day 3 ($P < 0.05$); however, in contrast to the subjects with modest gastric injury, these subjects showed a further prominent increase in MLS to 4.0 (IQR 0) on day 10 ($P < 0.05$) (Fig. 1c; Table 2).

Gastric Secretory Parameters

The analysis of all the participants showed that the gastric secretory volume and acid secretion level were unchanged after 10 days of LDA administration, but that the mucus secretion level steadily increased during this time, although it showed a relatively skewed distribution. Overall, the median LDA-induced increase in gastric mucus output rose from 0.8 (IQR 1.7) mg hexose/10 min on day 0 to 1.1 (IQR 1.2) mg hexose/10 min on day 3 and to 1.6 (IQR 1.6) mg hexose/10 min on day 10, with the difference reaching statistical significance on day 10 compared with day 0 ($P = 0.02$) (Fig. 2a; Table 1). Together with the increase in mucus secretion, LDA administration resulted in a consistent decrease in the gastric acid/mucus ratio, from 4.3 (IQR 5.2) on day 0, to 3.6 (IQR 5.6) on day 3, and finally to 2.9 (IQR 4.7) on day 10, with the difference reaching statistical significance on day 10 compared with day 0 ($P = 0.008$) (Fig. 2b; Table 1).

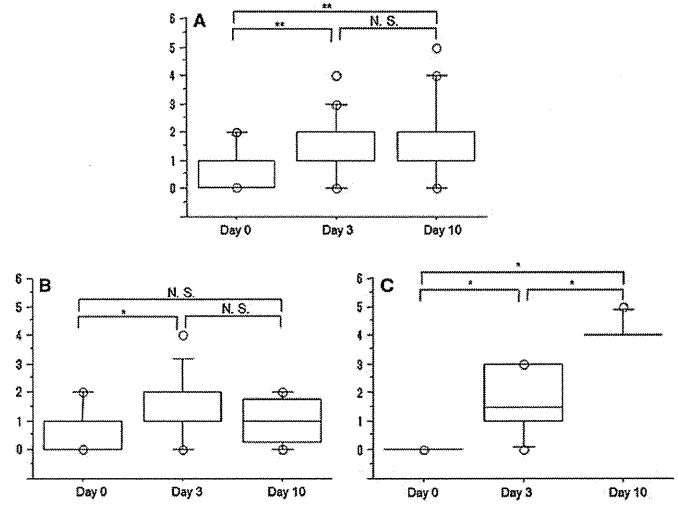

Fig. 1 Time-course of modified Lanza score during the 10-day administration of 100 mg aspirin. Data are shown for the entire subject cohort (**a**), for those with modest gastric injury on day 10 (**b**), and for those with severe gastric injury on day 10 (**c**). $*P < 0.05$, $**P < 0.01$, *N.S.* not significant

Table 1 Change in various parameters during the 10-day administration of low-dose aspirin among the entire study cohort

Parameters	Day 0	Day 3	Day 10	*P* value
Epigastric pain score	1.0 (0.3)	1.0 (0.4)	1.0 (0.7)	Day 0 vs. day 3: $P = 0.82$
				Day 3 vs. day 10: $P = 0.65$
				Day 0 vs. day 10: $P = 0.31$
Modified Lanza score	0.0 (1.0)	1.0 (1.0)	1.0 (1.3)	Day 0 vs. Day 3: $P = 0.002$
				Day 3 vs. day 10: $P = 0.31$
				Day 0 vs. day 10: $P = 0.002$
Secretion volume (mL)	36.0 (8.9)	33.0 (13.0)	33.0 (7.0)	Day 0 vs. day 3: $P = 0.60$
				Day 3 vs. day 10: $P = 0.39$
				Day 0 vs. day 10: $P = 0.010$
Total acid output (mEq/10 min)	4.3 (1.5)	4.3 (1.2)	4.4 (1.1)	Day 0 vs. day 3: $P = 0.68$
				Day 3 vs. day 10: $P = 0.97$
				Day 0 vs. day 10: $P = 0.49$
Total mucus output (mg hexose/10 min)	0.8 (1.7)	1.1 (1.2)	1.6 (1.6)	Day 0 vs. day 3: $P = 0.80$
				Day 3 vs. day 10: $P = 0.12$
				Day 0 vs. day 10: $P = 0.02$
Gastric acid/mucus ratio	4.3 (5.2)	3.6 (5.6)	2.9 (4.7)	Day 0 vs. day 3: $P = 0.38$
				Day 3 vs. day 10: $P = 0.14$
				Day 0 vs. day 10: $P = 0.009$

All data are expressed as the median, with the inter-quartile range (IQR) given in parenthesis

When the same analyses of gastric secretory parameters were repeated in the two subgroups described above, the changes in the parameters showed a pattern that differed depending on the extent of injury. Subjects with modest gastric injury showed a steadily increasing trend in gastric mucus output during the observation period, with the median value increasing from 0.8 (IQR 1.6) mg hexose/10 min on day 0, to 1.1 (IQR 1.1) on day 3, and finally to 1.4 (IQR 1.7) on day 10, with the difference reaching statistical significance on day 10 compared with day 0 ($P = 0.02$). In contrast, mucus output in subjects with severe gastric injury remained at a similar level during the observation period, with median values of 1.9 (IQR 1.1) mg hexose/10 min on day 0, 1.8 (IQR 0.9) mg hexose/10 min on day 3, and 1.9 (IQR 0.8) mg hexose/10 min on day 10 (Table 2). There was no significant alteration in the gastric acid secretion level during the observation period regardless of the extent of gastric mucosal injury. Consequently, the gastric acid/mucus ratio steadily decreased in subjects with modest gastric injury during the 10-day administration of LDA, with median ratios of 5.2 (IQR 7.6) on day 0, 3.8 (IQR 5.6) on day 3, and 2.9 (IQR 4.9) on day 10; the difference was statistically significant between day 0 and day 10 ($P = 0.009$). In contrast, there was no trend of alteration in the acid/mucus ratio in subjects with severe gastric injury during the observation period; the median ratio was 3.1 (IQR 0.5) on day 0, 2.1 (IQR 1.9) on day 3, and 3.0 (IQR 1.8) on day 10 (Table 2). Due to the small number of subjects with severe gastric injury ($n = 6$) and the relatively scattered data, we failed to find any significant difference in the relative change in mucus output or acid/mucus ratio between subjects with and without severe gastric injury [1.1 (IQR 1.1) vs. 1.3 (IQR 0.9), respectively, for mucus output on day 10 relative to the pre-treatment value of 0.9 (IQR 0.5) vs. 0.7 (IQR 0.5) for acid mucus ratio; $P > 0.2$ for each].

In addition, there was no significant difference in the pre-treatment (day 0) values of the gastric secretory parameters between those with and those without severe gastric mucosal injury, although gastric acid secretion and mucus output tended to be higher in those with severe gastric injury compared to those without the injury (Table 2).

Symptom Score

There was no discernible change in the epigastric pain score or other abdominal symptom scores during the observation period irrespective of the extent of endoscopic gastric mucosal injury (Tables 1, 2).

Discussion

In this study, we sought to determine the relationship between LDA-induced gastropathy and gastric secretory function, especially gastric mucus secretion, by monitoring these parameters using an endoscopic aspiration technique

Table 2 Changes in various parameters during the 10-day administration of low-dose aspirin among subgroups based on the manifestation of endoscopic gastric mucosal injury

Parameters	Subjects with modest gastropathy ($n = 23$)				Subjects with severe gastropathy ($n = 6$)			
	Day 0	Day 3	Day 10	*P* value	Day 0	Day 3	Day 10	*P* value
Epigastric pain score	1.0 (0.6)	1.0 (0.6)	1.0 (0.7)	Day 0 vs. day 3: $P = 0.93$	1.0 (0.3)	1.0 (0)	1.0 (0.7)	Day 0 vs. day 3: $P = 0.99$
				Day 3 vs. day 10: $P = 0.75$				Day 3 vs. day 10: $P = 0.65$
				Day 0 vs. day 10: $P = 0.50$				Day 0 vs. day 10: $P = 0.29$
Modified Lanza score	0.0 (1.0)	1.0 (1.0)	1.0 (1.5)	Day 0 vs. day 3: $P = 0.02$	0.0 (1.0)	1.5 (2.0)	4.0 (0)	Day 0 vs. day 3: $P = 0.04$
				Day 3 vs. day 10: $P = 0.11$				Day 3 vs. day 10: $P = 0.03$
				Day 0 vs. day 10: $P = 0.08$				Day 0 vs. day 10: $P = 0.02$
Secretion volume (mL)	36.0 (9.4)	31.5 (12.1)	31.5 (7.1)	Day 0 vs. day 3: $P = 0.83$	40.0 (7.5)	33.5 (15.5)	39.0 (9.0)	Day 0 vs. day 3: $P = 0.23$
				Day 3 vs. day 10: $P = 0.11$				Day 3 vs. day 10: $P = 0.34$
				Day 0 vs. day 10: $P = 0.08$				Day 0 vs. day 10: $P = 0.60$
Total acid output (mEq/10 min)	4.1 (1.4)	4.3 (1.1)	4.2 (1.0)	Day 0 vs. day 3: $P = 0.96$	5.3 (1.9)	4.5 (2.9)	5.2 (1.3)	Day 0 vs. day 3: $P = 0.46$
				Day 3 vs. day 10: $P = 0.55$				Day 3 vs. day 10: $P = 0.46$
				Day 0 vs. day 10: $P = 0.93$				Day 0 vs. day 10: $P = 0.17$
Total mucus output (mg hexose/10 min)	0.8 (1.6)	1.1 (1.1)	1.4 (1.7)	Day 0 vs. day 3: $P = 0.92$	1.9 (1.1)	1.8 (0.9)	1.9 (0.8)	Day 0 vs. day 3: $P = 0.46$
				Day 3 vs. day 10: $P = 0.12$				Day 3 vs. day 10: $P = 0.92$
				Day 0 vs. day 10: $P = 0.02$				Day 0 vs. day 10: $P = 0.60$
Gastric acid/mucus ratio	5.2 (7.6)	3.8 (5.6)	2.9 (4.9)	Day 0 vs. day 3: $P = 0.48$	3.1 (0.5)	2.1 (1.9)	3.0 (1.8)	Day 0 vs. day 3: $P = 0.34$
				Day 3 vs. day 10: $P = 0.09$				Day 3 vs. day 10: $P = 0.92$
				Day 0 vs. day 10: $P = 0.009$				Day 0 vs. day 10: $P = 0.46$

All data are expressed as the median, with the IQR given in parenthesis

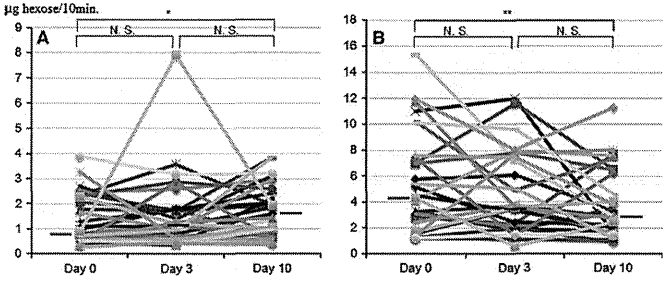

Fig. 2 Time-course of gastric mucus output (**a**) and gastric acid/mucus ratio (**b**) during the 10-day administration of 100 mg aspirin among the entire study cohort. * $P < 0.05$, ** $P < 0.01$. *N.S.* not significant. *Dark horizontal bars* Median values

in a cohort of healthy volunteers receiving daily doses of LDA for 10 days. In the study cohort overall, LDA administration significantly increased gastric mucus secretion with a concomitant significant decrease in the gastric acid/mucus ratio. Interestingly, such changes in gastric secretory parameters were observed exclusively in those subjects without severe gastric injury, while there was no alteration in these parameters in the subjects with severe gastric injury. Thus, our results suggest that a reactive increase in gastric mucus secretion may be physiologically significant as an adaptive defense mechanism against LDA-induced gastropathy.

Earlier studies with high doses of aspirin demonstrated that gastric injury peaked at 3 days of treatment at an intensive level, followed by a tendency for the lesions to resolve despite continued administration of the drug [9–11, 14]. However, in more recent observational studies with LDA of 7-day duration, gastric injury was found to be relatively modest and to reach a plateau at 3 days of treatment [15, 16]. Our results are consistent with those of these more recent studies [15, 16] and demonstrate that, overall, LDA induced a modest but significant increase in gastric mucosal injury on day 3 in our healthy volunteers, with the level of injury remaining relatively constant up to day 10. Nonetheless, we could recognize two different subgroups of subjects based on the time-course of the gastric injury during LDA administration. Although both groups showed an initial, significant increase in gastric injury on day 3, the patterns were different thereafter. One group, comprising the majority (79 %) of subjects, showed a tendency for resolution of the injury between day 3 and day 10, while the other group, comprising a minority (21 %) of the subjects, showed a further exacerbation of the injury during the same period. This finding that not all subjects exhibited sufficient adaptive resolution of the injury is consistent with the results of an earlier study [13]. Hence, this study reveals that whereas the stomach of the majority of our subjects adapted to repeated administration of LDA, this typical gastric response failed in a small number of our subjects.

Early animal model studies produced controversial results with respect to the effect of aspirin administration on gastric mucus secretion, with some studies showing that the thickness of the gastric gel mucus significantly increased following exposure to aspirin [23, 24], while others showed that aspirin inhibited the biosynthesis of mucus [25], gastric mucosal mucus content [26, 27], and/or secretion of the gastric mucus [28]. Yet another study demonstrated recovery of the gastric mucosal mucin several hours after a significant decrease in secretion immediately following instillation of aspirin [29]. Different methodological approaches could be partly responsible for these inconsistent results. Nonetheless, these animal model studies are difficult to extrapolate to the human situation of repeated doses of the drug, because other than the potential inter-specific differences, most of these animal studies dealt with the results from observations of only several hours after a single instillation of aspirin [23–29]. Meanwhile, there have been very few human studies that have investigated the relationship between repeated aspirin intake and gastric mucus secretion [14]. To our knowledge, this is the first study that has examined the effect of repeated LDA administration and gastric mucus secretion in human subjects. Our results reveal a significant, overall increase in gastric mucus secretion in our healthy volunteers following 10 days of LDA administration.

The substantial increase in gastric mucus secretion by day 10 of LDA administration in our healthy volunteers seems to contradict a previous study describing that a 1-week administration of naproxen decreased total mucin output in *H. pylori*-negative healthy volunteers [30]. Differences in the doses of the employed drugs might explain these different results, as would the different type of drugs [aspirin vs. other nonsteroidal anti-inflammatory drugs (NSAIDs)]. Although both aspirin and other NSAIDs similarly deplete gastric prostaglandin synthesis by the inhibition of the COX enzyme [12, 13, 21], these aspirin and other NSAIDs are known to differently affect two types of COX enzyme isoforms. While ordinary NSAIDs inhibit both COX-1 and COX-2, aspirin preferentially inhibits COX-1. There is a possibility that, unlike other NSAIDs, aspirin can uniquely affect gastric mucus secretion.

Our analysis of the two subgroups of study subjects based on the extent of gastric mucosal injury revealed a significant increase in gastric mucus secretion only in those who showed an adaptive response that resolved the mucosal injury in the later phase of the study; in contrast, mucus secretion was unchanged in those subjects who failed to show the adaptive response and experienced further gastric injury. This finding seems to be consistent with those of previous studies in rats showing that the initial aspirin-induced reduction in gastric mucosal mucin was followed by subsequent recovery of the mucin [29] and that the mucin content was significantly correlated with the extent of aspirin-induced gastric damage [31]. In contrast, baseline values (day 0) of gastric mucus secretion tended to be paradoxically lower in subjects without severe gastric injury compared to those with injury, although not significantly so. Thus, a reactive increase in gastric mucus secretion following exposure to LDA—rather than a steady-state secretory level—is more likely to be important in terms of acting as a protective covering that inhibits further deterioration of focally injured areas. However, this process may fail in some individuals who go on to develop severe mucosal injury, even ulcers, and related

complications. Additionally, previous studies have shown that NSAIDs and aspirin increase gastric epithelial cell exfoliation [32, 33]. Since gastric epithelial cells contain large amounts of mucus, one may consider that an increased amount of mucus in the collected gastric juice in our study subjects was due to the exfoliation by the aspirin administration rather than any increase in mucus secretion. However, such scenario is refuted by the findings that the amount of mucus tended to increase more in subjects with the least endoscopically assessed injury.

Of relevance to our findings in healthy volunteers receiving LDA over the short term (10 days), we recently demonstrated in the clinical setting of long-term LDA-takers (>2 years of intake in the majority of cases) that the gastric mucus secretion level was significantly higher in aspirin-takers than in the non-aspirin controls and that the increased level of mucus secretion was more prominent in aspirin-takers without severe gastric injury than in those with severe gastric injury [8]. Taken together, it is likely that the changes in gastric secretory function in response to apparent gastric mucosal injury observed in the present short-term study could persist during prolonged administration, suggesting that our findings have clinical implications. To date, the intricate mechanisms by which gastric mucosal adaptation to aspirin and other NSAIDs occurs are not fully understood, although the process entails an increase in mucosal blood flow [11, 13, 34] and enhancement in mucosal defense by increased cellular proliferation [14, 35, 36]. Otherwise, the results of our study suggest that an increase in mucus secretion in response to damaging agents could have an important role in gastric adaptation. Since our study cohort consisted of a relatively homogenous population of healthy volunteers (relatively young, male, *H. pylori*-negative, non-smokers), further studies regarding genetic polymorphisms responsible for gastric mucus secretion independently of prostaglandin synthesis are warranted to determine the susceptibility of LDA-takers to drug-induced gastropathy. Among prostaglandin-independent pathways, growth factors, such as transforming growth factor-alpha and epidermal growth factor, trefoil peptides, and/or other regenerating proteins, could be potential candidates responsible for the higher level of mucus secretion observed in the LDA-takers of this study because these molecules are known to act as protective agents for the gastric epithelium and to be induced by the administration of aspirin or other NSAIDs [37–39].

In conclusion, our results suggest the potential contribution of a reactive increase in gastric mucus secretion to the gastric adaptive response to repeated doses of LDA. Therapeutically, these results also suggest that, in addition to the inhibition of gastric acid secretion, the potentiation of gastric mucus secretion could be another promising approach for the prevention of LDA-induced gastropathy.

Conflict of interest None.

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CASE REPORT

Cystic lesion within the infraspinatus muscle caused by a partial-thickness rotator cuff tear

Hirotaka Sano · Shin Hitachi · Akira Oizumi · Nobuyuki Yamamoto · Eiji Itoi

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Introduction

A cystic lesion within the rotator cuff muscle is a relatively rare pathological condition that can be diagnosed by magnetic resonance (MR) imaging [1–4]. In the literature, the presence of cystic lesions has been confirmed mainly in shoulders with partial- or full-thickness rotator cuff tears [1, 3]. However, the true etiology of intramuscular cystic lesions has not yet been completely clarified.

Lunn et al. [5] reported isolated lesions of the infraspinatus tendon or its musculotendinous junction associated with muscle edema in 19 shoulders. In these patients, severe infraspinatus muscle edema was observed followed by wasting as well as fatty infiltration of this muscle. However, they did not mention whether a cystic lesion was observed in the infraspinatus muscle or not; therefore, the relationship between this lesion and the intramuscular cyst remained unclear [5].

The patient reported in this article developed edema in the infraspinatus muscle associated with a partial-thickness rotator cuff tear that was subsequently replaced by an intramuscular cystic lesion. Furthermore, the pathogenesis of an intramuscular cystic lesion in this patient was discussed based on both MR and surgical findings. Consent for publication of all the data was obtained from the patient.

Case report

A 33-year-old, right-hand-dominant male, who was a construction company employee, injured his right shoulder in a car accident. The right side of his car had collided with a dumpster, and his right shoulder had collided with the door of the car. He had been suffering from motion pain in his right shoulder since the accident. The primary physician did not detect any bony abnormalities from the plain radiographs. Since the patient's shoulder pain was not relieved by non-steroidal anti-inflammatory drugs, he was referred to our clinic 3 months later.

On initial examination, the impingement test and the empty can test were positive. However, muscle weakness was not observed in the manual muscle test. Neither the supraspinatus nor the infraspinatus tendon showed a tear on MR images, although a small amount of effusion was seen in the subacromial bursa. The distal portion of the infraspinatus muscle was depicted with high intensity in T2-weighted images, which also suggested the presence of a small amount of effusion in the muscle belly (Fig. 1). The subacromial bursa was injected with corticosteroids three times by the principal author; this successfully relieved the patient's shoulder pain for several months.

H. Sano (✉) · N. Yamamoto · E. Itoi
Department of Orthopaedic Surgery,
Tohoku University School of Medicine,
1-1 Seiryo-machi, Aobaku, Sendai 980-8574, Japan
e-mail: sanohirotaka@med.tohoku.ac.jp

N. Yamamoto
e-mail: koyomoc@med.tohoku.ac.jp

E. Itoi
e-mail: itoi-eiji@med.tohoku.ac.jp

S. Hitachi
Department of Diagnostic Radiology, Tohoku University School of Medicine, 1-1 Seiryo-machi, Aobaku, Sendai 980-8574, Japan
e-mail: hitachi@rad.med.tohoku.ac.jp

A. Oizumi
Department of Orthopaedic Surgery, Kurihara Central Hospital, 3-1-1 Miyano-chuo, Tsukidate, Kurihara, Miyagi 987-2205, Japan
e-mail: akirazaru@syd.odn.ne.jp

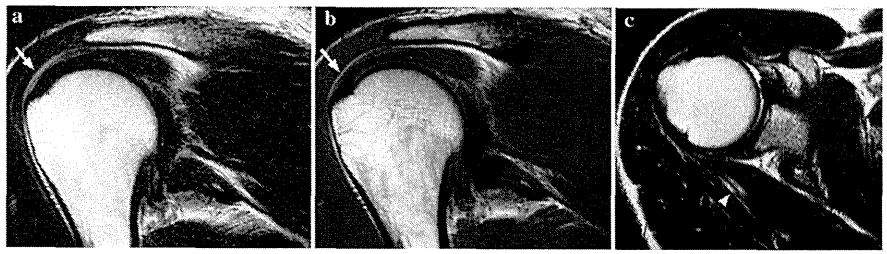

Fig. 1 MR findings 3 months after the injury (**a** T2-weighted oblique coronal image; **b** T1-weighted oblique coronal image, **c** T2-weighted axial image). In the T2-weighted image, the subacromial bursa was depicted with high intensity (**a** *arrow*). On the other hand, it was depicted with low intensity in the T1-weighted image (**b** *arrow*). The distal portion of the infraspinatus muscle represented high intensity, which suggested the presence of a small amount of effusion in the muscle belly (**c** *arrowhead*). An obvious tear was not observed in the supraspinatus or infraspinatus tendon

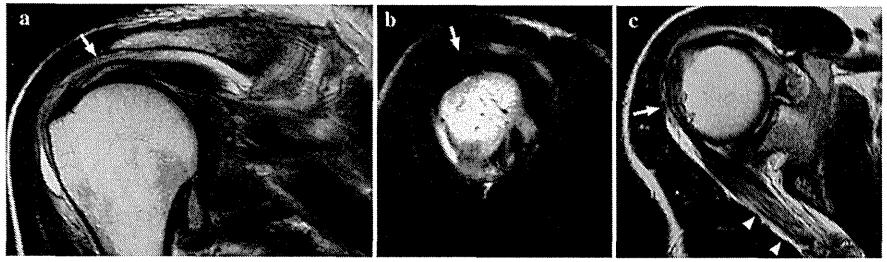

Fig. 2 MR findings 17 months after the injury (T2-weighted images: **a** oblique coronal plane; **b** oblique sagittal plane, **c** axial plane). A marked edema (**a** *arrow*) with the collection of effusion was seen in the subacromial bursa. Proliferation of the synovium with fluid collection was also seen in the inferior pouch. Both the thinning of the infraspinatus tendon substance and an articular side partial-thickness tear with a deep delamination were confirmed (**b**, **c** *arrows*). Intramuscular edema extended to the upper part of the infraspinatus muscle (**c** *arrowheads*)

Seventeen months after the injury, severe motion pain as well as nocturnal pain around his right shoulder recurred, particularly in the scapular region. He was bothered by the severe motion pain when he elevated or externally rotated his right shoulder; however, he did not complain about spontaneous pain throughout the daytime. Physical examinations revealed marked atrophy of the infraspinatus muscle, and the strength of the external rotation was reduced to the F level. No restriction was seen for either the active flexion or abduction angle. MR images showed that there was a partial-thickness tear with deep delamination in the articular side of the infraspinatus tendon. Atrophy and edema were seen in the infraspinatus muscle belly (Fig. 2). At this time point, a cystic lesion appeared in the infraspinatus muscle belly (Fig. 3). Interestingly, this cystic lesion was localized within the upper portion of infraspinatus muscle. The lower portion of this muscle remained intact. On electromyography, the magnitude of motor unit potentials in the infraspinatus muscle was slightly reduced, which could be interpreted as an incomplete injury of the suprascapular nerve. To wait for the spontaneous healing of this nerve, the patient was conservatively treated for further several months.

Surprisingly, the intramuscular cystic lesion was dramatically enlarged in the MR images, which were taken 23 months after the injury. A partial-thickness tear in the infraspinatus tendon became more evident. On T2-weighted images, a high-intensity area extended from the site of the partial-thickness tear to the cystic lesion in the infraspinatus muscle. Joint fluid collection as well as proliferation of synovium was also confirmed inside the glenohumeral joint (Fig. 4). However, the patient's clinical symptoms were almost the same as before, although both active flexion and abduction were limited up to 90° because of severe motion pain. For the differential diagnosis of the tumorous lesion, a needle biopsy was performed under ultrasound guidance. In the histological specimen obtained from the wall of the cystic lesion, the presence of chronic

inflammation was confirmed, but no tumorous cells were observed.

Based on these findings, surgery was performed both for the exploration and the repair of the partial-thickness rotator cuff tear. Arthroscopic observation revealed the presence of a partial-thickness tear in the articular side of the infraspinatus tendon (Fig. 5). Marked proliferation of synovium was seen both in the rotator interval and in the inferior pouch. Pathological diagnosis of the harvested synovium was non-specific chronic synovitis. No tears could be identified on the bursal surface of the infraspinatus tendon. In the open procedure, the infraspinatus tendon was detached once from the middle facet of the greater tuberosity. A small but deep hole was observed on the cutting surface; the hole extended proximally toward the muscle belly, constituting a narrow tunnel (Fig. 6). The depth of this tunnel was approximately 5 cm. The tunnel was enlarged with a scalpel to divide the infraspinatus tendon into two layers, which were then reattached together to the middle facet by the transosseous suture technique.

The intramuscular cystic lesion diminished quickly after surgical treatment. In the follow-up MR imaging study performed 12 months after surgery, no re-tear was observed in the infraspinatus tendon (Fig. 7). Although the patient complained of neither rest pain nor nocturnal pain, the muscle strength of external rotation continued to be limited to the G level.

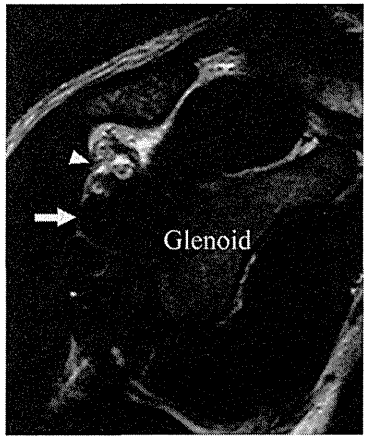

Fig. 3 T2-weighted oblique sagittal MR image at the glenoid level (17 months after the injury). A cystic lesion appeared in the upper portion of the infraspinatus muscle belly at this level (*arrowhead*). On the other hand, the lower portion of the infraspinatus muscle remained intact (*arrow*)

Discussion

With advances in MR imaging, intramuscular edema in the rotator cuff muscles is being confirmed more accurately than before. Previous authors hypothesized that joint fluid enters into the substance of rotator cuff tendon either from the bursal or the joint side, and tracks along the sheath or in the substance of the muscle to form the intramuscular cystic lesion [1, 2]. However, this hypothesis has been developed only based on the radiological findings. None of the authors surgically confirmed the communication between the partial-thickness tear and the intramuscular cystic lesion.

The MR findings in the present patient were consistent with those described in the previous reports. The partial-thickness tear of the infraspinatus tendon seemed to communicate with intramuscular edema as well as a cystic lesion. Moreover, surgical findings also supported the relationship between the partial-thickness tear and intramuscular edema or the cystic lesion. A small partial-thickness tear was observed arthroscopically on the articular side of the infraspinatus tendon. A narrow tunnel was found on the stump of the infraspinatus tendon, which extended medially to the muscle belly. The delamination of the tendon in the limited area seemed to form this narrow tunnel with a one-way valve. Although the pathogenesis in the present case was still controversial, we presumed that the glenohumeral joint fluid might flow into the tendon substance through the articular-side tear, which contributed to the development of edema and cystic lesion in the muscle belly.

It was reported that the denervation of the infraspinatus muscle due to isolated suprascapular nerve palsy or Parsonage-Turner syndrome (neuralgic amyotrophy) showed similar MR findings to those seen in the present case [6–8]. However, the entire infraspinatus muscle belly was usually involved in these pathological conditions. In the present case, the lower portion of the infraspinatus muscle remained intact even 17 months after the injury. Moreover, the intramuscular cystic lesion diminished immediately

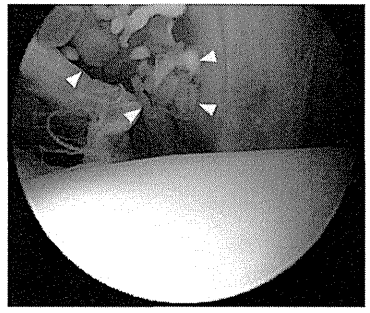

Fig. 5 Arthroscopic findings in the glenohumeral joint. A partial-thickness tear was observed on the articular side of the infraspinatus tendon (*arrowheads*)

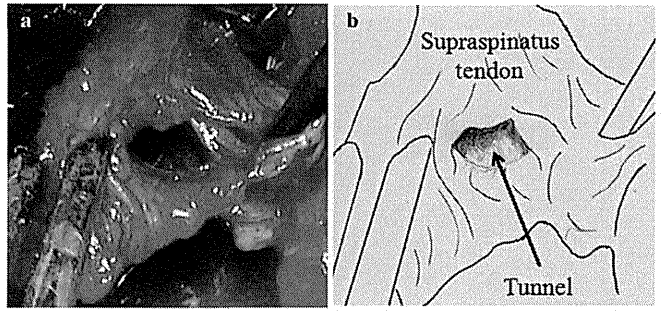

Fig. 6 Surgical findings of the infraspinatus tendon (**a** photograph, **b** schematic illustration). A small tunnel was observed on the cutting surface of the detached infraspinatus tendon that extended proximally to the muscle belly. The depth of this tunnel was approximately 5 cm

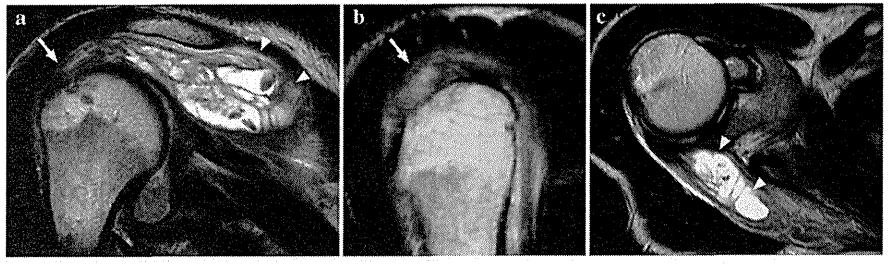

Fig. 4 MR findings 23 months after the injury (T2-weighted images: **a** oblique coronal plane; **b** oblique sagittal plane, **c** axial plane). An intramuscular cystic lesion was dramatically enlarged (**a**, **c** *arrowheads*). Note that no capsular structure was seen around the lesion. The presence of a partial-thickness tear in the infraspinatus tendon (**a**, **b** *arrows*) as well as the synovial proliferation with fluid collection in the inferior pouch became more evident

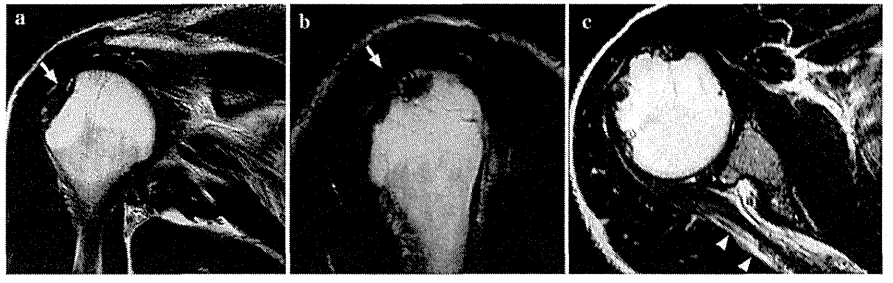

Fig. 7 MR findings 12 months after surgery (T2-weighted images: **a** oblique coronal plane; **b** oblique sagittal plane, axial plane). The infraspinatus tendon healed to the middle facet of the humeral head (**a**, **b** *arrows*). No effusion was observed in the subacromial bursa or glenohumeral joint. However, marked atrophy with fatty degeneration was observed in the infraspinatus muscle (**c** *arrowheads*)

after the repair of the tendinous portion. Based on these findings, we assumed that there was less possibility of Parsonage-Turner syndrome in the present case.

At the time of follow-up, the muscle strength of the infraspinatus had not recovered completely even 12 months after surgery. Atrophy of this muscle continued to be visible on MR images. Early surgical repair of the torn infraspinatus tendon may have provided a better functional outcome in the present case.

Conflict of interest The authors declare that they have no conflict of interest related to the publication of this manuscript.

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